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M.E. Raichle. ..... No Abstract

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3. The Computational Neuron
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J.G. Hildebrand ..... No Abstract

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444. Learning and memory: systems and functions— misc. II 1061
445. Learning and memory: physiology IV 1064
446. Ingestive behavior: taste aversion and neural mechanisms 1066
447. Monoamines and behavior: behavioral effects of dopamine 1070
448. Drugs of abuse: alcohol, barbiturates and benzodiazepines III 1072
449. Drugs of abuse: stimulants I 1074
450. Drugs of abuse: cocaine and dopamine neuronal systems 1076
451. Degenerative disease: Parkinson's II 1080
452. Neuromuscular diseases I 1082

82. History of neuroscience 180

83. Teaching of neuroscience: undergraduate and graduate education 183

84. Teaching of neuroscience: computer-assisted instruction 186

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85. Teaching of neuroscience: elementary and secondary grades 189

THURSDAY, OCTOBER 29

Symposia — 8:30 a.m.

453. Molecular Analyses of Neuronal Physiology and Potential for Gene Therapy Using Herpes Simplex Virus Vectors
Chaired by: A. Geller 1085
454. Thalamic Mechanisms of Nociception
Chaired by: F.A. Lenz 1085

Presidential Special Lecture — 11:45 a.m.

455. Neurobiological Foundations of Speech and Language
P.A. Tallal ..... No Abstract

Slide Sessions — 8:30 a.m.

456. Neuroendocrine regulation II 1085
457. Trauma 1087
458. Neuroglia and myelin IV 1089
459. Gene structure and function VI 1090
460. Potassium channel expression and localization 1092
461. Process outgrowth, growth cones and sprouting V 1094
462. Pattern formation, compartments and boundaries III 1096
463. Nerve growth factor VI 1098
464. Visual cortex: motion processing II 1100
465. Neurotoxicity 1102
466. Transmitters in invertebrates III 1104

Poster Sessions — 8:30 a.m.

467. Genesis of neurons and glia III 1107
468. Cell lineage and determination III 1109
469. Formation and specificity of synapses VI 1112
470. Nutritional and prenatal factors: alcohol 1114
471. Glia and other non-neuronal cells IV 1116
472. Development of cerebral cortex and limbic system II 1119
473. Transplantation: cortex 1121
474. Transplantation: retina, olfactory bulb, SCN 1123
475. Staining, tracing and imaging techniques III 1125
476. Blood-brain barrier I 1128
477. Presynaptic mechanisms VI 1130
478. Sodium channels 1133
479. Calcium channel molecular biology 1138
480. Acetylcholine: CNS II 1140
481. Acetylcholine: muscarinic receptors II 1142
482. Excitatory amino acids: excitotoxicity V 1145
483. Excitatory amino acids: anatomy and physiology III 1148
484. Excitatory amino acids: pharmacology V 1151
485. Excitatory amino acids: receptors VI 1154
486. GABA receptors: function IV 1157
487. GABA receptors: function V 1160
488. Peptides: receptors IV 1162
489. Peptides: physiological effects IV 1165
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491. Catecholamines: receptors III 1170
492. Cardiovascular regulation: forebrain mechanisms 1173
493. Cardiovascular regulation: upper brainstem mechanisms 1176
494. Cardiovascular regulation: spinal cord and medulla 1178
495. Cardiovascular regulation: cardiac control and sympathetic rhythms 1180
496. Cardiovascular regulation: hypertension, reflexes and peripheral autonomics 1183
497. Cardiovascular regulation: lower brainstem II 1186
498. Subcortical somatosensory pathways: trigeminal 1189
499. Auditory system: cochlea 1190
500. Auditory system: anatomy II 1193
501. Chemical senses: peripheral mechanisms—olfaction, carotid body 1196
502. Chemical senses: central pathways—olfaction 1199
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504. Cerebellum IV 1206
505. Human cognition II 1207
506. Learning and memory: systems and functions—models 1210
507. Learning and memory: systems and functions—neuropsychology II 1212
508. Learning and memory: physiology V 1215
509. Learning and memory: pharmacology—excitatory amino acids 1218
510. Biological rhythms and sleep V 1220
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513. Ingestive behavior: water and salt intake 1229
514. Ingestive behavior: nutrients, serotonin and insulin 1232
515. Drugs of abuse: ethanol and GABA 1235
516. Drugs of abuse: cocaine's interaction with non-dopamine systems 1237
517. Epilepsy: basic mechanisms IV 1240
518. Degenerative disease: Alzheimer's—neuropharmacology and neurotransmitters III 1243
519. Degenerative disease: Parkinson's III 1247
520. Degenerative disease: other 1249
521. Ischemia: drug treatment I 1252
522. Ischemia: drug treatment II 1254
523. Ischemia: glucose 1257

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524. Ischemia: gene or protein induction 1258
525. Ischemia: free radicals 1261
526. Ischemia: vasculature 1262
527. Ischemia: imaging 1264
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85. Teaching of neuroscience: elementary and secondary grades 189

Symposia — 1:00 p.m.

528. Molecular Biology of Olfaction
Chaired by: G.M. Shepherd 1264
529. Regulation of Neuronal Peptide Gene Expression: Lessons from the Neurohypophyseal System
Chaired by: C. Sladek 1264

Special Lecture — 1:00 p.m.

530. The Molecular Genetics of Cell-Cell Interactions Regulating Cell Fate in the *Drosophila* Visual System
L. Zipursky ..... No Abstract

Special Lecture — 4:15 p.m.

531. Sensory Information Processing in Electric Fish: Computational Rules and Their Neuronal Implementation
W. Heiligenberg ..... No Abstract

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532. Brain metabolism and blood flow IV 1266
533. Ischemia I 1267
534. Cardiovascular regulation II 1268
535. Calcium channel modulation 1270
536. Axon guidance mechanisms and pathways V 1272
537. Visual cortex: behavior and behavioral correlates 1274
538. Regulation of autonomic function 1276
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544. Nerve growth factor VII 1288
545. Other factors and trophic agents: glia 1291
546. Other factors and trophic agents: injury 1294
547. Other factors and trophic agents: cytokines 1297
548. Nutritional and prenatal factors 1299
549. Glia and other non-neuronal cells V 1301
550. Motor systems II 1303
551. Visual development: striate cortex I 1305
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554. Visual development: mammalian thalamus and midbrain 1310
555. Visual development: retinal ganglion cells 1312
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558. Regeneration IV 1320
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561. Gene structure and function VII 1330
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567. Long-term potentiation IV 1347
568. Synaptic pharmacology I 1352
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571. Excitatory amino acids: excitotoxicity VI 1360
572. Excitatory amino acids: receptors VII 1363
573. Peptides: receptors V 1366
574. Peptides: biosynthesis and metabolism III 1368
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577. Catecholamines: norepinephrine 1374
578. Catecholamines: tyrosine hydroxylase 1377
579. Serotonin receptors: behavioral actions 1379
580. Serotonin: receptor modulation 1381
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587. Visual behavior: psychophysics and eye movements 1396
588. Auditory, vestibular and lateral-line hair cells 1398
589. Reflex function I 1402
590. Control of posture and movement III 1405

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591. Control of posture and movement IV 1408
592. Invertebrate motor function 1411
593. Other systems of the CNS: hypothalamus 1415
594. Association cortex and thalamocortical relations 1417
595. Learning and memory: systems and functions—spatial I 1421
596. Learning and memory: systems and functions—spatial II 1424
597. Ingestive behavior: peptides 1427
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599. Drugs of abuse: cocaine and biochemistry 1432
600. Aging and behavior II 1434
601. Degenerative disease: Alzheimer's—ß-amyloid V 1437
602. Degenerative disease: Alzheimer's—ß-amyloid VI 1440
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604. Developmental disorders 1445
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FRIDAY, OCTOBER 30

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605. $GABA_B$ Receptors and Their Role in Neurotransmission, Neuromodulation and Neuropathology
Chaired by: N.G. Bowery 1448
606. Neurobehavioral Mechanism of Salt Intake Behavior
Chaired by: E. Stellar and B.S. McEwen 1448

Special Lecture — 10:00 a.m.

607. The Translation of Sensory Signals Into Commands for the Control of Saccadic Eye Movements: The Role of the Primate Superior Colliculus
D. Sparks ..... No Abstract

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609. Peptides: receptors VI 1450
610. Ischemia II 1452
611. Visual development: critical period and deprivation 1454
612. Drugs of abuse: cocaine and other stimulants 1456
613. Excitatory amino acids: pharmacology VI 1458
614. Process outgrowth, growth cones and sprouting VIII 1460
615. Regeneration V 1462
616. Degenerative disease: Alzheimer's—ß-amyloid VII 1464
617. Regional localization of receptors and transmitters 1466

Poster Sessions — 8:30 a.m.

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627. Blood-brain barrier III 1491
628. Long-term potentiation V 1494
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631. Acetylcholine: neuronal nicotinic receptors III 1506
632. Excitatory amino acids: receptors VIII 1509
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625.	Aging III	Poster					fAM
22.	Axon guidance mechanisms and pathways I	Poster	mAM				
105.	Axon guidance mechanisms and pathways II	Poster	mPM				
249.	Axon guidance mechanisms and pathways III	Slide		tuPM			
264.	Axon guidance mechanisms and pathways IV	Poster		tuPM			
536.	Axon guidance mechanisms and pathways V	Slide				thPM	
311.	**Cell Biology of the Growth Cone**	**SYMP**			**wAM**		
14.	Cell lineage and determination I	Slide	mAM				
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390.	Cell migration and motility I	Slide			wPM		
541.	Cell migration and motility II	Poster				thPM	
21.	Cell shape and differentiation I	Poster	mAM				
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330.	Development of cerebral cortex and limbic system I	Poster			wAM		
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23.	Formation and specificity of synapses I	Poster	mAM				
98.	Formation and specificity of synapses II	Slide	mPM				
182.	Formation and specificity of synapses III	Poster		tuAM			
250.	Formation and specificity of synapses IV	Slide		tuPM			
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469.	Formation and specificity of synapses VI	Poster				thAM	
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327.	Genesis of neurons and glia II	Poster			wAM		
467.	Genesis of neurons and glia III	Poster				thAM	
185.	Glia and other non-neuronal cells I	Poster		tuAM			
270.	Glia and other non-neuronal cells II	Poster		tuPM			
405.	Glia and other non-neuronal cells III	Poster			wPM		
471.	Glia and other non-neuronal cells IV	Poster				thAM	
549.	Glia and other non-neuronal cells V	Poster				thPM	
608.	Glia and other non-neuronal cells VI	Slide					fAM
109.	Hormones and development: gonadal hormones I	Poster	mPM				
110.	Hormones and development: gonadal hormones II	Poster	mPM				
108.	Hormones and development: non-gonadal hormones	Poster	mPM				
24.	Ion channels: development	Poster	mAM				
560.	Membrane composition and cell-surface macromolecules	Poster				thPM	
32.	Molecular and pharmacological correlates of development I	Poster	mAM				
111.	Molecular and pharmacological correlates of development II	Poster	mPM				
184.	Molecular and pharmacological correlates of development III	Poster		tuAM			
269.	Molecular and pharmacological correlates of development IV	Poster		tuPM			
406.	Motor systems I	Poster			wPM		

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12.	Nerve growth factor I	Slide	mAM				
183.	Nerve growth factor II	Poster		tuAM			
266.	Nerve growth factor III	Poster		tuPM			
329.	Nerve growth factor IV	Poster			wAM		
401.	Nerve growth factor V	Poster			wPM		
463.	Nerve growth factor VI	Slide				thAM	
544.	Nerve growth factor VII	Poster				thPM	
247.	Neuronal death	Slide		tuPM			
25.	Neuronal death: apoptosis	Poster	mAM				
27.	Neuronal death: axotomy	Poster	mAM				
31.	Neuronal death: cellular response	Poster	mAM				
29.	Neuronal death: morphology	Poster	mAM				
28.	Neuronal death: retina	Poster	mAM				
30.	Neuronal death: sympathetic neurons	Poster	mAM				
26.	Neuronal death: toxins	Poster	mAM				
548.	Nutritional and prenatal factors	Poster				thPM	
470.	Nutritional and prenatal factors: alcohol	Poster				thAM	
387.	Other factors and trophic agents: BDNF, NT3	Slide			wPM		
106.	Other factors and trophic agents: BDNF, NT3, NT4	Poster	mPM				
267.	Other factors and trophic agents: CNTF	Poster		tuPM			
403.	Other factors and trophic agents: FGF	Poster			wPM		
402.	Other factors and trophic agents: IGF	Poster			wPM		
547.	Other factors and trophic agents: cytokines	Poster				thPM	
170.	Other factors and trophic agents: general I	Slide		tuAM			
620.	Other factors and trophic agents: general II	Poster					fAM
621.	Other factors and trophic agents: general III	Poster					fAM
545.	Other factors and trophic agents: glia	Poster				thPM	
546.	Other factors and trophic agents: injury	Poster				thPM	
107.	Other factors and trophic agents: *trks*	Poster	mPM				
268.	Pattern formation, compartments and boundaries I	Poster		tuPM			
404.	Pattern formation, compartments and boundaries II	Poster			wPM		
462.	Pattern formation, compartments and boundaries III	Slide				thAM	
104.	Process outgrowth, growth cones and sprouting I	Poster	mPM				
262.	Process outgrowth, growth cones and sprouting II	Poster		tuPM			
263.	Process outgrowth, growth cones and sprouting III	Poster		tuPM			
399.	Process outgrowth, growth cones and sprouting IV	Poster			wPM		
461.	Process outgrowth, growth cones and sprouting V	Slide				thAM	
542.	Process outgrowth, growth cones and sprouting VI	Poster				thPM	
543.	Process outgrowth, growth cones and sprouting VII	Poster				thPM	
614.	Process outgrowth, growth cones and sprouting VIII	Slide					fAM
618.	Process outgrowth, growth cones and sprouting IX	Poster					fAM
186.	Regeneration I	Poster		tuAM			
271.	Regeneration II	Poster		tuPM			
407.	Regeneration III	Poster			wPM		
558.	Regeneration IV	Poster				thPM	
615.	Regeneration V	Slide					fAM
163.	**Regeneration of Vertebrate Sensory Receptors**	**SYMP**		**tuAM**			
33.	Sensory development: auditory and olfactory	Poster	mAM				
34.	Sensory development: somatosensory	Poster	mAM				
393.	Transplantation I	Slide			wPM		
559.	Transplantation II	Poster				thPM	
473.	Transplantation: cortex	Poster				thAM	
331.	Transplantation: gene therapy and cell lines	Poster			wAM		
36.	Transplantation: Parkinson's—adrenal, PC12	Poster	mAM				
35.	Transplantation: Parkinson's—fetal tissue	Poster	mAM				

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474.	Transplantation: retina, olfactory bulb, SCN	Poster				thAM	
622.	Transplantation: spinal cord	Poster					fAM
556.	Visual development: abnormal development of cortex	Poster				thPM	
611.	Visual development: critical period and deprivation	Slide					fAM
554.	Visual development: mAMmalian thalamus and midbrain	Poster				thPM	
389.	Visual development: neuronal and synaptic development	Slide			wPM		
553.	Visual development: optic tectum	Poster				thPM	
557.	Visual development: retina	Poster				thPM	
555.	Visual development: retinal ganglion cells	Poster				thPM	
551.	Visual development: striate cortex I	Poster				thPM	
552.	Visual development: striate cortex II	Poster				thPM	
THEME B: CELL BIOLOGY							
476.	Blood-brain barrier I	Poster				thAM	
540.	Blood-brain barrier II	Slide				thPM	
627.	Blood-brain barrier III	Poster					fAM
38.	Cytoskeletal transport and membrane targeting	Poster	mAM				
39.	Gene structure and function I	Poster	mAM				
112.	Gene structure and function II	Poster	mPM				
113.	Gene structure and function III	Poster	mPM				
254.	Gene structure and function IV	Slide		tuPM			
333.	Gene structure and function V	Poster			wAM		
459.	Gene structure and function VI	Slide				thAM	
561.	Gene structure and function VII	Poster				thPM	
562.	Gene structure and function VIII	Poster				thPM	
87.	**Motilities of the Auditory Periphery**	**SYMP**	**mPM**				
37.	Neuroglia and myelin I	Poster	mAM				
272.	Neuroglia and myelin II	Poster		tuPM			
332.	Neuroglia and myelin III	Poster			wAM		
458.	Neuroglia and myelin IV	Slide				thAM	
626.	Neuroglia and myelin V	Poster					fAM
243.	**New Waves in Cell Calcium**	**SYMP**		**tuPM**			
529.	**Regulation of Neuronal Peptide Gene Expression: Lessons from the Neurohypophyseal System**	**SYMP**				**thPM**	
179.	Staining, tracing and imaging techniques I	Slide		tuAM			
408.	Staining, tracing and imaging techniques II	Poster			wPM		
475.	Staining, tracing and imaging techniques III	Poster				thAM	
THEME C: EXCITABLE MEMBRANES AND SYNAPTIC TRANSMISSION							
535.	Calcium channel modulation	Slide				thPM	
479.	Calcium channel molecular biology	Poster				thAM	
10.	Calcium channel toxins I	Slide	mAM				
409.	Calcium channel toxins II	Poster			wPM		
188.	Calcium channels: pharmacology and effects of transmitters	Poster		tuAM			
187.	Calcium channels: physiology I	Poster		tuAM			
410.	Calcium channels: physiology II	Poster			wPM		
40.	Calcium-activated potassium channels	Poster	mAM				
275.	Chloride and other channels	Poster		tuPM			
253.	Ion channel modulation I	Slide		tuPM			
336.	Ion channel modulation II	Poster			wAM		
629.	Ion channel modulation III	Poster					fAM
570.	Ion channels: cell function	Poster				thPM	
115.	Ligand-gated ion channels	Poster	mPM				

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274.	Long-term potentiation II	Poster		tuPM			
323.	Long-term potentiation III	Slide			wAM		
567.	Long-term potentiation IV	Poster				thPM	
628.	Long-term potentiation V	Poster					fAM
565.	Postsynaptic mechanisms I	Poster				thPM	
566.	Postsynaptic mechanisms II	Poster				thPM	
460.	Potassium channel expression and localization	Slide				thAM	
388.	Potassium channel permeation and gating	Slide			wPM		
335.	Potassium channels: pharmacology and effects of transmitters	Poster			wAM		
41.	Potassium channels: physiology and molecular biology	Poster	mAM				
114.	Presynaptic mechanisms I	Poster	mPM				
248.	Presynaptic mechanisms II	Slide		tuPM			
273.	Presynaptic mechanisms III	Poster		tuPM			
318.	Presynaptic mechanisms IV	Slide			wAM		
334.	Presynaptic mechanisms V	Poster			wAM		
477.	Presynaptic mechanisms VI	Poster				thAM	
478.	Sodium channels	Poster				thAM	
568.	Synaptic pharmacology I	Poster				thPM	
569.	Synaptic pharmacology II	Poster				thPM	
563.	Synaptic structure and function I	Poster				thPM	
564.	Synaptic structure and function II	Poster				thPM	
3.	**The Computational Neuron**	**SYMP**	**mAM**				
	THEME D: NEUROTRANSMITTERS, MODULATORS AND RECEPTORS						
411.	Acetylcholine: CNS I	Poster			wPM		
480.	Acetylcholine: CNS II	Poster				thAM	
630.	Acetylcholine: choline acetyltransferase and cholinesterase	Poster					fAM
117.	Acetylcholine: muscarinic receptors I	Poster	mPM				
481.	Acetylcholine: muscarinic receptors II	Poster				thAM	
189.	Acetylcholine: muscle nicotinic receptors	Poster		tuAM			
42.	Acetylcholine: neuronal nicotinic receptors I	Poster	mAM				
337.	Acetylcholine: neuronal nicotinic receptors II	Poster			wAM		
631.	Acetylcholine: neuronal nicotinic receptors III	Poster					fAM
259.	Acetylcholine: receptors	Slide		tuPM			
116.	Acetylcholine: release	Poster	mPM				
342.	Behavioral pharmacology: benzodiazepines, et al.	Poster			wAM		
641.	Behavioral pharmacology: dopamine, serotonin, NE	Poster					fAM
51.	Behavioral pharmacology: ethanol, GABA, et al.	Poster	mAM				
196.	Catecholamine receptors: adrenergic I	Poster		tuAM			
255.	Catecholamine receptors: adrenergic II	Slide		tuPM			
9.	Catecholamine receptors: dopamine I	Slide	mAM				
281.	Catecholamine receptors: dopamine II	Poster		tuPM			
17.	Catecholamines	Slide	mAM				
282.	Catecholamines: dopamine I	Poster		tuPM			
634.	Catecholamines: dopamine II	Poster					fAM
125.	Catecholamines: dopamine—electrophysiology	Poster	mPM				
577.	Catecholamines: norepinephrine	Poster				thPM	
124.	Catecholamines: receptors I	Poster	mPM				
418.	Catecholamines: receptors II	Poster			wPM		
491.	Catecholamines: receptors III	Poster				thAM	
197.	Catecholamines: release	Poster		tuAM			
578.	Catecholamines: tyrosine hydroxylase	Poster				thPM	
191.	Excitatory amino acids: anatomy and physiology I	Poster		tuAM			

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483.	Excitatory amino acids: anatomy and physiology III	Poster				thAM	
43.	Excitatory amino acids: excitotoxicity I	Poster	mAM				
190.	Excitatory amino acids: excitotoxicity II	Poster		tuAM			
276.	Excitatory amino acids: excitotoxicity III	Poster		tuPM			
321.	Excitatory amino acids: excitotoxicity IV	Slide			wAM		
482.	Excitatory amino acids: excitotoxicity V	Poster				thAM	
571.	Excitatory amino acids: excitotoxicity VI	Poster				thPM	
44.	Excitatory amino acids: pharmacology I	Poster	mAM				
118.	Excitatory amino acids: pharmacology II	Poster	mPM				
192.	Excitatory amino acids: pharmacology III	Poster		tuAM			
277.	Excitatory amino acids: pharmacology IV	Poster		tuPM			
484.	Excitatory amino acids: pharmacology V	Poster				thAM	
613.	Excitatory amino acids: pharmacology VI	Slide					fAM
45.	Excitatory amino acids: receptors I	Poster	mAM				
119.	Excitatory amino acids: receptors II	Poster	mPM				
172.	Excitatory amino acids: receptors III	Slide		tuAM			
278.	Excitatory amino acids: receptors IV	Poster		tuPM			
412.	Excitatory amino acids: receptors V	Poster			wPM		
485.	Excitatory amino acids: receptors VI	Poster				thAM	
572.	Excitatory amino acids: receptors VII	Poster				thPM	
632.	Excitatory amino acids: receptors VIII	Poster					fAM
175.	GABA receptors: function I	Slide		tuAM			
279.	GABA receptors: function II	Poster		tuPM			
413.	GABA receptors: function III	Poster			wPM		
486.	GABA receptors: function IV	Poster				thAM	
487.	GABA receptors: function V	Poster				thAM	
120.	GABA receptors: structure	Poster	mPM				
8.	GABA receptors: structure and function	Slide	mAM				
605.	**$GABA_B$ Receptors and Their Role in Neurotransmission, Neuromodulation and Neuropathology**	**SYMP**					**fAM**
199.	Histamine and other biogenic amines	Poster		tuAM			
47.	Interaction between neurotransmitters I	Poster	mAM				
126.	Interactions between neurotransmitters II	Poster	mPM				
385.	Interactions between neurotransmitters III	Slide			wPM		
640.	Interactions between neurotransmitters IV	Poster					fAM
453.	**Molecular Analyses of Neuronal Physiology and Potential for Gene Therapy Using Herpes Simplex Virus Vectors**	**SYMP**				**thAM**	
100.	Molecular biology of serotonin receptors	Slide	mPM				
340.	Ontogeny of serotonin receptors	Poster			wAM		
633.	Opiate receptor ligands	Poster					fAM
575.	Opiate receptors: interactions with other systems	Poster				thPM	
16.	Opioid and sigma receptors	Slide	mAM				
490.	Opioid receptors: coupling and biochemistry	Poster				thAM	
417.	Opioids: anatomy and physiology I	Poster			wPM		
576.	Opioids: anatomy and physiology II	Poster				thPM	
280.	Opioids: behavior	Poster		tuPM			
339.	Peptides: anatomical localization—human and primate	Poster			wAM		
121.	Peptides: anatomical localization—non-primates	Poster	mPM				
122.	Peptides: biosynthesis and metabolism I	Poster	mPM				
414.	Peptides: biosynthesis and metabolism II	Poster			wPM		
574.	Peptides: biosynthesis and metabolism III	Poster				thPM	
91.	Peptides: biosynthesis, metabolism, biochemistry I	Slide	mPM				
324.	Peptides: physiological and behavioral effects	Slide			wAM		
123.	Peptides: physiological effects I	Poster	mPM				

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415.	Peptides: physiological effects II	Poster			wPM		
416.	Peptides: physiological effects III	Poster			wPM		
489.	Peptides: physiological effects IV	Poster				thAM	
174.	Peptides: receptors I	Slide		tuAM			
193.	Peptides: receptors II	Poster		tuAM			
194.	Peptides: receptors III	Poster		tuAM			
488.	Peptides: receptors IV	Poster				thAM	
573.	Peptides: receptors V	Poster				thPM	
609.	Peptides: receptors VI	Slide					fAM
86.	**Protein Phosphates and the Regulation of Neuronal Excitability**	**SYMP**	**mPM**				
420.	Purines: ATP and guanosine	Poster			wPM		
419.	Purines: adenosine	Poster			wPM		
162.	**Recent Advances in Neuropeptide Biosynthesis: Molecular and Cellular Biology of Neuropeptide-processing Enzymes**	**SYMP**		**tuAM**			
173.	Receptor modulation, up and down regulation I	Slide		tuAM			
343.	Receptor modulation, up and down regulation II	Poster			wAM		
391.	Receptor modulation, up and down regulation III	Slide			wPM		
642.	Receptor modulation, up and down regulation IV	Poster					fAM
617.	Regional localization of receptors and transmitters	Slide					fAM
48.	Regional localization of receptors and transmitters: biogenic amines, misc.	Poster	mAM				
49.	Regional localization of receptors and transmitters: peptides and GABA	Poster	mAM				
635.	Regulation of serotonin receptors	Poster					fAM
50.	Second messengers I	Poster	mAM				
127.	Second messengers II	Poster	mPM				
203.	Second messengers III	Poster		tuAM			
260.	Second messengers IV	Slide		tuPM			
341.	Second messengers V	Poster			wAM		
422.	Second messengers VI	Poster			wPM		
317.	Serotonin neuropharmacology	Slide			wAM		
46.	Serotonin receptors: 5HT1A subtypes	Poster	mAM				
637.	Serotonin receptors: action on neurotransmission	Poster					fAM
579.	Serotonin receptors: behavioral actions	Poster				thPM	
198.	Serotonin receptors: molecular biology	Poster		tuAM			
636.	Serotonin receptors: pharmacologic characterization	Poster					fAM
638.	Serotonin: pharmacology I	Poster					fAM
639.	Serotonin: pharmacology II	Poster					fAM
580.	Serotonin: receptor modulation	Poster				thPM	
195.	Sigma receptors and ligands	Poster		tuAM			
200.	Transmitters in invertebrates I	Poster		tuAM			
201.	Transmitters in invertebrates II	Poster		tuAM			
466.	Transmitters in invertebrates III	Slide				thAM	
202.	Uptake, storage, secretion and metabolism I	Poster		tuAM			
251.	Uptake, storage, secretion and metabolism II	Slide		tuPM			
421.	Uptake, storage, secretion and metabolism III	Poster			wPM		
581.	Uptake, storage, secretion and metabolism IV	Poster				thPM	
	THEME E: ENDOCRINE AND AUTONOMIC REGULATION						
320.	Cardiovascular regulation I	Slide			wAM		
534.	Cardiovascular regulation II	Slide				thPM	
495.	Cardiovascular regulation: cardiac control and sympathetic rhythms	Poster				thAM	
492.	Cardiovascular regulation: forebrain mechanisms	Poster				thAM	

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496.	Cardiovascular regulation: hypertension, reflexes and peripheral autonomics	Poster				thAM	
426.	Cardiovascular regulation: lower brainstem I	Poster			wPM		
497.	Cardiovascular regulation: lower brainstem II	Poster				thAM	
494.	Cardiovascular regulation: spinal cord and medulla	Poster				thAM	
493.	Cardiovascular regulation: upper brainstem mechanisms	Poster				thAM	
319.	Hypothalamic-pituitary-adrenal regulation	Slide			wAM		
283.	Hypothalamic-pituitary-adrenal regulation: CRF	Poster		tuPM			
284.	Hypothalamic-pituitary-adrenal regulation: POMC, ACTH, other factors	Poster		tuPM			
204.	Hypothalamic-pituitary-adrenal regulation: glucocorticoid and mineralocorticoid receptors	Poster		tuAM			
643.	Hypothalamic-pituitary-adrenal regulation: stress	Poster					fAM
423.	Hypothalamic-pituitary-adrenal regulation: stress—developmental aspects	Poster			wPM		
90.	Hypothalamic-pituitary-gonadal regulation	Slide	mPM				
55.	Hypothalamic-pituitary-gonadal regulation: LHRH and LH I	Poster	mAM				
285.	Hypothalamic-pituitary-gonadal regulation: LHRH and LH II	Poster		tuPM			
52.	Hypothalamic-pituitary-gonadal regulation: neuropeptides	Poster	mAM				
344.	Hypothalamic-pituitary-gonadal regulation: other	Poster			wAM		
53.	Hypothalamic-pituitary-gonadal regulation: steroids	Poster	mAM				
288.	Neural control of immune functions	Poster		tuPM			
326.	Neural-immune interactions	Slide			wAM		
287.	Neural-immune interactions: behavior and stress	Poster		tuPM			
424.	Neural-immune interactions: innervation and other	Poster			wPM		
425.	Neural-immune interactions: interleukin-1	Poster			wPM		
206.	Neural-immune interactions: other interleukins and cytokines	Poster		tuAM			
392.	Neuroendocrine regulation I	Slide			wPM		
456.	Neuroendocrine regulation II	Slide				thAM	
56.	Neuroendocrine regulation: hypothalamus/pituitary	Poster	mAM				
57.	Neuroendocrine regulation: limbic system	Poster	mAM				
286.	Neuroendocrine regulation: osmotic regulation I	Poster		tuPM			
346.	Neuroendocrine regulation: osmotic regulation II	Poster			wAM		
345.	Neuroendocrine regulation: other	Poster			wAM		
54.	Neuroendocrine regulation: prolactin	Poster	mAM				
538.	Regulation of autonomic function	Slide				thPM	
207.	Regulation of autonomic function: gastrointestinal and visceral afferents	Poster		tuAM			
59.	Regulation of autonomic function: genitourinary	Poster	mAM				
347.	Regulation of autonomic function: other	Poster			wAM		
58.	Respiratory regulation: central networks/patterns	Poster	mAM				
348.	Respiratory regulation: chemoreception	Poster			wAM		
208.	Respiratory regulation: transmitters/receptors	Poster		tuAM			
209.	Temperature regulation and fever	Poster		tuAM			
205.	Water and osmotic regulation	Poster		tuAM			
THEME F: SENSORY SYSTEMS							
435.	Auditory system: anatomy I	Poster			wPM		
500.	Auditory system: anatomy II	Poster				thAM	
67.	Auditory system: central physiology I	Poster	mAM				
166.	Auditory system: central physiology II	Slide		tuAM			
353.	Auditory system: central physiology III	Poster			wAM		
499.	Auditory system: cochlea	Poster				thAM	
434.	Auditory system: neurochemistry	Poster			wPM		
588.	Auditory, vestibular and lateral-line hair cells	Poster				thPM	

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502.	Chemical senses: central pathways—olfaction	Poster				thAM	
436.	Chemical senses: pathways—gustation	Poster			wPM		
258.	Chemical senses: peripheral mechanisms	Slide		tuPM			
501.	Chemical senses: peripheral mechanisms—olfaction, carotid body	Poster				thAM	
354.	Chemical senses: peripheral mechanisms—taste	Poster			wAM		
135.	Invertebrate sensory systems	Poster	mPM				
528.	**Molecular Biology of Olfaction**	**SYMP**				**thPM**	
430.	Pain modulation: behavior	Poster			wPM		
289.	Pain modulation: brainstem and thalamus	Poster		tuPM			
212.	Pain modulation: cognitive, autonomic and endocrine	Poster		tuAM			
290.	Pain modulation: descending	Poster		tuPM			
130.	Pain modulation: dorsal horn	Poster	mPM				
62.	Pain modulation: hyperalgesia	Poster	mAM				
292.	Pain modulation: peripheral	Poster		tuPM			
129.	Pain modulation: peripheral neuropathy	Poster	mPM				
291.	Pain modulation: pharmacology and endocrinology	Poster		tuPM			
428.	Pain modulation: spinal I	Poster			wPM		
429.	Pain modulation: spinal II	Poster			wPM		
351.	Pain modulation: supraspinal	Poster			wAM		
211.	Pain pathways: dorsal horn	Poster		tuAM			
128.	Pain pathways: hyperalgesia	Poster	mPM				
350.	Pain pathways: supraspinal	Poster			wAM		
94.	Pain: pharmacology	Slide	mPM				
167.	Pain: physiology and behavior	Slide		tuAM			
63.	Retina and photoreceptors I	Poster	mAM				
171.	Retina and photoreceptors II	Slide		tuAM			
431.	Retina and photoreceptors: amacrine, ganglion and glial cells	Poster			wPM		
352.	Retina and photoreceptors: photoreceptors, horizontal and bipolar cells	Poster			wAM		
60.	Somatic and visceral afferents I	Poster	mAM				
210.	Somatic and visceral afferents II	Poster		tuAM			
349.	Somatic and visceral afferents: touch	Poster			wAM		
582.	Somatosensory cortex and thalamocortical relationships: anatomy	Poster				thPM	
644.	Somatosensory cortex and thalamocortical relationships: development and plasticity	Poster					fAM
583.	Somatosensory cortex and thalamocortical relationships: physiology	Poster				thPM	
645.	Somatosensory cortex and thalamocortical relationships: psychophysics and networks	Poster					fAM
584.	Somatosensory cortex and thalamocortical relationships: thalamus	Poster				thPM	
316.	Somatosensory system	Slide			wAM		
61.	Spinal cord	Poster	mAM				
427.	Subcortical somatosensory pathways: lemniscal and spinothalamic	Poster			wPM		
498.	Subcortical somatosensory pathways: trigeminal	Poster				thAM	
101.	Subcortical visual pathways	Slide	mPM				
64.	Subcortical visual pathways: LGN	Poster	mAM				
432.	Subcortical visual pathways: cortico-tectal, Accessory Optic System, centrifugals	Poster			wPM		
65.	Subcortical visual pathways: retina and colliculus	Poster	mAM				
454.	**Thalamic Mechanisms of Nociception**	**SYMP**				**thAM**	
586.	Visual behavior: clinical studies	Poster				thPM	
587.	Visual behavior: psychophysics and eye movements	Poster				thPM	
169.	Visual cortex: anatomical studies	Slide		tuAM			
132.	Visual cortex: anatomy of extrastriate cortex	Poster	mPM				
134.	Visual cortex: anatomy of striate cortex	Poster	mPM				
537.	Visual cortex: behavior and behavioral correlates	Slide				thPM	
585.	Visual cortex: brain imaging techniques	Poster				thPM	

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11.	Visual cortex: functional circuits and oscillations I	Slide	mAM				
131.	Visual cortex: functional circuits and oscillations II	Poster	mPM				
99.	Visual cortex: functional organization of striate cortex	Slide	mPM				
433.	Visual cortex: motion processing I	Poster			wPM		
464.	Visual cortex: motion processing II	Slide				thAM	
256.	Visual cortex: neuronal response properties	Slide		tuPM			
66.	Visual cortex: physiology of extrastriate cortex	Poster	mAM				
133.	Visual cortex: physiology of striate cortex	Poster	mPM				
313.	Visual cortex: theoretical studies	Slide			wAM		
	THEME G: MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION						
136.	Basal ganglia and thalamus I	Poster	mPM				
137.	Basal ganglia and thalamus II	Poster	mPM				
293.	Basal ganglia and thalamus III	Poster		tuPM			
294.	Basal ganglia and thalamus IV	Poster		tuPM			
356.	Basal ganglia and thalamus V	Poster			wAM		
437.	Basal ganglia and thalamus VI	Poster			wPM		
503.	Basal ganglia and thalamus VII	Poster				thAM	
178.	Cerebellum I	Slide		tuAM			
357.	Cerebellum II	Poster			wAM		
438.	Cerebellum III	Poster			wPM		
504.	Cerebellum IV	Poster				thAM	
140.	Circuitry and pattern generation I	Poster	mPM				
442.	Circuitry and pattern generation II	Poster			wPM		
539.	Circuitry and pattern generation III	Slide				thPM	
18.	Control of posture and movement I	Slide	mAM				
441.	Control of posture and movement II	Poster			wPM		
590.	Control of posture and movement III	Poster				thPM	
591.	Control of posture and movement IV	Poster				thPM	
218.	Control of posture and movement: arm movement I	Poster		tuAM			
647.	Control of posture and movement: arm movement II	Poster					fAM
360.	Control of posture and movement: clinically related studies	Poster			wAM		
648.	Control of posture and movement: locomotion	Poster					fAM
592.	Invertebrate motor function	Poster				thPM	
103.	Motor systems and sensorimotor integration: cortex I	Slide	mPM				
213.	Motor systems and sensorimotor integration: cortex II	Poster		tuAM			
214.	Motor systems and sensorimotor integration: cortex III	Poster		tuAM			
355.	Motor systems and sensorimotor integration: cortex IV	Poster			wAM		
397.	Motor systems and sensorimotor integration: cortex V	Slide			wPM		
649.	Muscle I	Poster					fAM
650.	Muscle II	Poster					fAM
19.	Oculomotor I	Slide	mAM				
102.	Oculomotor II	Slide	mPM				
296.	Oculomotor III	Poster		tuPM			
358.	Oculomotor IV	Poster			wAM		
295.	Oculomotor system: saccades	Poster		tuPM			
589.	Reflex function I	Poster				thPM	
646.	Reflex function II	Poster					fAM
138.	Spinal cord and brainstem I	Poster	mPM				
139.	Spinal cord and brainstem II	Poster	mPM				
217.	Spinal cord and brainstem III	Poster		tuAM			
359.	Spinal cord and brainstem IV	Poster			wAM		
440.	Spinal cord and brainstem V	Poster			wPM		

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244.	**The Role of Sensory Information in the Guidance of Voluntary Movement**	**SYMP**		**tuPM**			
439.	Vestibular system: behavioral responses	Poster			wPM		
216.	Vestibular system: morphology and physiology	Poster		tuAM			
215.	Vestibular system: neurochemistry	Poster		tuAM			
	THEME H: OTHER SYSTEMS OF THE CNS						
594.	Association cortex and thalamocortical relations	Poster				thPM	
68.	Brain metabolism and blood flow I	Poster	mAM				
177.	Brain metabolism and blood flow II	Slide		tuAM			
361.	Brain metabolism and blood flow III	Poster			wAM		
532.	Brain metabolism and blood flow IV	Slide				thPM	
143.	Comparative neuroanatomy	Poster	mPM				
2.	**Emerging Principles of Organization Within the Midbrain Periaqueductal Gray Matter**	**SYMP**	**mAM**				
141.	Limbic system I	Poster	mPM				
142.	Limbic system II	Poster	mPM				
219.	Limbic system III	Poster		tuAM			
386.	Limbic system IV	Slide			wPM		
593.	Other systems of the CNS: hypothalamus	Poster				thPM	
	THEME I: NEURAL BASIS OF BEHAVIOR						
376.	Aging and behavior I	Poster			wAM		
600.	Aging and behavior II	Poster				thPM	
6.	Biological rhythms and sleep I	Slide	mAM				
92.	Biological rhythms and sleep II	Slide	mPM				
365.	Biological rhythms and sleep III	Poster			wAM		
366.	Biological rhythms and sleep IV	Poster			wAM		
510.	Biological rhythms and sleep V	Poster				thAM	
511.	Biological rhythms and sleep VI	Poster				thAM	
512.	Biological rhythms and sleep VII	Poster				thAM	
382.	**Cognitive and Neurobiological Consequences of Normal Aging: From Rats to Primates**	**SYMP**			**wPM**		
227.	Drugs of abuse: alcohol, barbiturates and benzodiazepines I	Poster		tuAM			
322.	Drugs of abuse: alcohol, barbiturates and benzodiazepines II	Slide			wAM		
448.	Drugs of abuse: alcohol, barbiturates and benzodiazepines III	Poster			wPM		
154.	Drugs of abuse: amphetamine and other stimulants	Poster	mPM				
656.	Drugs of abuse: behavioral effects of cocaine	Poster					fAM
153.	Drugs of abuse: benzodiazepines and barbiturates	Poster	mPM				
157.	Drugs of abuse: cannabinoids and opioids	Poster	mPM				
599.	Drugs of abuse: cocaine and biochemistry	Poster				thPM	
155.	Drugs of abuse: cocaine and development	Poster	mPM				
450.	Drugs of abuse: cocaine and dopamine neuronal systems	Poster			wPM		
612.	Drugs of abuse: cocaine and other stimulants	Slide					fAM
516.	Drugs of abuse: cocaine's interaction with non-dopamine systems	Poster				thAM	
228.	Drugs of abuse: cocaine—other studies	Poster		tuAM			
515.	Drugs of abuse: ethanol and GABA	Poster				thAM	
598.	Drugs of abuse: ethanol and monoamines	Poster				thPM	
375.	Drugs of abuse: interaction of cocaine, DA-altering drugs and the VTA	Poster			wAM		
229.	Drugs of abuse: nicotine, cocaine, et al.	Poster		tuAM			
156.	Drugs of abuse: opioids	Poster	mPM				
302.	Drugs of abuse: pharmacology of cocaine	Poster		tuPM			
449.	Drugs of abuse: stimulants I	Poster			wPM		

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657.	Drugs of abuse: stimulants II	Poster					fAM
369.	Hormonal control of reproductive behavior I	Poster			wAM		
370.	Hormonal control of reproductive behavior II	Poster			wAM		
371.	Hormonal control of reproductive behavior III	Poster			wAM		
372.	Hormonal control of reproductive behavior IV	Poster			wAM		
144.	Human cognition I	Poster	mPM				
505.	Human cognition II	Poster				thAM	
394.	Human cognition: blood flow/metabolism	Slide			wPM		
145.	Human cognition: electrophysiology I	Poster	mPM				
252.	Human cognition: electrophysiology II	Slide		tuPM			
315.	Human cognition: neuropsychology	Slide			wAM		
396.	Ingestive behavior: NPY, galanin and insulin	Slide			wPM		
514.	Ingestive behavior: nutrients, serotonin and insulin	Poster				thAM	
597.	Ingestive behavior: peptides	Poster				thPM	
314.	Ingestive behavior: salt appetite and food intake	Slide			wAM		
446.	Ingestive behavior: taste aversion and neural mechanisms	Poster			wPM		
513.	Ingestive behavior: water and salt intake	Poster				thAM	
13.	Invertebrate learning and behavior I	Slide	mAM				
224.	Invertebrate learning and behavior II	Poster		tuAM			
299.	Invertebrate learning and behavior III	Poster		tuPM			
398.	Invertebrate learning and behavior IV	Slide			wPM		
147.	Learning and memory: pharmacology—acetylcholine	Poster	mPM				
654.	Learning and memory: pharmacology—benzodiazepines	Poster					fAM
509.	Learning and memory: pharmacology—excitatory amino acids	Poster				thAM	
222.	Learning and memory: pharmacology—monoamines	Poster		tuAM			
221.	Learning and memory: pharmacology—opioids	Poster		tuAM			
362.	Learning and memory: pharmacology—other I	Poster			wAM		
655.	Learning and memory: pharmacology—other II	Poster					fAM
146.	Learning and memory: physiology I	Poster	mPM				
220.	Learning and memory: physiology II	Poster		tuAM			
297.	Learning and memory: physiology III	Poster		tuPM			
445.	Learning and memory: physiology IV	Poster			wPM		
508.	Learning and memory: physiology V	Poster				thAM	
651.	Learning and memory: systems and functions—conditioning I	Poster					fAM
652.	Learning and memory: systems and functions—conditioning II	Poster					fAM
653.	Learning and memory: systems and functions—conditioning III	Poster					fAM
443.	Learning and memory: systems and functions—misc. I	Poster			wPM		
444.	Learning and memory: systems and functions—misc. II	Poster			wPM		
506.	Learning and memory: systems and functions—models	Poster				thAM	
168.	Learning and memory: systems and functions—neuropsychology I	Slide		tuAM			
507.	Learning and memory: systems and functions—neuropsychology II	Poster				thAM	
595.	Learning and memory: systems and functions—spatial I	Poster				thPM	
596.	Learning and memory: systems and functions—spatial II	Poster				thPM	
226.	Monoamines and behavior: 8-OH-DPAT and noradrenaline	Poster		tuAM			
301.	Monoamines and behavior: accumbens, striatum and frontal cortex	Poster		tuPM			
447.	Monoamines and behavior: behavioral effects of dopamine	Poster			wPM		
7.	Monoamines and behavior: human disease and animal models	Slide	mAM				
151.	Monoamines and behavior: serotonin	Poster	mPM				
368.	Monoamines and behavior: striatum and DA receptors	Poster			wAM		
298.	Motivation and emotion I	Poster		tuPM			
364.	Motivation and emotion II	Poster			wAM		
148.	Neural plasticity I	Poster	mPM				
257.	Neural plasticity II	Slide		tuPM			
363.	Neural plasticity III	Poster			wAM		
606.	**Neurobehavioral Mechanism of Salt Intake Behavior**	**SYMP**					**fAM**
223.	Neuroethology: avian vocalization and audition	Poster		tuAM			

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149.	Neuroethology: invertebrates, electric fish	Poster	mPM				
367.	Neuroethology: tetrapods—bat echolocation	Poster			wAM		
152.	Neuropeptides and behavior I	Poster	mPM				
373.	Neuropeptides and behavior II	Poster			wAM		
374.	Neuropeptides and behavior III	Poster			wAM		
95.	Psychotherapeutic drugs	Slide	mPM				
158.	Psychotherapeutic drugs: clozapine and dopamine antagonists	Poster	mPM				
303.	Psychotherapeutic drugs: lithium, benzodiazepines and antidepressants	Poster		tuPM			
230.	Psychotherapeutic drugs: sigma receptors and antipsychotics	Poster		tuAM			
96.	Stress	Slide	mPM				
225.	Stress and neuronal systems	Poster		tuAM			
150.	Stress: chronic	Poster	mPM				
300.	Stress: general	Poster		tuPM			

THEME J: DISORDERS OF THE NERVOUS SYSTEM

669.	Affective disorders	Poster					fAM
670.	Affective disorders: serotonin	Poster					fAM
603.	Degenerative disease: Parkinson's IV	Poster				thPM	
15.	Degenerative disease: Alzheimer's—ß-amyloid I	Slide	mAM				
72.	Degenerative disease: Alzheimer's—ß-amyloid II	Poster	mAM				
305.	Degenerative disease: Alzheimer's—ß-amyloid III	Poster		tuPM			
325.	Degenerative disease: Alzheimer's—ß-amyloid IV	Slide			wAM		
601.	Degenerative disease: Alzheimer's—ß-amyloid V	Poster				thPM	
602.	Degenerative disease: Alzheimer's—ß-amyloid VI	Poster				thPM	
616.	Degenerative disease: Alzheimer's—ß-amyloid VII	Slide					fAM
236.	Degenerative disease: Alzheimer's—CSF	Poster		tuAM			
235.	Degenerative disease: Alzheimer's—animal models	Poster		tuAM			
239.	Degenerative disease: Alzheimer's—clinical observations	Poster		tuAM			
306.	Degenerative disease: Alzheimer's—cognition	Poster		tuPM			
240.	Degenerative disease: Alzheimer's—etiologic toxins	Poster		tuAM			
234.	Degenerative disease: Alzheimer's—growth factors	Poster		tuAM			
307.	Degenerative disease: Alzheimer's—neuropharmacology and neurotransmitters	Poster		tuPM			
97.	Degenerative disease: Alzheimer's—neuropharmacology and neurotransmitters	Slide	mPM				
518.	Degenerative disease: Alzheimer's—neuropharmacology and neurotransmitters	Poster				thAM	
93.	Degenerative disease: Alzheimer's—other	Slide	mPM				
238.	Degenerative disease: Alzheimer's—plaques and tangles	Poster		tuAM			
233.	Degenerative disease: Alzheimer's—postmortem analysis	Poster		tuAM			
237.	Degenerative disease: Alzheimer's—tau	Poster		tuAM			
73.	Degenerative disease: Huntington's disease	Poster	mAM				
395.	Degenerative disease: Parkinson's I	Slide			wPM		
451.	Degenerative disease: Parkinson's II	Poster			wPM		
519.	Degenerative disease: Parkinson's III	Poster				thAM	
658.	Degenerative disease: Parkinson's V	Poster					fAM
520.	Degenerative disease: other	Poster				thAM	
604.	Developmental disorders	Poster				thPM	
159.	Epilepsy: anticonvulsant drugs	Poster	mPM				
71.	Epilepsy: basic mechanisms I	Poster	mAM				
232.	Epilepsy: basic mechanisms II	Poster		tuAM			
378.	Epilepsy: basic mechanisms III	Poster			wAM		
517.	Epilepsy: basic mechanisms IV	Poster				thAM	
70.	Epilepsy: human studies and animal models I	Poster	mAM				

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
231.	Epilepsy: human studies and animal models II	Poster		tuAM			
377.	Epilepsy: human studies and animal models III	Poster			wAM		
69.	Genetic models I	Poster	mAM				
304.	Genetic models II	Poster		tuPM			
667.	Infectious diseases	Poster					fAM
533.	Ischemia I	Slide				thPM	
610.	Ischemia II	Slide					fAM
659.	Ischemia: acidosis	Poster					fAM
661.	Ischemia: animal models	Poster					fAM
665.	Ischemia: calcium	Poster					fAM
662.	Ischemia: cellular mechanisms	Poster					fAM
666.	Ischemia: clinical studies	Poster					fAM
521.	Ischemia: drug treatment I	Poster				thAM	
522.	Ischemia: drug treatment II	Poster				thAM	
525.	Ischemia: free radicals	Poster				thAM	
524.	Ischemia: gene or protein induction	Poster				thAM	
663.	Ischemia: glia	Poster					fAM
523.	Ischemia: glucose	Poster				thAM	
527.	Ischemia: imaging	Poster				thAM	
660.	Ischemia: neurophysiology	Poster					fAM
241.	Ischemia: neurotransmitters	Poster		tuAM			
664.	Ischemia: temperature effect	Poster					fAM
526.	Ischemia: vasculature	Poster				thAM	
381.	**Mechanisms of HIV-related Injury in the Central Nervous System**	**SYMP**			**wPM**		
452.	Neuromuscular diseases I	Poster			wPM		
668.	Neuromuscular diseases II	Poster					fAM
465.	Neurotoxicity	Slide				thAM	
380.	Neurotoxicity: amphetamine	Poster			wAM		
671.	Neurotoxicity: biological	Poster					fAM
672.	Neurotoxicity: excitotoxins	Poster					fAM
674.	Neurotoxicity: metals	Poster					fAM
675.	Neurotoxicity: metals—aluminum	Poster					fAM
673.	Neurotoxicity: miscellaneous toxins	Poster					fAM
242.	Schizophrenia	Poster		tuAM			
379.	Schizophrenia: neurochemistry	Poster			wAM		
310.	**Strategies for the Study of Alzheimer Amyloidosis Using Animal Models**	**SYMP**			**wAM**		
457.	Trauma	Slide				thAM	
74.	Trauma: behavioral studies	Poster	mAM				
77.	Trauma: cellular reaction	Poster	mAM				
79.	Trauma: drug treatment	Poster	mAM				
80.	Trauma: glia/blood-brain barrier	Poster	mAM				
81.	Trauma: hypothermia	Poster	mAM				
75.	Trauma: mechanisms of injury and healing	Poster	mAM				
78.	Trauma: metabolic changes	Poster	mAM				
76.	Trauma: transmitters	Poster	mAM				

OTHER:

82.	History of neuroscience	Poster	mAM, PM	tuAM, PM	wAM, PM	thAM, PM	fAM
84.	Teaching of neuroscience: computer-assisted instruction	Poster	mAM, PM	tuAM, PM	wAM, PM	thAM, PM	fAM
85.	Teaching of neuroscience: elementary and secondary grades	Poster	mAM, PM	tuAM, PM	wAM, PM	thAM, PM	fAM
83.	Teaching of neuroscience: undergraduate and graduate education	Poster	mAM, PM	tuAM, PM	wAM, PM	thAM, PM	fAM

390.3

ADHESION, ANTI-ADHESION AND MIGRATION OF OLFACTORY NEURONS AND NEURONAL PRECURSORS ARE REGULATED BY DISTINCT MOLECULAR DOMAINS OF LAMININ. A.L. Calof*[1], P.D. Yurchenco[2], J.J. O'Rear[2], and A.D. Lander[3]. [1]U. of Iowa, Iowa City, IA; [2]R.W. Johnson Med. Sch., Piscataway, NJ; [3]Mass. Inst. of Tech., Cambridge, MA.

Neuronal precursors and immature neurons of the mouse olfactory epithelium (OE) are motile *in vivo*, and can be stimulated to migrate and are guided *in vitro* by the ECM protein laminin (LN) and its homologue merosin (MN). LN and MN are also anti-adhesive, i.e. they cause OE neuronal cells to adhere weakly to substrata that would otherwise be strongly adhesive (Calof and Lander (1991) J. Cell Biol. 115:779). Investigations into the domains of laminin responsible for its effects on OE cells have revealed the following: The **anti-adhesive** activity of LN is highly heat stable and maps to the E1' fragment of the molecule. Although integrin $\alpha1\beta1$ is a cell-surface receptor known to interact with this region of LN, function-blocking antibodies directed against this integrin do not inhibit anti-adhesion. The **migration-promoting** activity of LN is distinct from its anti-adhesive activity, and is not the result of adhesion-altering effects of LN. Migration-promoting activity is heat-labile, maps to the E8 fragment of LN, and can be completely blocked by a monoclonal antibody directed against integrin subunit $\alpha6$. Migration promoted by MN, however, is only partially blocked by this antibody. Surprisingly, although LN is not detectably **adhesive** for OE neuronal cells, some of its domains are. E8 is weakly adhesive, and a recombinant G-domain (rG) is strongly adhesive. Adhesion to rG was not dependent on $\alpha6$-containing integrins, nor could it be blocked by a peptide contained in rG that is thought to represent the binding site of integrin $\alpha3\beta1$. Adhesion to rG was blocked, however, by low concentrations of heparin (4 μg/ml) and by antibodies directed against LN fragment E3 (which is contained in rG). These data suggest that neuronal adhesion, anti-adhesion and migration can be independently regulated by distinct domains of LN and distinct receptors.

390.4

AVIAN NEURAL CREST CELLS RECOGNIZE LAMININ THROUGH ITS E8 AND T8 DOMAINS USING $\alpha_1\beta_1$ INTEGRINS Thomas Lallier*, Roberto Perris, Reiner Deutzmann, Mats Paulsson and Marianne Bronner-Fraser
Developmental Biology Center, University of California, Irvine, Ca. 92717

We have localized the sites of neural crest cell interaction with laminin by examining their ability to migrate on and attach to proteolitic fragments of the molecule. Neural crest cells preferentially migrate on and bind to the E8 domain on the long arm of laminin, but not the E1', E3, E4, or P1 fragments. When the E8 domain of laminin was further digested with trypsyn to yeild T8, E8R, T8R, T8', T8'A, G1-3 or 25kD fragments, neural crest cells migrated on and attached to the T8 - T8' fragments. Interestingly, combinations of the T8'A and 25kD fragments supported neural crest cell interactions, though neither was active alone. This suggests that simultaneous addition of the two fragments reconstitutes the active site within the T8' domain. The presence or absence of Ca^{2+} in the plating buffer appears to alter the mechanisms of neural crest cell attachment to laminin and its fragments. When substrata were plated in the presence of Ca^{2+}, these cells utilize the $\alpha_1\beta_1$ integrin heterodimer for "early" attachment to laminin and the E8 - T8' fragments. In contrast, when substrata were coated in the absence of Ca^{2+}, these cells utilize a β_1 integrins for "early" attachment only to laminin and the E8 and T8 fragments. With prolonged contact time, however, neural crest cells appear to modify their substrata such that "later" attachment was mediated by $\alpha_1\beta_1$ integrins to laminin and the E8 - T8' fragments regardless of the coating conditions. Our results suggest that neural crest cells preferentially migrate and attach to the T8' region within the E8 domain of laminin and that they modify laminin substrata as a result of prolonged cell-matrix interactions.

390.5

NEURONAL MIGRATION AND LAMININ - STUDIES ON THE ROLE OF LAMININ,THE NEURITE OUTGROWTH DOMAIN OF THE B2 CHAIN, AND CELLULAR MOVEMENTS AS REVEALED BY INFRARED VIDEO MICROSCOPY. P.Liesi*,E.Trenkner[2],H-U. Dodt[3],G. Hager[3], and W. Zieglgänsberger[3]. Institutes of Biotechnology*(Helsinki) and Basic Res.(New York)[2],and Max-Planck-Institut for Psychiatry[3](Munich).

Low magnification video microscopy on cerebellar neurons growing on a laminin substrate showed that neurons migrated by first sending out a process that contacted other neurites, which was followed by nuclear movement inside a preformed process.This was also verified on living slices of cerebellum by a novel technique of infrared video microscopy. This technique showed that external granule cells were bipolar cells attaching to the basement membrane and extending out another process towards the presumptive granule cell layer.These cells showed a back-and-forth movement that closely resembled that on a laminin substrate. The role of the neurite outgrowth domain of the B2 chain of laminin on granule cell migration was studied in the cable culture system. Neuronal movement in these cultures was totally inhibited by antibodies against this domainof laminin. Immunocytochemistry localized this domain in intimate cell-to-cell contacts between the migrating neurons and other neurons and glia. Studies on weaver granule cells on a laminin substrate revealed that these cells were proteolytically more active than their normal counterparts and deposited large amounts of the neurite outgrowth promoting peptide antigen on their surfaces. These results indicate that neuronal migration on laminin simulates that visualized in living slices of newborn rodent cerebellum in situ. The B2 chain of laminin may be essential for neuronal migration, and as weaver granule cells are proteolytically active, and over express the neurite outgrowth domain of the B2 chain of laminin, the reported neurotoxicity of this domain may provide a mechanism for granule cell death occuring in this mutation.

390.6

MINIMAL DISPERSION OF NEUROEPITHELIAL CELLS AND THEIR PROGENY DURING GENERATION OF THE CORTICAL PREPLATE

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Thalamocortical input promotes the differentiation of the developing cortical plate into architecturally and functionally specialized areas. Thalamic axons show remarkable specificity in targeting to appropriate cortical regions and invasion of the cortical plate, indicating the operation of an effective marking system. One way to generate this marking system would be for neuroepithelial cells to impart positional information to their progeny. However, the marking system is probably not found in the cortical plate since clonally related cells become widely dispersed across diverse regions (Walsh & Cepko, Science, '92). A candidate structure to contain the marking system is the subplate, a derivative of the preplate which is comprised of the earliest generated cortical neurons and is distinct from the later generated cortical plate. To evaluate this possibility, we investigated the dispersion of neuroepithelial cells and their progeny as the preplate develops. In rat, preplate neurons are generated from E12 to E14. Dorsal telencephalon from E12 or E13 rat embryos was cultured with the pial surface apposed to a Millicell membrane. A small group of neuroepithelial cells was labeled with DiI. A SIT camera was used to take fluorescence images of selected explants at 15 to 30 minute intervals over 48 hrs. Other explants were imaged at 1, 12, 24, 36 & 48 hrs after labeling. The great majority of labeled cells remain tightly clustered, but a small fraction move short distances from the main cluster. These observations were confirmed in the same explants sectioned transversely. Use of the neuron-specific marker TuJ1 (Lee et al, Cell Motil Cytoskel '90) shows that a preplate does form in the explants; DiI labeled cells are present in both the neuroepithelium and the preplate. Similar results were obtained with the ventricular surface apposed to the membrane, or in floating intact forebrains. These findings show that neuroepithelial cells and their progeny that form the preplate tend to remain clustered, indicating that positional information could be effectively imparted from the neuroepithelium to the preplate.

390.7

INVOLVEMENT OF THE T61 ANTIGEN IN CNS NEURONAL MIGRATION. S. Henke-Fahle* and S. Meyer. Dept. of Ophthalmology, University of Tübingen, and Max-Planck-Institut für Entwicklungsbiologie, W-7400 Tübingen, Germany.

Neuronal migration in various parts of the central nervous system depends on the motility of a leading process along a radial glia fiber. In order to investigate whether a monoclonal antibody that had previously been shown to inhibit outgrowth of retinal neurites by arresting their growth cones *in vitro* (Henke-Fahle and Bonhoeffer, Nature 303, 65, 1983) also interfered with neuronal migration, we performed perturbation experiments in living embryos.

Hybridoma cells producing antibodies against T61 antigen were injected into the mesencephalic ventricle of chick embryos at embryonic day 6 to provide a permanent source of antibody. After further incubation (4-9 days), the distribution of antibodies and the tectal lamination in sagittal brain sections were examined. The antibodies had penetrated the tectal wall completely, whereas the hybridoma cells remained confined to the ventricular cavity. Tectal lamination was disturbed in the following way: 1) The neuroepithelial cell layer appeared thicker and consisted of more cells when compared to controls. 2) The Stratum album centrale (SAC) was dislocated towards the pial surface. 3) The developing Stratum griseum et fibrosum superficiale (SGFS) was drastically reduced in size. These results suggest that young neurons were impaired in their ability to migrate radially towards the future target layers in the presence of T61 antibody.

Western blot analysis showed two bands of apparent molecular weight 380 and 170 kD. Solubilization properties of the antigen indicated that T61 is not a transmembrane protein.

390.8

GENERATION OF WIDESPREAD CEREBRAL CORTICAL CLONES C. Walsh & C. Cepko, Dept of Neurology, Mass. Gen. Hosp. and Dept. of Genetics, Harvard Med. Sch., Boston, MA 02115

Cell lineage in the cerebral cortex can be traced using a library of retroviruses that carry distinct DNA inserts as genetic tags. When the tags are analyzed using the polymerase chain reaction (PCR), they mark clones of cells independent of migration patterns of sibling cells. Cortical clones marked in this way often show widespread dispersion, in some cases covering much of the neocortex (Walsh and Cepko, 1992, Science 255: 434). Analyzing rats that received viral injections at E14-E15 6 days (E20-21) or 10 days (P3) later can show how this widespread dispersion occurs. Inoculations were made such that ≤5 clones per hemisphere were labeled. The brains were sectioned, reacted for ß-galactosidase histochemistry, and labelled cells were plotted using a three-dimensional computerized reconstruction program, CARP (Austin and Cepko, 1990, Development 110: 713). Labeled cells from tissue sections were processed using PCR to analyze the genetic tags and determine clonal patterns.

Widespread cortical clones were very common after short survival times. At E21, 32% of all cortical clones showed wide dispersion, whereas at P3 57% of cortical clones were widely dispersed, accounting for the overwhelming majority of labelled cortical neurons. At E21, there was extensive clonal dispersion in the rostro-caudal or caudo-rostral direction, and clones included cells in the cortex and the proliferative zones. This suggests movement of proliferating cells essentially parallel to the ventricle. At P3, dispersion in the medial-lateral plane was greater than at embryronic stages, which may reflect dispersion of postmitotic neurons in the intermediate zone (O'Rourke et al, 1991, Soc. Neurosci. Abst. 17:533). Thus, widespread clonal dispersion reflects several phenomena occurring in sequence. Supported by the Howard Hughes Medical Institute and the NIH.

390.9

LATERAL DISPERSION OF PREMIGRATORY, NEURAL PROGENITORS WITHIN THE VENTRICULAR ZONE OF CEREBRAL CORTEX G. Fishell*, C.A. Mason and M.E. Hatten. Dept. of Pathology in the Center for Neurobiology, College of Physicians and Surgeons, Columbia University, New York, N.Y., 10032.

In cortical regions of developing brain, neural precursors are generated in compact ventricular zones along the inner surface of the neural tube, after which postmitotic progeny migrate along the glial fiber system to establish the cortical laminae. Although recent evidence suggests significant dispersion of clonally related neural cells during development, the relative contribution of movements within the germinal zone and tangential movement across the radial glial fiber system have not been determined. To visualize the dispersion of neural progenitor cells within the ventricular zone of the mouse cerebral cortex, the lateral wall of the telencephalic vesicle was removed on embryonic day 15, and a random population of cells on the ventricular surface was labeled with a dilute solution of the lipophilic dye DiI. Immunostaining with the neural antigen nestin and with BrdU demonstrated that labeled cells were within the VZ. By correlated video-enhanced, fluorescence and phase-contrast microscopy short bursts of motility at rates between 10-100μm/h rapidly dispersed labeled cells across the ventricular zone. Labeled, dividing cells gave rise to daughter cells which separated and moved independently across wide areas of the cortical VZ. To determine whether cell dispersion was confined to the cortical VZ, we examined the behavior of labeled cells approaching the boundary with the lateral ganglionic eminence. Labeled cells did not cross from one proliferative zone to the other. Instead they contacted the boundary with filopodial extensions and moved rostro-caudally along the interface. These experiments suggest that boundaries establish and maintain a regional pattern of neurogenesis in developing brain. Within the germinal zone of the cerebral cortex, neural progenitors disperse widely prior to radial migration along the glial fiber system.

390.10

RESCUE OF *WEAVER* GRANULE NEURON DIFFERENTIATION BY TRANSPLANTATION OF THEIR PROGENITORS INTO WILD-TYPE DEVELOPING CEREBELLAR CORTEX. W.-Q. Gao* and M. E. Hatten. Dept. of Pathology, Center for Neurobiology and Behavior, College of Physicians and Surgeons, Columbia University, New York, NY 10032

The migration of postmitotic neurons away from compact, germinal zone in developing brain is a critical step in CNS neuronal differentiation. To examine the autonomy of expression of molecular signals for cerebellar granule cell migration *in vivo*, neuronal progenitors purified from the neurological mutant mouse *weaver*, an animal with phenotypic defects in migration, were tranplanted into the external germinal layer (EGL) of wild-type developing cerebellar cortex. In wild-type cerebellar cortex, dye-labeled, *weaver* EGL progenitors progressed through all of the classical stages of granule neuron differentiation, including the extension of parallel fibers, migration through the molecular and Purkinje cell layers, final posioning in the internal granule cell layer, and extension of dendrites. These observations provide evidence that *weaver* gene acts non-autonomously *in vivo*, and suggest that local interactions in the cerebellar EGL induces initial steps in neuronal differentiation required for granule cell migration.

390.11

GRANULE NEURON MIGRATION IN DEVELOPING CEREBELLAR CORTEX: A CORRELATED *IN VITRO* AND *IN VIVO* ANALYSIS. R.J. Rivas* W.-Q. Gao, and M.E. Hatten. Dept. of Pathology and Center for Neurobiology and Behavior, Columbia Univ., New York, NY 10032.

In order to compare the mechanism of neuronal migration with axon extension in vitro, granule neurons were purified from early postnatal cerebellar cortex (P4-P6) and labeled with the fluorescent lipophilic dye, PKH-26. Labeled neurons were co-cultured with unlabeled astroglial cells and neuronal migration was examined by a combination of time-lapse video and fluorescence microscopy using a light-intensified CCD camera. Examination of labeled neurons revealed frequent extension and retraction of the leading process during migration, as well as lamellopodial and filopodial extension along the entire length of the leading process. The dynamics of leading process motility differed from that of the growing granule neuron axon, where motility was confined primarily to the growth cone, which moved forward steadily without withdrawing. In order to compare migration and axon extension *in situ*, PKH-labeled neurons were transplanted back into the external granule layer (EGL) of P5 mice. The animals were then allowed to develop normally. After 1-10 days, coronal sections of cerebellar cortex were prepared and the disposition of labeled neurons was examined by confocal microscopy of fixed preparations or by time-lapse fluorescence microscopy. By 1-2 d after implantation, labeled neurons moved into the deeper aspect of the EGL and extended long parallel fiber axons (100-300 um in length) prior to migration along the Bergmann glial fibers. Migratory neurons with a leading process were observed after 2-4 d. 4-6 d after implantation, labeled neurons detached from the Bergmann glial fibers and entered the internal granule layer. These results suggest that cell soma migration and leading process motility differ from parallel fiber growth in both the timing of extension and in motile behavior.

RECEPTOR MODULATION, UP AND DOWN REGULATION III

391.1

THE EFFECT OF PHARMACOLOGIC INCREASE IN ENDOGENOUS DOPAMINE ON 11C-RACLOPRIDE BINDING MEASURED WITH POSITRON EMISSION TOMOGRAPHY (PET) IN THE BABOON BRAIN

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Recent PET studies have demonstrated the sensitivity of ^{18}F-N-methylspiroperidol (a high affinity D2 dopamine ligand) binding to pharmacologic alterations in endogenous dopamine (Dewey et al., 1990, 1991, Logan et al., 1991). The present study was undertaken to further examine drug induced changes in dopamine using ^{11}C-raclopride, a lower affinity D2 ligand, and two drugs which increase dopamine by different mechanisms. PET studies were conducted in anesthetized, adult female baboons (Papio anubis) using the CTI 931 tomograph. Two ^{11}C-raclopride scans were performed, two hours apart, prior to and following drug intervention with either d-amphetamine, which releases cytosolic dopamine (1.0 mg/kg, IV, 5 minutes pre-injection) or GBR 12909, the selective dopamine reuptake inhibitor (1.5 or 3.0 mg/kg, IV, 20 minutes pre-injection). The data were analyzed with the distribution volume method (Logan et al., 1990). After both interventions, ^{11}C-raclopride binding was decreased, bilaterally, in the striatum (specific binding), but not in the cerebellum (non-specific binding). These decreases exceeded the test-retest variability of the ligand, determined in the same animals. The rate of metabolism of the radiotracer was unaltered. Therefore, ^{11}C-raclopride binding is sensitive to alterations in endogenous dopamine. This finding provides further support for the utility of PET in assessing the functional responsiveness of the dopamine system, in vivo. Supported by DOE/OHER, NIH: NS15638, NS15380.

391.2

SIMILARITIES AND DIFFERENCES IN DOPAMINE RECEPTOR SUBTYPE REGULATION: MOLECULAR MECHANISMS. Ian Creese* and S.-X. Xu, Center for Molecular & Behavioral Neuroscience, Rutgers-Newark, NJ 07102.

Steady state levels of cell surface receptors are determined by many distinct processes, each of which may be subject to different regulatory mechanisms. Both striatal D_{1A} and D_{2A} receptors show a similar developmental pattern where receptor number increases by 6-14 fold over the first 30 days postnatal. However, mRNA levels for both receptors increase by only 2 fold over this period. Whereas denervation, by a 6-OHDA lesion of the nigro-striatal pathway, increased D_{2A} receptor mRNA levels by 53% and receptor level by 38%, D_{1A} receptors and their mRNA were unchanged. In contrast, a 3 week administration of the D_1 receptor antagonist SCH23390 (0.5mg/kg/day s.c.) increased both D_{1A} receptor density and mRNA levels. However, treatment with the D_2 receptor antagonist haloperidol (0.5 mg/kg/day, s.c) had no effect on striatal D_{2A} mRNA levels, in spite of significant increases in receptor B_{max}. This was not the result of the intermittent receptor blockade following drug injection, as chronic infusion of haloperidol via osmotic mini-pump also did not increase D_{2A} mRNA, in spite of a 59% increase in receptor B_{max}. Interestingly, chronic dopamine depletion with reserpine did not affect mRNA levels for D_{1A} or D_{2A} receptors, with only D_{2A} receptors being significantly upregulated. These findings indicate that the various molecular mechanisms involved in receptor regulation (gene transcription, receptor degradation etc.) are differentially involved in the regulation of each dopamine receptor subtype.

Supported by grants from the Stanley Foundation and NIMH.

391.3

INCREASED 5-HT2 AND NK-1 RECEPTOR BINDING ASSOCIATED WITH SEROTONIN/SUBSTANCE P HYPERINNERVATION IN RAT INFERIOR OLIVE. M. Paré, L. Descarries* and R. Quirion. Centre de recherche en sciences neurologiques (Département de physiologie), Université de Montréal, and Douglas Hospital Research Center and Department of Psychiatry, McGill University, Montréal and Verdun, Québec, CANADA.

Serotonin/5-HT2 and substance P (SP)/NK-1 receptors were measured by ligand binding autoradiography with [3H]ketanserin and [125I]BH-SP, in the inferior olive (IO) of adult rats previously subjected to cerebroventricular administration of 5,6-dihydroxytryptamine. This experimental treatment was previously shown to be followed by marked 5-HT hyperinnervation in most subdivisions of IO (e.g. thrice the normal number of 5-HT axon varicosities in the lateral dorsal accessory olive, lat DAO). It is also known to induce a parallel augmentation of the number of SP-immunoreactive varicosities in several parts of IO, which suggests a co-localization of both transmitters in the same hyperinnervating terminals. In normal IO, the density of 5-HT2 sites was relatively low and rather homogeneous. NK-1 binding appeared denser and more heterogeneously distributed. After 5-HT/SP hyperinnervation, considerable increases in the density of both classes of binding sites were observed. Specific [3H]ketanserin binding was now stronger in most IO subnuclei, including some in which it had not been detected in the normal. [125I]BH-SP binding showed even greater elevations or became detectable in a few subnuclei, remained unchanged in others, and was slightly decreased in the lat DAO. The normal and altered distributions of both ligands did not match the respective patterns of 5-HT and/or SP innervation and hyperinnervation. In view of current information on the cellular localization of 5-HT2 and NK-1 receptors in IO, it seemed likely that both increases in the hyperinnervated state were largely the result of an up-regulation, and not the mere reflection of an augmented number as autoreceptors on 5-HT and/or SP terminals. (Supported by MRC grants MT-3544 and MA-8580).

391.4

MUSCARINIC ANTAGONISTS INHIBIT THE PROLIFERATION AND EGF RECEPTOR EXPRESSION OF HUMAN OCULAR & NIH/3T3 FIBROBLASTS

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Protein kinase C (PKC) and immediate early gene expression are increased by muscarinic agonists via G_p protein and diacyl glycerol. PKC reduces the binding of EGF and EGF-R synthesis in fibroblasts by phosphorylation of the epidermal growth factor receptor (EGF-R). Additionally, muscarinic antagonists retard scleral growth in avian form-deprivation and human physiological myopia. We hypothesize an inhibitory effect of muscarinic antagonists on the growth of ocular fibroblasts, possibly by modulating their EGF-R expression. Using NIH/3T3, human scleral, and corneal fibroblasts (keratocytes) in culture, we investigated the effect of muscarinic agents on cell proliferation, protooncogene expression, and EGF-R density. When confluent monolayers of fibroblasts were incubated with muscarinic agents (10nM to 10μM), atropine and pirenzepine caused a dose-dependent reduction of proliferation and the number of cells in the G2/M phase of the cell cycle. *c-fos* expression was induced by carbachol, an effect partly blocked by atropine. EGF-R were quantitated by labelling with biotinylated anti-EGF-R antibody and the fluorescence measured following the addition of streptavidin-R-phycoerythrin. Carbachol (1-10μM) caused an early reduction in EGF-R, followed by an increase to 1.5x the resting level, by 18 hours. Oxotremorine showed the opposite effect on EGF-R levels. When human EGF (1ng/ml) was added, downregulation of EGF-R in keratocytes was observed at 2 hours, followed by upregulation to resting levels by 12 hours. With atropine (100μM), EGF caused an identical downregulation, but the EGF-R density remained at 40% of resting levels even after 12 hours. We also demonstrated the inhibition of fibroblast proliferation by atropine *in-vivo*, using rabbit models of corneal wound healing.

391.5

AGE-DEPENDENT REGULATION OF CORTICAL AMINO ACID RECEPTORS. R.A. Lanius* and C. Shaw, Depts. of Neuroscience, Ophthalmology and Physiology, University of British Columbia.

We have used a 'living' rat cortical slice preparation to examine the regulation of $GABA_A$ ([3H]-SR 95531), AMPA ([3H]-CNQX), NMDA ([3H]-CGP 39653) and kainate ([3H]-kainate) receptor populations during postnatal development. Regulation of these receptor populations was acheived using either stimulation with the appropriate agonist or using veratridine and glutamate (V+G) to increase neural electrical activity. In adult cortex, V+G resulted in an up-regulation of $GABA_A$ receptors in contrast to a down-regulation of AMPA, NMDA, and kainate receptors. During postnatal development V+G showed age-dependant effects: in animals younger than 25d, $GABA_A$ receptor number was decreased while AMPA receptor number was increased; NMDA and kainate receptors decreased. After 40d, V+G treatment led to an increase in $GABA_A$ receptor number; at ages greater than 60 d AMPA receptors decreased. NMDA and kainate receptors showed decreases to V+G at all ages. Agonist stimulation led to down-regulation of all four receptor populations. Quisqualate stimulation of AMPA receptors increased down-regulation with increasing postnatal age, an opposite effect to that observed for $GABA_A$ and kainate receptors. The differing effects with age on receptor regulation for either changes in cell electrical activity or agonist stimulation may suggsest a role for such age-dependent receptor regulation in the events leading to neuronal critical period plasticity.

391.6

REVERSAL OF ENZYMATIC CONTROL OF CORTICAL AMINO ACID RECEPTOR REGULATION IN DEVELOPMENT. C. Shaw* and R. A. Lanius. Departments of Ophthalmology, Neuroscience, and Physiology, University of British Columbia, Vancouver, Canada.

We have used a rat neocortical slice preparation to examine the effects of phosphorylating / dephosphorylating reactions by alkaline phosphatase (AP) or protein kinase (PK) on $GABA_A$ and AMPA receptors in different stages of postnatal development. Cortical slices were frozen then thawed before exposure to varying concentrations of AP or PK. In adult rats, the addition of AP led to increases in binding of [3H]-SR 95531 ($GABA_A$) and [3H]-CNQX (AMPA) with a significant 20% increase at $2.5x10^{-7}$mg/mL in both receptor populations. In contrast, PK led to significant 30% decreases for both receptors at $5x10^{-6}$ pM/μg protein/mL. The effects of AP or PK on [3H]-SR 95531 binding were reversed in 12 to 14 day old rats. AP resulted in significant 24% and 28% decreases in [3H]-SR 95531 binding at $2.5x10^{-6}$ and $2.5x10^{-7}$ mg/mL, respectively. Conversely, PK led to significant 29% and 55% increases in [3H]-SR 95531 binding at $5x10^{-6}$ and 5x10-7pM/μg protein/mL, respectively. Similar effects of AP or PK on [3H]-CNQX binding were observed in 19 to 21 day old rats. AP led to a significant 37% decrease in [3H]-CNQX binding at $2.5x10^{-4}$mg/mL whereas PK resulted in significant 23% and 43% increases in [3H]-CNQX binding at $5x10^{-8}$ and $5x10^{-9}$ pM/μg protein/mL. These results suggest an age-dependent role for phosphatase and kinase reactions leading to amino acid receptor regulation in neocortex during postnatal development.

391.7

NMDA RECEPTOR (NMDAR1) ANTISENSE (AS) OLIGODEOXYNUCLEOTIDE HAS NEUROPROTECTIVE PROPERTIES IN RAT CORTICAL NEURONS. C.Wahlestedt, S.Regunathan, L.Lyandvert, F. Yee and D.J.Reis*. Div. Neurobiology, Dept. Neurol. & Neurosci., Cornell Univ. Med. Coll., N.Y., N.Y. 10021.

Based on the nucleotide sequence of rat NMDAR1 (Moriyoshi et al., *Nature* 354:31, 1991), we designed several AS, and corresponding sense (S), oligodeoxynucleotides (D-oligos) in an attempt to suppress synthesis of the NMDAR1 receptor protein. Herein described data were obtained with aminoterminally directed AS and matching S 18-mer D-oligos. These D-oligos were added under serum-free conditions to primary cultures of rat cortical neurons which were studied with respect to: (1) ^{3}H-MK-801 binding; (2) basal and NMDA-induced lactate dehydrogenase (LDH) release; and (3) basal and NMDA-induced $^{45}Ca^{2+}$ influx. A reduction of ^{3}H-MK-801 sites by 30-50% (*vs.* S or no D-oligo) could be achieved by 3-5 days incubation of the neurons with 1 μM of the AS D-oligo; this down-regulation was less dramatic than with an aminoterminal AS D-oligo of the NPY Y1-receptor (Yee *et al.*, this meeting). The AS D-oligo induced a concentration dependent (0.1-10 μM) reduction of spontaneous LDH release (measured 1-3 days after removal of serum) and evoked release (5 min exposure of 9-day old cultures to 100 μM NMDA). Finally, we found that spontaneous, but not NMDA-induced, $^{45}Ca^{2+}$ influx (measured over 2 min) was elevated, up to 2-fold, in neurons exposed to AS D-oligos, possibly also reflecting increased degrees of neuronal survival in these cultures. However, there may exist a "window" of optimum AS D-oligo concentration, since the S D-oligo, at 10 μM but not at 1 μM, showed minor effects *per se*. In conclusion, the present study indicates the usefulness of the antisense approach for down-regulation of (receptor) protein, such as the NMDAR1. It also lends direct support to the hypothesis that excitation through activation of the NMDA-receptor is associated with neuronal cell death.

391.8

ANXIOLYTIC AND ANTICONVULSANT DOSES OF ABECARNIL FAIL TO INDUCE TOLERANCE AND DEPENDENCE IN MICE AND CATS. M. Serra, C.A. Ghiani, M.C. Foddi, C. Motzo and G. Biggio*, Department of Experimental Biology, Chair of Pharmacology, University of Cagliari, Italy.

In mice, [35S]TBPS binding and exploratory motility were dramatically reduced by the acute administration of abecarnil (AB) (0.2 - 1 mg/kg i.p.). Chronic administration of this drug (1 mg/kg 3 times at day) induced in two weeks a significant increase in [35S]TBPS binding in the mice cerebral cortex with no change in motor behaviour. This biochemical effect was paralleled by the failure of a challenge dose of AB (1 mg/kg) to modify both [35S]TBPS binding and motor activity. On the contrary, in mice chronically treated with a lower but pharmacological effective dose (0.2 mg/kg) the exploratory motility was reduced (-30%) by a challenge dose of AB (0.2 mg/kg). This evidence may suggest that tolerance develops only with very high doses of AB. This conclusion is consistent with the finding that in the brain of this chronic treated mice the binding of [35S]TBPS was unchanged compared to control. Moreover, while in mice treated with the high dose of AB (1 mg/kg) the binding of [35S]TBPS was altered after drug discontinuation (increased by 26% at 48 h and decreased by 15% at 96 h), we found not change in mice chronically treated with 0.2 mg/kg at the same times. Our data indicate that chronic administration of AB is associated with the development of tolerance and with discontinuation syndrome only at very high doses while is devoid of these effects at lower, but pharmacologically effective, doses. This conclusion is also consistent with the finding that in cats chronically treated with AB (7 mg/kg 3 times for 15 days) the intraperitoneal administration of flumazenil (20 mg/kg) failed to precipitate an abstinence syndrome.

391.9

EFFECT OF LESIONS OF THE DORSAL RAPHE NUCLEUS ON SEROTONIN TRANSPORTER, 5-HT_{1A} AND 5-HT$_2$ BINDING SITES IN RAT.
M.D. Underwood, M.J. Bakalian, M.L. Miller, C.K. Katz, T.F. Lagattuta, J.J. Mann and V. Arango.* Labs of Neuropharm., Univ. of Pittsburgh, Pittsburgh, PA 15213

Decreases in the serotonin (5-HT) transporter and increases in 5-HT_{1A} binding are found in restricted cortical areas of suicide victims. We sought to determine whether 5-HT binding following lesions of the dorsal raphe nucleus (DRN) in rat parallel the 5-HT binding changes reported in man and to examine the extent of any subcortical changes. Three groups of rats were studied: unlesioned controls (n=5), DRN lesion (1 mA for 45 sec; n = 5) and sham-operated controls (n=5). Binding to the 5-HT transporter (^{3}H-cyanoimipramine; 0.4 nM), 5-HT_{1A} (^{3}H-DPAT; 2nM) and 5-HT$_2$ (^{3}H-ketanserin; 2 nM) sites were quantified in 25 regions by image analysis. 5-HT and 5-HIAA content in frontal lobe were determined by HPLC. In controls, specific 5-HT transporter binding was greatest in the DRN (409±66 fmol/mg tissue), least in the corpus callosum (8±3) and did not differ from sham-operated controls (p>0.05). DRN lesions reduced 5-HT and 5-HIAA by 78% and 92%, respectively, and reduced (p<0.05) transporter binding in all regions examined from 90% (perirhinal cortex) to 29% (rostroventrolateral medulla). 5-HT_{1A} binding was greatest in presubiculum (101±8) and least in the caudate nucleus (1±0.3). DRN lesions had restricted effects on 5-HT_{1A} binding with increases in frontal cortex (119% of control, p=0.06), inferior colliculus (137%, p=0.09) and hippocampus (115%, p=0.01). ^{3}H-ketanserin binding ranged from 31±10 fmol/mg (frontal cortex) to 2±1 fmol/mg (corpus callosum) and was unaffected by DRN lesions (p>0.05) in any brain region. We conclude that DRN lesions produce: 1) profound effects on 5-HT projections to the forebrain and brainstem; 2) restricted changes in ^{3}H-DPAT binding and 3) no effect on ^{3}H-ketanserin binding. These findings parallel those in suicides in several respects: 1) 5-HT transporter binding is reduced; 2) 5-HT_{1A} binding is increased and 3) ^{3}H-ketanserin binding is unchanged. Notable differences include: 1) widespread *vs.* localized reductions in transporter sites and 2) marked reduction *vs* no change in 5-HT and 5-HIAA. The cortical 5-HT receptor changes in suicide are consistent with a partial lesion of the DRN. Supported by NARSAD award to MDU and MH46745.

391.10

EFFECTS OF PARA-CHLOROAMPHETAMINE LESIONS ON PLATELET [^{3}H]-PAROXETINE BINDING IN RATS.
M.J. Owens,*D.L. Knight and C.B. Nemeroff. Lab. of Neuropsychopharmacology, Dept. of Psychiatry & Behavioral Sciences, Emory Univ. Sch. Med., Atlanta GA 30322.

Considerable evidence has accumulated to implicate serotonergic neuronal dysfunction in the pathophysiology of major depression. One such line of evidence is the reduction in the density of binding sites for [^{3}H]-imipramine or [^{3}H]-paroxetine, markers of the 5-HT transporter, in platelets of patients with major depression. In an attempt to determine whether changes in platelet [^{3}H]-paroxetine binding represents changes in CNS 5-HT function, we investigated whether depletion of 5-HT and destruction of 5-HT nerve terminals by para-chloroamphetamine alters [^{3}H]-paroxetine binding to the platelet 5-HT transporter

Rats received a single injection of para-chloroamphetamine (10 mg/kg, sc) or water. Because platelets have a lifetime of several weeks in plasma, groups of rats were killed at various time points (0, 1, 2, 4, and 10 weeks post-injection) following the lesion in order to allow for several complete turnovers of new platelet production. [^{3}H]-paroxetine binding (0.6 nM) in prefrontal cortex and platelets was determined in triplicate using 1 μM fluoxetine to determine non-specific binding. Para-chloroamphetamine administration decreased cortical [^{3}H]-paroxetine binding 40-50% at all time points studied. Although platelet [^{3}H]-paroxetine binding was highly variable, a small increase in binding was observed at the two week time point only. These preliminary findings suggest that at the magnitude of lesion produced here (50% destruction of 5-HT terminals), platelet 5-HT transporter binding is not greatly effected.

NEUROENDOCRINE REGULATION I

392.1

AMPLIFIED PULSATILE SECRETION OF GROWTH HORMONE (GH) IN CHRONIC RENAL FAILURE.
J.D. Veldhuis*, M.L. Wilkowski, A. Iranmanesh, W.K. Bolton, Dept. Internal Medicine, NSF Science Center in Biological Timing, Univ. Virginia Health Sciences Center, Charlottesville, VA 22908, and Salem Veterans Affairs Hospital, Salem, VA 24153.

Uremia evokes alterations in multiple neuroendocrine axes, including the gonadotropic, corticotropic, and somatotropic (GH) axis. We have studied the neuroendocrine control of episodic GH secretion and simultaneously estimated GH half-life in 7 middle-aged men (mean ages 39 ± 5.7 years) with endstage renal failure (ESRF) compared to a group of 7 age-matched controls (mean ages 42 ± 4.2 years). Multiparameter deconvolution analysis (PNAS 84:7686-90, 1987) of 24 hour serum immunoradiometric GH concentrations revealed a calculated half-life of endogenous GH of 21 ± 1.8 min in the men with ESRF compared to 17 ± 2.0 in controls. Uremia was accompanied by an increased frequency of pulsatile GH release namely 16 ± 1.0 secretory bursts/24 hour versus 12 ± 1.8 (control). The mass of GH secreted per burst was increased in uremia (4.5 ± 1.2 ng/mL versus 3.0 ± 1.1). Consequently, the 24 hour endogenous GH production rate was approximately 2-fold higher (73 ± 1.9 ng/mL/day) in uremics versus 36 ± 18 in age-matched controls. In contrast, the duration of GH secretory bursts was not altered in uremia. The mean 24-hr serum GH concentration was 1.5 ± 0.38 (uremia) versus 0.62 ± 0.28 ng/mL (control). In summary, uremia is accompanied by a small increase in the half-life of putatively intact immunoradiometric GH as well as an increased frequency and mass of pulsatile GH secretion. We therefore hypothesize that somatostatin withdrawal, and to a lesser extent decreased GH clearance, contribute to elevated plasma GH concentrations observed in uremia in humans.

392.2

pGLUTAMYLGLUTAMYLPROLINEAMIDE (EEP) MODULATION OF GROWTH HORMONE SECRETION IN THE CHICKEN.
V.L. Trudeau*, S. Harvey, R.J. Ashworth and S.M. Cockle. Dept. of Physiol., Univ. Alberta, Edmonton, Canada and Biochem. and Physiol., Univ. Reading, U.K.

A TRH-like tripeptide, EEP, originally isolated and characterized from rabbit prostate, also present in significant concentrations (63% total TRHir) in rat anterior pituitary, has no identified function. TRH is a physiological stimulator of GH secretion in birds; it is more effective in egg-laying than in meat-type broiler strains. EEP is present in low concentrations (6% total TRHir) in the pituitary of broiler but not layer strains. Given the structural similarities of these peptides, EEP may compete for pituitary TRH receptors (TRH-R) and affect GH release. EEP (10^{-5}-10^{-3}M) inhibited ^{3}H-Me-TRH binding to chicken pituitary membrane TRH-R but did not affect basal GH release from whole pituitaries in vitro, suggesting that EEP action is dependent on other (hypothalamic?) factors. Unlike TRH stimulation, EEP (1-100µg/kg; i.v.) decreased basal plasma GH levels by >50% within 10 min in anaesthetized immature males. Paradoxically, EEP potentiated submaximal TRH-induced GH release without changes in TRH-R, an effect very similar to that of benzodiazepine-type TRH antagonists. These studies demonstrate for the first time that EEP participates in regulation of GH release in the chicken possibly by interactions with the pituitary TRH-R. In addition, the relative lack of EEP in the avian pituitary may explain why TRH more effectively stimulates GH release in birds than in mammals. (Supported by NSERC).

392.3

PATCH CLAMP ANALYSIS OF GROWTH HORMONE-RELEASING FACTOR (1-44) EFFECTS IN HUMAN GROWTH HORMONE SECRETING CELLS IN CULTURE.
C. Chen*, P. Heyward, P. McNeill, J. Cummins and I. J. Clarke. Prince Henry's Institute of Medical Research, P.O. Box 152, Clayton (Melbourne), Victoria 3168, Australia

We have previously reported that local application of human growth hormone-releasing factor 1-44 (hGRF) in rat pituitary somatotropes causes a depolarization of cell membrane and growth hormone (GH) secretion that depends upon Ca influx (Chen et al., Neuroendocrinology 50:679, 1989). This effect can be obtained by conventional microelectrode intracellular recording but not by standard whole-cell patch-clamp recording. Alternatively in the present experiment, using the Nystatin-perforated patch-clamp technique in the whole-cell configuration, this effect has been observed in identified human growth hormone (GH) secreting tumour cells from acromegalic patients. With local application of hGRF (10nM), a slow depolarization occurs with the generation of multiple action potentials. Current-induced depolarization in the absence of hGRF causes a single action potential but in the presence of hGRF multiple action potentials are seen. The characteristics of the action potentials, in terms of Ca and Na flux, are similar to those previously seen in rat somatotropes (Chen et al., Life Sci. 46:983, 1990). In particular, hGRF increased the action potential due to Ca influx. The Ca current was enhanced by hGRF in cells treated with TEA (20mM) and TTX (1uM) in the bath solution and Cs in the electrode solution to eliminate K and Na currents. The voltage-dependent kinetics of Ca currents were also modified by hGRF application. These results show that hGRF can increase influx of Ca in human tumour derived somatotrophs *via* its effects on Ca channels in a manner similar to that seen in normal rat somatotropes. Supported by NH & MRC and Eli Lilly Growth Grant.

392.4

INHIBITORY REGULATION OF PULSATILE GnRH RELEASE FROM GT_1 GnRH CELL LINES BY VASOPRESSIN AND GABA .
R. I. Weiner and G. Martínez de la Escalera*. Reproductive Endocrinology Center, University of California San Francisco, CA 94143, and Instituto de Investigaciones Biomédicas, National University of México, México City 04510, México.

Arginine vasopressin (AVP) containing nerve terminals synapse with GnRH neurons and may play a role in stress-induced inhibition of LH secretion. γ-aminobutyric acid (GABA) neurons also synapse with GnRH neurons and substantial evidence support their role on GnRH release. GnRH cell lines (GT_1) release GnRH with a pulse frequency similar to that observed in castrated mice. Superfusion of GT_{1-1} cells with AVP (100 nM) inhibited pulsatile release of GnRH. The mechanism by which AVP inhibits GnRH release is not clear since 10-100 nM AVP for 1-10 min does not affect tyrosine phosphorylation (assessed by phosphotyrosine Western blots) nor adenylate cyclase activity (assessed by RIA of intracellular cyclic AMP) in GT_{1-7} cells. On the other hand, GABA (10 µM) showed a biphasic effect on GnRH release, with a brief and rapid stimulation followed by a long and sustained inhibition. The effect of GABA is correlated with a bicuculline- and saclofen-sensitive inhibition of the basal and stimulated intracellular levels of cAMP. These findings demonstrate that two putative inhibitory neurotransmitters in the regulation of GnRH release are capable of exerting their actions directly on GnRH neurons. (Work supported by NIH Grant HD 08924 and The Rockefeller Foundation).

392.5

PROLACTIN INHIBITION OF GnRH RELEASE FROM GT1 GnRH CELL LINE VIA PROLACTIN RECEPTORS. L. Milenkovic, G. D'Angelo and R.I. Weiner*, Reproductive Endocrinology Center, University of California San Francisco, CA 94143

Numerous observations have demonstrated an inverse relationship between prolactin (PRL) and LH secretion. We tested whether PRL directly acts on GT1 GnRH neurons to affect GnRH release. Prolactin (rat or mouse, 10^{-10} - 10^{-8} M) suppressed GnRH release in a dose-dependent fashion from both GT1-1 and GT1-7 cell lines in static culture. The maximal concentration of PRL suppressed 40% of GnRH released during 1 hour. Both the mRNA and protein for PRL receptors were shown to be present in GT1-7 cell line. A monoclonal antibody to rat liver PRL receptor (U5) stained a 42 kDa dublet, consistent with the short form of the PRL receptor, in western blot of GT1-7 cell membranes. Western blots of mouse liver membranes indicated a major band at 42 kDa, and a minor band at approximately 80 kDa. A cDNA probe which recognized all known forms of the PRL receptor hybridized to the bands of 2.4, 3.2 and 4.2 kb in northern blots of GT1-7 cell RNA. In conclusion PRL inhibits GnRH release via prolactin receptors expressed on GnRH neuronal cell lines. Further work is necessary to identify the PRL receptor form mediating this action. (This work was supported by NIH Grant HD 08924.)

392.6

SOMATIC AFFERENT REGULATION OF PLASMA PROLACTIN IN ANESTHETIZED RATS.
H. Hotta[1], A. Sato*[1], Y. Sato[2] and A. Suzuki[1]. [1]Department of Autonomic Nervous System, Tokyo Metropolitan Institute of Gerontology, Tokyo 173, [2]Laboratory of Physiology, Tsukuba College of Technology, Tsukuba 305, Japan.

These experiments examined the effects of noxious and non-noxious mechanical stimulation of various skin areas (forelimb and forepaw, abdomen, hindlimb and hindpaw) on levels of plasma prolactin in anesthetized rats. The experiments were performed on female Wistar rats on the day of estrus, which had been anesthetized with urethane (1.1 g/kg, i.p.) and artificially ventilated. Blood samples, for the determination of plasma prolactin by RIA, were collected from a femoral artery every 20 min. Noxious mechanical stimulation of the skin was delivered by pinching, using a surgical clamp, while non-noxious mechanical stimulation was provided by brushing with a tooth brush. Pinching of a hindpaw for 6 min significantly increased plasma prolactin during stimulation, while pinching of a forepaw or abdomen had no significant effect. Brushing of skin areas for 6 min did not significantly change plasma prolactin. These results indicate that when emotional factors were eliminated by anesthesia, cutaneous nociceptive sensory information contributes to the reflex regulation of prolactin secretion from the anterior pituitary, and also that this effect is highly dependent on the site of stimulation.

392.7

CHEMICAL PLASTICITY OF HYPOTHALAMIC TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS : ROLE OF PROLACTIN. P. Ciofi*, W.R. Crowley [a], D. Croix, J.C. Beauvillain, V. Mitchell, G. Tramu[b] and M. Mazzuca. U.156 INSERM, 59045 Lille (France); [a]Dept. Pharmacol., Univ. Ten. Memphis, Memphis TN 38163; [b]Lab. Neurocyto. Fonct., URA CNRS 339, Univ. Bordeaux I, Talence 33405 (France).

Reports of increased immunoreactivity (IR) for enkephalin (ENK) (Soc. Neurosci. Abs. 15:702, 1989) and neuropeptide Y (NPY) (Endo.128:823, 1991) in TIDA neurons of lactating rats and mice led us to determine with immunohistochemistry 1) whether the expression of other peptides in the median eminence (ME) is also altered during lactation in rats and mice, and 2) whether prolactin (PRL) might be involved in these changes. IRs for NPY, ENK and neurotensin (NT) were dramatically decreased in the ME of post-partum (PP) day 8 lactating mice that were pup-deprived for two days, compared to continually nursing PP8 mice. Fluorescent triple-staining showed colocalization of these IRs in all tyrosine-hydroxylase-IR endings. Preliminary ultrastructural double-staining experiments suggest a nonhomogenous distribution of these neuropeptides among neurosecretory vesicles in the same nerve terminals. On PP-8, NPY- and ENK-IRs were visible in putative ME TIDA endings of both intact and PP1-ovariectomized lactating rats. Changes in NT-IR could not be discriminated. Considerable interanimal variation in NPY-IR suggested the influence of an underlying pulsatile phenomenon. When intact lactating rats were separated from their litters for four hours and then reunited, we detected a trend for increased NPY-IR, but not for ENK- or NT-IR, in the ME during the 30-60min following the onset of suckling, associated with elevated circulating PRL levels. NPY-IR, but not ENK- or NT-IR, was also expressed in putative TIDA endings in orchidectomized rats made hyperprolactinemic from implants of estradiol-filled capsules.

These results suggest that 1) an ovarian influence may not be required for the expression of NPY- and ENK-IRs in the TIDA neurons during lactation, 2) PRL may be responsible for inducing NPY expression in these cells, and 3) additional factors may regulate expression of ENK- and NT-IRs in TIDA neurons with both sex and species differences in their actions.

392.8

A TROPHOBLAST-SPECIFIC FACTOR(S) INCREASES TYROSINE HYDROXYLASE (TH) ACTIVITY IN FETAL HYPOTHALAMIC CELL CULTURES. L.A. Arbogast*, M.J. Soares, M.C. Robertson and J.L. Voogt, Physiology Dept., Univ. of Kansas Med. Ctr., Kansas City, KS 66103 and Physiology Dept., Univ. of Manitoba, Winnipeg, Manitoba.

We previously reported that a factor(s) from rat choriocarcinoma (Rcho) cells *in vivo* suppresses PRL and increases tuberoinfundibular dopaminergic neuronal activity. Rcho cells can differentiate into trophoblast giant cells which produce members of the placental PRL family. The aims of this study were: 1) to establish a neuronal culture system to characterize the Rcho factor(s) which increase TH activity and 2) to assess the ability of lactogenic hormones to alter TH activity. Ventral hypothalamic cells of fetal day 20 rats were dispersed with trypsin, plated at 200,000 cells/well and maintained in culture for 14 days. TH-immunopositive neurons were observed in the cultures. Cell contents of dopamine and dihydroxyphenylalanine (DOPA) were 186 pg and 50 pg, respectively, per 200,000 cells. TH activity was determined by a 1h incubation with Earles' Balanced Salts containing 100 µM brocresine, a DOPA decarboxylase inhibitor, and assessing DOPA in the medium. Medium DOPA was linear between 0-4h with or without brocresine, but was 3-fold higher with brocresine. Hypothalamic cells were co-cultured for 24h with 100,000 Rcho, HRP (placental line not expressing known placental PRLs), or MMQ (a PRL-secreting cell line) cells. Control DOPA accumulation was 94±17 pg DOPA/ 200,000 cells/h. Co-culture with Rcho cells increased DOPA accumulation 4-fold, whereas HRP and MMQ cells had no effect. A 2-fold or 3-fold increase in TH activity was observed with 25,000 or 50,000 Rcho cells, respectively. α-Methyl-p-tyrosine (1mM), a specific inhibitor of TH, markedly reduced DOPA accumulation in both control and Rcho-stimulated cultures. Incubation for 24 h with 1µg/ml of rPRL or recombinant rat placental lactogen I did not alter TH activity, whereas 1h incubation with 5 mM dibutyrl cAMP increased DOPA accumulation 3-fold. Conclusion: A trophoblast-specific factor(s), likely not placental lactogen I, specifically and in a dose-dependent fashion increases TH activity in hypothalamic cells *in vitro*. Supported by HD24190 (JLV), HD20676 (MJS), HD07368 (LAA).

392.9

SUPPRESSED TYROSINE HYDROXYLASE (TH) AND INCREASED NPY GENE EXPRESSION IN THE ARCUATE NUCLEUS OF LACTATING RATS. H.-J. Wang and M.S. Smith*. Department of Physiology, University of Pittsburgh, Pittsburgh, PA 15261.

The suckling stimulus alters hypothalamic function, resulting in suppressed GnRH neuronal activity and increased prolactin secretion. To understand mechanisms responsible for these effects of suckling, we examined whether lactation was associated with changes in gene expression in the arcuate nucleus (AN). Animals were studied during diestrus-1, and on day 10 postpartum with 8 pups suckling or 24 hr after pup removal. *In situ* hybridization (ISH) was performed to assess mRNA levels for TH and NPY. For single label ISH,TH and NPY riboprobes were labeled with ^{35}S. For double label ISH, TH riboprobe was labeled with ^{35}S and NPY riboprobe with digoxigenin. TH mRNA (grain area/AN area) levels in lactating rats were suppressed to 65 % in the rostral AN and were undetectable in the remainder of AN, as compared to diestrous controls. In contrast, TH mRNA in the zona incerta did not differ between the two groups of animals. At 24 hr after pup removal, TH mRNA levels had increased to nearly 200% of diestrous controls. NPY mRNA levels were similar in diestrous and lactating animals except in the caudal area of the AN (at the level of the dorsomedial hypothalamic nucleus) where mRNA levels increased nearly two-fold in lactating rats. The change in NPY gene expression was reversed 24 hr after pup removal. Using double label ISH, we found TH and NPY expressing neurons in close proximity in some areas of the AN but no evidence for expression of TH and NPY in the same neurons. In summary, the suckling stimulus changes gene expression in specific subpopulations of dopamine and NPY neurons in the AN. These changes may possibly alter the dynamics of the transmitter released. Supported by Grant HD14643.

392.10

FOS-RELATED ANTIGENS AS MARKERS OF BASELINE NEURONAL ACTIVITY: EFFECTS OF LACTATION ON ARCUATE NUCLEUS DOPAMINE NEURONS. G.E. Hoffman*, W-S.Lee, R. Abbud and M.S. Smith. Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

In many areas of the brain, neurons express Fos-related antigens (FRAs), but not cFos, under baseline conditions. These studies assessed whether FRA expression can serve as a marker for both decreased as well as increased baseline neuronal activity. To test this hypothesis, we examined FRA expression in tuberoinfundibular (TI) dopamine (DA) neurons of the arcuate nucleus in cycling female rats and in pregnant and lactating animals. The TIDA neurons comprise the principal prolactin inhibitory system. Their activity tonically inhibits prolactin release. However, in the lactating rat, TIDA neuron activity is presumably inhibited, thereby facilitating prolactin release. Double label immunocytochemistry localized tyrosine hydroxylase (TH) and either FRAs or c-Fos. FRAs and c-Fos were distinguished using antisera generated against either the M-peptide region of c-Fos (capable of recognizing most FRAs as well as c-Fos) or the N-terminal of c-Fos (selective for c-Fos). Under resting conditions, cycling rats and pregnant rats showed FRA but not c-Fos expression in their TIDA neurons. Animals suckling 8 pups for 10 days had no FRA or cFos activity in TIDA neurons. FRA staining returned 3 hours after pup removal and was restored to full expression by 24 hours. The changes in FRA expression in the presence of the suckling stimulus and upon pup removal parallel changes we observed in TH gene expression in TIDA neurons of lactating animals (See abstract by Wang, H.-J. et al). These data suggest that FRA expression can effectively serve as a marker of baseline activity in identified populations of neurons. *Supported by NIH Grants HD 14643 and NS 28730.*

392.11

RAPID CHANGES IN OXYTOCIN AND VASOPRESSIN mRNA CONCENTRATIONS IN THE EARLY POSTPARTUM PERIOD: EVIDENCE FOR A ROLE OF FACTORS OTHER THAN SUCKLING. R.S. Crowley and J.A. Amico*. University of Pittsburgh School of Medicine and VA Medical Center, Pittsburgh, PA 15261.

Oxytocin (OT) and vasopressin (AVP) gene expression are enhanced in the rat hypothalamus in late gestation and during the second and third weeks of lactation. Increased OT gene expression during lactation is believed to be due to suckling, which is known to activate OT neurons resulting in coordinated electrophysiological activity of these neurons and release of OT from the posterior pituitary. We hypothesized that other factors such as ovarian steroids might modulate responses to stimulation of these neurons. We therefore investigated expression of hypothalamic OT and AVP mRNAs using Northern analysis and in situ hybridization during the first ten days of lactation when rats are continuously suckling but ovarian steroid concentrations are known to change markedly. We report that during the first three postpartum days, OT and AVP mRNAs decreased dramatically reaching less than one fifth of peak gestation levels by day 2 postpartum in both supraoptic (SON) and paraventricular (PVN) nuclei. OT and AVP mRNA returned to levels comparable to late gestation on or about day 10 lactation, and remained elevated after 15 and 23 days of lactation. We have also compared OT mRNA isolated from lactating day 3 rats to cohorts which had litters removed at the time of parturition. Lactating rats had significantly lower OT mRNA levels than their non-lactating cohorts. These data refute the hypothesis that lactation is characterized by persistently elevated OT and AVP mRNAs produced as a result of continuous stimulation by suckling.

392.12

OXYTOCIN (OT) AND VASOPRESSIN (AVP) GENE EXPRESSION IN THE OSMOTICALLY-STIMULATED FEMALE RAT. J.A. Amico, R.S. Crowley, S.M. Challinor*. Univ. of Pittsburgh Sch. of Med. and VA Medical Center, Pittsburgh, PA 15261.

Our finding that ovarian steroids, estradiol (E_2) and progesterone (P), modulate hypothalamic OT and AVP gene expression in the lactating rat, led us to question whether similar modulation occurs in the osmotically-stimulated female rat. Sustained hyperosmolality (4-14 days of oral 2% NaCl) is reported to enhance OT and AVP mRNA concentrations in intact male rats. Similar studies have not been done in female rats. If E_2 and P modulate signals for up-regulation of the OT and AVP genes during osmotic activation, the response of these genes to sustained hypernatremia in the female rat would likely be heterogeneous because E_2 and P levels rapidly fluctuate during the estrous cycle. Moreover upregulation of OT and AVP gene expression should not occur in salt-loaded ovariectomized (OVX) rats.

Adult female cycling Sprague-Dawley rats matched for age and weight were sham OVX (intact) or OVX (3 wks prior to study), administered oral 2% NaCl or tap H_2O, and sacrificed on the 5th day of study. Salt-loaded OVX and salt-loaded intact rats developed comparable degrees of hypernatremia, depletion of posterior pituitary stores of OT and AVP, and weight loss (no significant differences, ANOVA). At sacrifice, E_2 and P concentrations were non-detectable in OVX rats and varied among intact rats, depending upon the stage of the estrous cycle. Salt-loaded OVX rats (n=8) showed no upregulation of OT or AVP mRNAs, whereas salt-loaded intact, cycling rats (n=12), showed both up- and downregulation compared to intact (n=12) and OVX (n=8) rats given tap H_2O. The data support ovarian steroid-mediated mechanisms for up- and downregulation of OT and AVP genes during osmotic activation.

TRANSPLANTATION I

393.1

IMPLANTED SYNTHETIC DOPAMINE (DA) AND NOREPINEPHRINE (NE) MICROSPHERES ATTENUATE APOMORPHINE INDUCED ROTATIONAL BEHAVIOR AND STIMULATE GROWTH OF DA FIBERS IN PARKINSONISM RATS. *A. McRae*[1], S. Hjorth[2], A. Dahlström[1], E.A. Ling[3], L. Dillon[4], D. Mason[4] and T. Tice[4]. University of Göteborg, Depts of Histology[1] and Pharmacology[2] Göteborg, Sweden; National University of Singapore, Dept. of Anatomy, Singapore[3]; Southern Res. Inst., Controlled Release Div., Birmingham, AL 35205, USA[4].*

Implanted chromaffin tissue alleviates some parkinsonism symptoms in humans and in experimental animals. Functional recovery could be due the release of large amounts of both NE and DA from this tissue. Implantable biodegradable controlled-release microspheres containing NE or DA provide a novel means to compare DA- or NE-induced restitution of subnormal DA function. Rats were unilaterally 6-OH-DA lesioned in the MFB. Six to eight weeks later, a suspension of 3µl of DA- or NE-containing microspheres was implanted in 2 sites in the DA denervated striatum. Contralateral rotational behavior induced by apomorphine was used as an index of lesion success and, following implantation of the microspheres, also as an index of functional recovery. Interestingly, both DA- and NE-microsphere implanted rats displayed a 30-50% reduction in the number of apomorphine-induced rotations up to 8 weeks postimplantation. Upon conclusion of the studies, immunocytochemical examination revealed growth of DA and tyrosine hydroxylase IR fibers in the striatum of DA and NE microsphere implanted rats. Preliminary EM studies showed signs of axonal growth cones and of axonal sprouting in the vicinity of the injected microspheres. Thus, both microencapsulated NE and DA have the capacity to assure functional recovery and to promote DA fiber (re)growth in parkinsonism rats. This novel means to deliver these substances to the CNS could be of therapeutic usefulness in Parkinson's disease.

393.2

ENCAPSULATED EMBRYONIC MESENCEPHALIC CELLS SURVIVE INTRACEREBRAL IMPLANTATION

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Polymeric cell encapsulation may be a useful technique for investigating the mechanism by which embryonic neural grafts ameliorate deficits in various animal models of CNS disease. Surrounding grafted cells with a biocompatible, semipermeable membrane that allows diffusional exchange of nutrients and metabolic products promotes sustained cell viability but also, isolates the enclosed cells preventing physical contact with cells of the host. This approach may reveal the relative importance of graft-derived reinnervation versus graft-derived molecular diffusion as mediators of graft efficacy. To test the feasibility of this approach, embryonic ventral mesenphalic cells were isolated from E17-19 day old fetuses and cultured in DMEM with 10 % FCS either on polystyrene culture plates directly or after being combined with an artificial extracellular matrix (Matrigel®). Semi-permeable capsules containing fetal mescencephalic cells seeded in Matrigel were implanted within the striatum of normal adult male Sprague-dawley rats within three hours of isolation. Animals were sacrificed at two (n=3) and four (n=4) weeks and analyzed histologically. The *in vitro* culture systems contained non-neuronal and neuronal cell types many of which stained positively for tyrosine hydroxylase (TH). Viable cells were present in all of the capsules *in vivo* explanted at two and four weeks which displayed morphological phenotypes similiar to those observed in *in vitro* cultures. Some of the cells stained positively for TH indicating that neuronal cells survived within the capsule over the four week period *in vivo*. Ongoing studies are aimed at optimizing neuronal cell survival and addressing functional efficacy.

393.3

SYNAPTOSOMAL UPTAKE OF [^{3}H]DOPAMINE IN THE STRIATUM OF WEAVER MUTANT MICE FOLLOWING INTRASTRIATAL TRANSPLANTATION OF MESENCEPHALIC CELL SUSPENSIONS. *L. C. Triarhou, B. Ghetti and J. R. Simon**. Indiana Univ. Sch. of Med., Indianapolis, IN 46202.

The weaver (*wv*) mutation induces a genetic nigrostriatal dopamine (DA) deficiency. Assays of [^{3}H]DA uptake were carried out *in vitro* in striatal synaptosomal fractions from wild-type mice (+/+) and from the two hemispheres of weaver mutant mice (*wv*/*wv*) that had received unilateral grafts of mesencephalic cell suspensions to the right side. Amphetamine-induced turning behavior was used to monitor graft survival. Recipient mice rotated by an average of 22 turns to the left and 7 turns to the right during the five 1-min sessions; the mean value L/(L+R) was 64%. Net [^{3}H]DA uptake, expressed as pmol/mg protein/2-min, was on the average 50 in the striatum of wild-type mice (n=10), 8 in the non-grafted, and 10 in the transplanted striatum of weaver mutants (n=11). Paired comparisons of right vs. left side in the striatum of the weaver recipients showed a 38% increase on the grafted side [mean value (R-L)/L]. In general, animals with a strong rotational bias to the left tended to have higher DA uptake values on the right. These findings attest to the functional effects of the grafts. Since DA uptake was measured in entire striatal preparations, the regional extent of DA effects in areas strictly associated with motoric activity may be underestimated. (Supported by USPHS R29-NS29283).

393.4

TRANSFORMING GROWTH FACTOR ALPHA: A POTENTIAL ROLE IN THE EFFICACY OF INTRASTRIATAL TRANSPLANTS S.E. Loughlin*, T. Lee, T. Ibrahim, D. Twardzik and J.H. Fallon. Department of Anatomy and Neurobiology, University of California Irvine, CA 92717

Intrastriatal transplants of fetal mesencephalon reverse behavioral deficits assoiciated with loss of dopaminergic innervation of the striatum. Transforming growth factor alpha precursor like immunoreactivity (TGFa-LI) is present in a subpopulation of astrocytes (Fallon, et al, 1990) which is increased by fetal transplants (Loughlin, et al, 1989). Transplants of adrenal medulla also ameliorate lesion induced deficits, even when dopaminergic cells do not survive (Bohn, et al, 1989). Adult adrenal medulla transplants caused an increase in striatal TGFa-LI astrocytes (Loughlin, et al, 1992). We therefore hypothesized that TGFa might play a role in the efficacy of transplants. To test this hypothesis, animals received unilateral 6-OHDA lesions of the dopaminergic projection to the striatum and apomorphine (0.25 mg/kg ip)-induced rotation behavior was quantified. One group of animals then received intrastriatal infusions of 200 ul TGFa (0.05 ug/ul) in artificial cerebrospinal fluid (CSF) via an Alzet minipump (2002) over a two week period. A control group received infusions of CSF alone. Rotation behavior was quantified and animals were sacrificed. Brains were processed for localization of TGFa-LI. CSF infusions produced a modest increase in endogenous TGFa-LI, while TGFa infusions caused a greater increase in TGFa-LI. Rotation behavior was unchanged in animals which received CSF infusions ($p > .4$). Infusions of TGFa, however, decreased rotation behavior by 40% ($p < 0.005$). Thus, TGFa may facilitate recovery from 6-OHDA lesions. Whether such recovery reflects regeneration of dopaminergic afferents or other compensatory changes is not known. Since endogenous TGFa-LI is increased by transplants which have been shown to ameliorate lesion induced deficits, it is possible that the efficacious effects of transplants are mediated through TGFa. These data have important implications for the development of new treatments for Parkinson's Disease. Supported by NS 26761 and the American Parkinson Disease Association SCC.

393.5

SUPERPARAMAGNETIC CONTRAST AGENTS FACILITATE MAGNETIC RESONANCE IMAGING OF NEURAL TRANSPLANTS IN RAT BRAIN IN VIVO. Andrew B. Norman*, Stephen R. Thomas, Ronald G. Pratt and Robert B. Norgren. Departments of Psychiatry and Radiology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

Although transplants of rat fetal striatal tissue can be observed in vivo using magnetic resonance (MR) imaging, the transplanted tissue is approximately isointense with the host brain, making it difficult to distinguish from host tissue. We have used superparamagnetic ferrite particles coupled to wheat germ agglutinin (WGA) to label and to visualize the transplant in vivo. Dissociated rat fetal striatal tissue (E 15-17) was incubated with WGA-ferrite particles for 15-20 min at 37°C and then transplanted unilaterally into rat striatum. Six days and 25 days following transplantation, rats were imaged at 0.14 T using a T_1 weighted protocol (TR=500 ms/TE=30 ms). After imaging, the rats were perfused and brain sections stained with cresyl violet or hematoxylin for iron. The labeled transplant was clearly visible on MR images as a feature characterized by very low signal intensity within the host brain. At higher concentrations of WGA-ferrite particles there was also a corona of high signal intensity representing a susceptibility artifact surrounding the area of low signal intensity. Histologically, the ferrite particles were observed to be mainly restricted to the area of the transplanted tissue and had a patchy distribution. The cells adjacent to the particles appeared to have normal morphology and cytochrome oxidase activity. These studies demonstrate that WGA-ferrite particles remain associated with transplanted cells for at least 3 weeks and permit the long-term monitoring of transplanted tissue using MR imaging in vivo. (Supported by NSF Grant BNS9015373)

393.6

IMPROVEMENTS IN THE KINEMATICS OF A TWO-DIMENSIONAL ARM MOVEMENT IN THE HEMIPARKINSONIAN MPTP MONKEY FOLLOWING ADRENAL MEDULLARY AUTOGRAFTS. P.J. Camarata, R.G. Parker, S. Park*, T.J. Ebner. Departments of Neurosurgery and Physiology, Univ. of Minnesota, Minneapolis, MN 55455, and Inje University, Seoul, Korea.

Most evaluations of hemiparkinsonian primates treated with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) have employed quantification of turning behavior or evaluation of simple reaching tasks. Objective measurements involving skilled volitional limb movements are necessary to adequately assess results of adrenal medullary grafting. In a visually guided reaching task requiring 6 directions and 5 distances of movement we measured reaction time, velocity, and time-to-peak velocity in three Rhesus monkeys before and after unilateral treatment with MPTP, involving some 20,000 movements for each animal. With each animal, reaction times significantly increase, peak velocities decrease, and the times-to-peak velocity increase after MPTP. Two of these animals have been studied after transplantation of autologous adrenal medulla to the striatum. One animal who underwent an open surgical graft to the caudate showed no improvement following transplantation. The animal who underwent stereotaxic transplantation to the caudate and putamen at several sites showed significant improvement in all parameters at most distances, at times returning to control values. Stereotaxic grafting of autologous adrenal medullary cells to the striatum may improve kinematic abnormalities in the hemiparkinsonian primate. Supported by the American College of Surgeons, the United Parkinson Foundation, and Mr. Hal Seth.

393.7

ASSESSMENT OF BEHAVIORAL IMPROVEMENT IN HEMIPARKINSONIAN MONKEYS FOLLOWING COGRAFTING OR SURGICAL CONTROLS. **R.A.E. Bakay*, L.D. Byrd, R.L. Watts, A. Mandir. Yerkes Regional Primate Research Center, Emory Univ., Atlanta, GA 30322.**

Eighteen rhesus monkeys are being evaluated quantitatively as to the effectiveness of CNS transplantation using adrenal medullary/peripheral nerve cografts. The monkeys were trained on one of two different operant behavioral tasks to give quantitative measures of disability produced by unilateral carotid administration of MPTP. MPTP produced marked deterioration of performance in the affected hand. None of the unoperated monkeys demonstrated spontaneous recovery (N=4). Five to nine months after MPTP administration, a transcortical intraventricular cograft was placed in the caudate (N=8) or the surgical equivalent without tissue (N=6). Increased speed of performance on operant task, and in some cases return of operant behavior, was observed in all cografted monkeys and only one of the surgical controls. The degree of recovery appeared to be directly correlated with the number of surviving chromaffin cells and inversely correlated with the degree of deficit prior to grafting. Supported by VAR&D, RO1 NS24340 and RR-00165.

393.8

COMPARISON OF AUTOLOGOUS ADRENAL MEDULLARY AND FETAL VENTRAL MESENCEPHALIC BRAIN TRANSPLANTATION STRATEGIES FOR THE TREATMENT OF PARKINSON'S DISEASE. I. Madrazo*, R.E. Franco-Bourland, H. Castrejon, C. Cuevas, F. Ostrosky-Solis, MC. Aguilera, E. Magallon, E. Grijalva, G. Guizar-Sahagun. IMSS, UNAM, INNSZ. Mexico, D.F. Mexico.

In the treatment of Parkinson's disease (PD) with dopamine rich grafts we have adopted 4 unilateral transplantation strategies: autologous unisite, multisite or peripheral nerve cografted adrenal medulla (AM) brain grafting, and fetal ventral mesencephalic (VM) homotransplantation. One to two years postsurgery, compared to non-transplanted parkinsonian subjects, most grafted individuals have shown various degrees of amelioration of their PD signs, and improved response to L-dopa. However, 3 to 4 years post-transplantation, homotransplanted patients have shown a notably more benign course of the disease, with a slower rate of progression, than most, but not all, of the AM grafted individuals. The benefits obtained from both AM and VM brain grafting procedures merit their further development.

393.9

HUMAN FETAL DOPAMINE CELL IMPLANTS IN EIGHT PATIENTS WITH ADVANCED PARKINSON'S DISEASE. CR Freed, RE Breeze, NL Rosenberg, SA Schneck, EH Kriek*, JX Qi, YB Zhang, T Lone, TH Wells, LO Ramig, L Thompson, JC Mazziotta, SC Huang, G Schroter, and A.A. Ansari. Univ. of Colo. Sch. Med., Colo. Neurologic Institute, Den. Ctr. Performing Arts, Neuropsych Labs., Denver, CO; UCLA Sch. of Med., Los Angeles, CA; Winship Cancer Ctr., Emory Univ. Sch. of Med., Atlanta, GA.

We have transplanted human embryonic mesencephalic tissue containing dopamine cells into the caudate and putamen of eight patients with advanced Parkinson's disease. Implants were unilateral in caudate and putamen (n=2) or bilateral into the putamen (n=6). Fetal tissue of 7 to 8 weeks' gestation was used. Alternate patients were immunosuppressed with cyclosporine A and prednisone. Results showed that at best, postural stability and walking were restored, hand movement became normal, and "off" episodes were eliminated. Drug doses were reduced as much as 50%. 18-F-6-fluorodopa PET scans have been compatible with continued growth and survival of the transplant for up to 33 months. Both immunosuppressed and non-immunosuppressed patients improved, although improvement was variable and some patients did not benefit. Fetal tissue implants may offer substantial long term clinical benefit to some patients with advanced Parkinson's disease.

393.10

SELECTIVE EFFECTS OF INTRAHIPPOCAMPAL LOCUS COERULEUS GRAFTS ON THE DEVELOPMENT BUT NOT EXPRESSION OF KINDLED SEIZURES. J. Bengzon*, M. Kokaia, Z. Kokaia and O. Lindvall. Restorative Neurology Unit, Department of Neurology, University Hospital, S-221 85 Lund, Sweden.

Intrinsic noradrenergic locus coeruleus (LC) neurons strongly suppress the development of seizures in the kindling model of epilepsy. We have previously shown (i) that transplantation of fetal LC tissue to the hippocampus or the amygdala-piriform cortex in 6-hydroxydopamine (6-OHDA) treated, hyperexcitable rats retards seizure development in hippocampal kindling; (ii) that kindling leads to a long-term decrease in basal hippocampal noradrenaline (NA) release from the intrinsic LC system as monitored by intracerebral microdialysis. We now report that this decrease in basal NA release can be reversed by LC grafts, but not by striatal tissue, implanted unilaterally into the previously kindled, non-denervated rat hippocampus. However, the LC grafts had no effect on the severity of the kindled convulsions despite a substantial noradrenergic hyperinnervation of the host hippocampus around the grafts. Bilateral implantation of fetal LC tissue into the hippocampus of previously 6-OHDA-treated, kindled rats, resulting in a normal or supranormal noradrenergic fiber density in major parts of the hippocampal formation, had no effect on the severity of fully developed generalized seizures. Finally, implantation of fetal LC tissue into the intact brain, producing a relatively dense noradrenergic hyperinnervation of the hippocampus up to about 1 mm from the grafts, did not influence the subsequent development of hippocampal kindling.

In conclusion, these data show that a graft-derived, noradrenergic hyperinnervation of the hippocampus fails to affect both kindling development and established seizures. Furthermore, LC grafts have no anticonvulsant effect when implanted into the hippocampus of noradrenaline-depleted, kindled rats, in contrast to the powerful antiepileptogenic effect exerted by the grafts during seizure development. The data thus point to a marked selectivity in the effects of noradrenergic transplants in the kindling model of epilepsy.

393.11

TRANSPLANTATION OF HCN-1A INTO RAT BRAIN. B. Morgan, D. Pizzo, K. Werrbach-Perez, L. Hutton, Y. Lu, K.N. Westlund, C. Hulsebosch, H.M. Eisenberg*, R. Perez-Polo. University of Texas Medical Branch at Galveston, TX 77555.

The HCN-1A is a human neural cell line that responds to the nerve growth factor (NGF) by fully differentiating and is a potential donor for transplantation into brain regions damaged by disease processes that have caused cell loss. It was developed from a child with megalencephaly. HCN-1A grown in 100% DMEM and 15% fetal calf serum with daily medium changes are differentiated in 10 ng/ml NGF, 1 mM forskolin, and 1 mM dibutyl cAMP (NGF cocktail). We have evaluated the cells using immunohistochemical methods and found the cells to contain GABA, glutamate, VIP, somatostatin, and cholecystokinin-8. Immunoreactivity of the cells for synaptophysin is increased with the differentiation of these cells. We have shown that these cells express $p75^{NGFR}$ mRNA using reverse transcription and the polymerase chain reaction (PCR) with primers for $p75^{NGFR}$ mRNA. The cells are also stained cytochemically with Me 20.4, an antibody to the human $p75^{NGFR}$. In preliminary experiments $5x10^5$ HCN-1A cells were implanted into rat motor cortex. Surviving cells were demonstrated two weeks after transplantation of HCN-1A differentiated with NGF cocktail and/or acetyl-L-carnitine arginyl amide into injured rat brain. Supported in part by a grant from the Moody Foundation and the Institute for Senescence, Pomezia, Italy.

393.12

AN ANATOMICAL STUDY OF FETAL RAT FRONTAL CORTICAL FRAGMENT GRAFTS TRANSPLANTED TO THE LESIONED MOTOR CORTEX OF ADULT RATS. B.W. Chopko*, T.J. Voneida. Dept. of Neurobiology, N.E. Ohio Univ. College of Medicine, Rootstown, Ohio 44272-9989, U.S.A.

The rostral motor cerebral cortical area of 13 adult rat hosts was unilaterally destroyed by aspiration. Within 3 hours after traumatization, fragments of embryonic day 15 (E15) fetal rat frontal cortical tissue were transplanted into the host lesion cavities. After a post-transplantation survival period of 2 to 33 weeks, grafts were anatomically investigated with Nissl, Weil, Bodian, HRP and Golgi techniques. Each graft was a viable, vascularized tissue mass attached to the host brain. Internal organization of graft parenchyma was never consistent with the laminar cytoarchitecture of normal cerebral cortex. Instead, graft cytoarchitecture was one of multiple subregions of varying cellular densities. Pyramidal and nonpyramidal shaped neurons were present. Dendrites both with and without spines were present, and typically, the apical dendrites of pyramidal shaped neurons were not oriented perpendicular to the pial surface. A spectrum of axonal morphologies developed, ranging from a loose feltwork of solitary axons to compact, curving fascicles. Beyond 2 weeks post-transplantation, all grafts contained myelin. Fetal cortical fragment grafts matured into tissue masses that contained cellular features consistent with cerebral cortex, but failed to develop the intercellular relationships, and hence the cytoarchitecture, of cortex. Further work is necessary in order to gain insight into the mechanisms responsible for the establishment of graft cellular organization.

393.13

TRANSPLANTED EMBRYONIC NEOCORTICAL NEURONS UNDERGO INCREASED DIFFERENTIATION IN SELECTIVELY NEURON-DEFICIENT CORTEX OF ADOLESCENT MICE. J.D. Macklis*, V.L. Sheen. Dept. Neurol., Prog in Neurosci, Harvard Medical School, Children's Hosp, Boston, MA, 02115

Embryonic day 17 (E17) neurons transplanted adjacent to photolytically induced pyramidal neuron-deficient cortex (lamina II/III) of early postnatal mice have revealed preferential migration and pyramidal phenotype within the lesioned zones. These results support involvement of potentially altered expression of environmental cues in guiding grafted neurons toward partial restoration of normal cytoarchitecture. The present experiments assess whether these normally developmentally age-specific cues may similarly guide directed repopulation in selectively lesioned older mice at times when non-specific developmental cues would no longer be expected.

Donor neocortical neurons from E14 or E17 mice prelabeled with combinations of fluorescent nanospheres and ^{3}[H] thymidine were transplanted into photolytically lesioned 4 or 6 week old mice (n=34). Photolysis of targeted pyramidal neurons followed unilateral injection of latex nanospheres (250 nl) containing the cytotoxic chromophore e_6, retrograde transport by callosal projection pyramidal neurons in contralateral laminae II/III and V, and transcranial laser illumination with a 670 nm continuous wave laser. After survival times of 1 to 6 weeks, serial sections were cut and processed for autoradiography, fluorescence, and routine histology. Preliminary results suggest that a subpopulation of transplanted neurons from both donor ages assume pyramidal morphology selectively within the neuron-deficient zone and extend processes. This suggests that E14 neurons "destined" to form deep cortical laminae may be influenced by a selectively altered environment to repopulate superficial laminae after normal development is completed. Supported by HD28478, MR Center grant HD18655, the Alzheimer's Association, and the Rita Allen Foundation.

HUMAN COGNITION: BLOOD FLOW/METABOLISM

394.1

PET STUDIES OF AUDITORY PROCESSING: PASSIVE PRESENTATION AND ACTIVE DETECTION. J.A. Fiez,*, P.A. Tallal, F.M. Miezin, S. Dobmeyer, M.E. Raichle, S.E. Petersen. Wash Univ., St. Louis, MO 63110, and Rutgers Univ. Newark, NJ 07102.

A positron emission tomography (PET) study of auditory processing was conducted using four classes of stimuli (vowels, syllables, words, and tone-triplets) and two auditory task conditions.

For each stimulus class, six different stimuli were presented ten times each. Activation of areas was assessed by defining areas of change on one group of subjects, and attempting to replicate these activations in other groups of subjects.

One set of subjects was instructed to listen passively to binaurally presented stimuli while fixating on a dot centered on a display monitor (passive task). Another set of subjects was trained to detect one of the stimuli within each set and to raise their left index finger whenever they heard the target stimulus, while also maintaining fixation (detection task). Maintaining fixation served as a control condition for all tasks (fixation task).

Areas which were more active during performance of the auditory detection than control task included: primary and surrounding auditory cortex, left prefrontal cortex, and the medial frontal cortex. A set of areas located bilaterally along the intraparietal sulcus were less active in the detection than control task. In contrast, none of the areas outside of auditory cortex were significantly changed during the passive task.

These results suggest that areas beyond the sensory processing regions are affected by task performance. Frontal regions might reflect processing necessary for accurate detection task performance; parietal regions may reflect a reduction due to shifting of attention from the visual fixation task to an active auditory task.

394.2

A PET STUDY OF VERBAL WORKING MEMORY. EA Raife, JA Fiez, ME Raichle, DA Balota, SE Petersen*. Wash. U., St. Louis, MO 63110.

A PET activation study was performed on 12 normal human subjects to examine areas related to working memory for verbal material. For the active tasks, subjects were serially presented 5 visual words or nonwords beneath a fixation cross prior to the start of the PET scan. Task instructions were to fixate while attempting to remember the items without verbalizing them or making mouth movements (monitored with EMG). Subjects recalled the items aloud when cued after the scan. Stimuli used in the active tasks were: 1) categorically-related nouns, 2) unrelated nouns, and 3) nonwords. Two control tasks were used: simple fixation, and a recitation control (subjects silently and slowly repeated the digits '12345' while maintaining fixation).

All active conditions relative to fixation showed increased flow in midline SMA and a right frontal area, and decreased flow at or near Rolandic cortex . The activations in the recitation control differed from those of the activation tasks; only a left Sylvian-insular region was activated in the recitation control as compared to fixation. While there was ceiling performance in the real word conditions, only half of the subjects recalled 100% of the nonwords. Activation differences found between good and poor performers included increased activation of a left premotor area in good performers in contrast to an anteromedial visual cortical area in the bad performers. There was a significant interaction between the two areas in the good vs. bad performers.

Several points are suggested by these results: 1) maintaining a representation of verbal material requires activation of certain areas (SMA, right prefrontal cortex) across many conditions; 2) inhibition of vocalization in these tasks produces decreases in Rolandic areas: 3) some areas differ with level of performance, suggesting a visual (bad performer) vs. phonological (good performer) strategy difference.

394.3

ACTIVATION OF LEFT POSTERIOR TEMPORAL CORTEX IN A VERBAL RESPONSE SELECTION TASK IS RATE DEPENDENT. ME Raichle*, JA Fiez, TO Videen, SE Petersen. Wash. U. Sch. Med., St. Louis, MO 63110.

The cerebral cortex in the area of the left temporoparietal boundary has been thought to play a significant role in word comprehension since the original observation of Wernicke in the 19th century. It was surprising, therefore, that initial PET activation studies, in which subjects were asked to say aloud an appropriate verb for visually-presented nouns, failed to detect activation in this area of cortex. As a control state, subjects were asked to repeat aloud visually-presented nouns, and the nouns were presented in both conditions at the rate of 1 per second. Five non-temporal areas showed significant activation: anterior cingulate, left prefrontal cortex and right cerebellum increased, while Sylvian-insular cortex decreased bilaterally.

A second study, using the same conditions, was performed at a rate of 1 word every 1.5 seconds. At this slower rate, there was a significant activation in the left posterior temporal cortex (x=59, y=-25, z=0); Talairach et.al, 1967) accompanied by the previously noted changes. Several explanations are suggested by these results including: 1) the left posterior temporal region acts as a short-term verbal memory buffer and is more active because the longer presentation time encourages longer storage times; 2) the region may be active because the slower presentation time allows more active and complete semantic processing above that demanded by the verb presentation task, per se. Other investigations have also suggested that slower word presentation rates produce greater temporal activation (Marrett et. al , Soc. Neuro. Abs, 16:27).

These data provide information into the role of left posterior temporal cortex in language processing, and also emphasize the crucial nature of paradigm design in the interpretation of imaging studies in the human brain.

394.4

REGIONAL CEREBRAL BLOOD FLOW CHANGES DURING STORY LISTENING. B. Mazoyer*, S. Dehaene, N. Tzourio, N. Murayama, L. Cohen, O. Levrier, G. Salamon, A. Syrota, J. Mehler. S.HF. Joliot, CEA, 91406 Orsay, and L.S.C.P., C.N.R.S. & E.H.E.S.S., Paris, France.

We have investigated regional cerebral blood flow (rCBF) changes during continuous speech listening in 10 right-handed French subjects. Each had six rCBF measurements using PET and [15O]-water, a series of three conditions being replicated twice: rest, listening to a text in a language unknown to them (Tamil), listening to a text in French (first protocol, N = 5); rest, listening to a list of French words, listening to a text in French (second protocol, N = 5). Auditory stimuli were presented binaurally over earphones. RCBF images were aligned with individual magnetic resonance images (MRI) and normalized CBF values within anatomically defined regions of interest were then compared across the three experimental conditions in each protocol (ANOVA).

Both left (LST) and right (RST) superior temporal gyri were activated in all conditions of auditory stimulation ($p<0.0005$). They were the only active regions during listening to stories in Tamil. A left inferior frontal gyrus activation (LIF, $p<0.01$) was found during word list listening. Listening to the stories in French in the first protocol activated the left and right temporal poles (LTP, $p<0.0001$; RTP, $p<0.0005$), and the left middle temporal gyrus (LMT, $p<0.005$). These activations were replicated in the second protocol (LTP: $p<0.001$; RTP: $p<0.005$; LMT: $p<0.005$). When pooling the two samples for this condition, extra-temporal activations were also found in LIF ($p<0.05$) and in left Brodmann's area 8 (LBA8, $p < 0.05$).

These results indicate that, besides regions devoted to single-word comprehension, story-level processing activates additional areas. Experiments are under way to separate the putative role of syntactic parsing, verbal memory and semantic integration.

394.5

FUNCTIONAL LOCALIZATION OF HUMAN OLFACTORY CORTEX WITH POSITRON EMISSION TOMOGRAPHY. R.J. Zatorre*, M. Jones-Gotman, A.C. Evans and E. Meyer. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A 2B4.

The cortical representation of human olfactory processing mechanisms was studied by examining regional cerebral blood flow changes with positron emission tomography during olfactory stimulation. Eleven normal right-handed subjects were presented with a series of eight odorants birhinally during the activation phase; a baseline condition consisted of inhalation with no odor present. We used the paired-image subtraction procedure, averaging the responses across subjects; anatomical localization was provided by matched magnetic resonance imaging. The principal result was strong activation ($t>3.6$ in all cases) at the junction of the inferior frontal and temporal lobes bilaterally, corresponding to the piriform cortex, and unilaterally, in the right orbitofrontal cortex. The results agree with anatomical and behavioral data implicating these regions in olfactory processing, and indicate a functional asymmetry favoring the right orbital area in olfaction.

394.6

NETWORK MODELS FOR MAPPING COGNITIVE BRAIN FUNCTION USING POSITRON EMISSION TOMOGRAPHY (PET) AND REGIONAL CEREBRAL BLOOD FLOW (rCBF). R. Horwitz* and P. Kirschner. Lab. Neurosci., Natl. Inst. on Aging, NIH, Bethesda, MD 20892.

Understanding how the brain mediates cognitive behavior has been aided by the use of PET and [O15]-water to measure rCBF during specific cognitive tasks. However, given that multiple regions often are activated during specific tasks, the complex interrelationships that occur during cognition need to be understood in terms of neural networks. The starting point for such analyses is to use correlations among rCBF in different brain loci to investigate brain interactions (e.g., Horwitz et al., J. Cogn. Neurosci., in press; Friston et al., Proc. R. Soc. London B, 1991). However, the appropriate way to perform such analyses, and to use the results to generate network models to account for the cognitive behavior under study, is not clear. We recently have developed an explicit network model for simulating rCBF/PET data that allows one to examine brain functional interactions (Horwitz, Abstr. Soc. Neurosci. 17, 540, 1991). Here, we use this simulation model to determine some of the conditions under which two different correlational approaches (Horwitz et al., which is a within-task design; Friston et al., which is an across-task design) produce similar or divergent results. Because the functional couplings between brain regions are specified in the model, we demonstrate for those cases with divergent results that the within-task correlational method better reflects the underlying functional configuration among the brain regions.

394.7

AGE-RELATED CHANGES IN REGIONAL CEREBRAL BLOOD FLOW (rCBF) ACTIVATION DURING VISUAL SELECTIVE ATTENTION. CL Grady*, B Horwitz, JA Salerno, E Wagner, SI Rapoport, MB Schapiro, LG Ungerleider, JV Haxby. Lab. of Neurosciences, Nat. Inst. on Aging, Bethesda, MD 20892.

Nine young (27 ± 3 yrs) and 6 old subjects (67 ± 3 yrs) performed visual tasks during positron emission tomographic measurements of rCBF using [15O]water: face and location matching without selective attention (NSA, where stimuli differed between tasks), and with selective attention (SA, where stimuli were the same for both tasks but task demands differed). Young subjects showed no change in reaction time in SA vs. NSA; old subjects were slower in both NSA tasks than the young ($p<0.001$) and showed further slowing during SA for faces ($p<0.02$). Image data were analyzed using Statistical Parametric Mapping. Neither group showed significant rCBF increase in SA vs. NSA for either face or location matching. However, a significant age by condition interaction was seen for face matching, in which the old group showed a larger SA-NSA difference in rCBF than the young ($p<0.005$) in left hemisphere lingual, fusiform and inferior occipital gyri, and right hemisphere fusiform, superior and mid temporal, and inferior frontal gyri, insula and cerebellum. During location matching, old subjects had a larger SA-NSA rCBF difference than did young subjects ($p<0.005$) in left hemisphere parahippocampal and superior temporal gyri, and cerebellum. Age-related differences in rCBF during visual SA thus are task dependent. During SA for faces, old subjects show greater use of ventral occipital areas known to mediate face perception. During SA for location, old subjects show involvement of parahippocampal cortex, an area not previously identified in perception of location, which may indicate the use of memory systems by older subjects to perform spatial attention tasks.

394.8

THE EFFECTS OF CLONIDINE ADMINISTRATION ON REGIONAL CEREBRAL BLOOD FLOW AND COGNITION IN THE ALCOHOLIC KORSAKOFF SYNDROME R.E. O'Carroll, A. Moffoot, K.P. Ebmeier, N. Dougall, C. Murray, G.M. Goodwin, G. Fink*. MRC Brain Metabolism Unit, Royal Edinburgh Hospital, Edinburgh, EH10 5HF, Scotland, UK.

McEntee & Mair (TINS 1990 13, 340-344) reported that administration of the α_2 agonist clonidine improved memory in the Alcoholic Korsakoff Syndrome (AKS). In an attempt to replicate and extend these findings, eighteen AKS subjects were recruited for a two phase trial. In phase 1, half the subjects received an acute infusion of 150μg/kg of clonidine in a single dose, half received an infusion of saline. All subjects underwent functional neuroimaging using Single Photon Emission Computerised Tomography (SPECT) before and 30 minutes following infusion. Clonidine infusion resulted in a highly significant increase in anterior cingulate cerebral blood flow, with an associated improvement in verbal fluency. Subjects then entered phase 2, a chronic double-blind placebo-controlled cross-over trial of clonidine 0.3mg twice daily for two weeks, and matched placebo for two weeks, incorporating an intervening two week 'wash-out' period. Detailed neuropsychological assessments were carried out at the end of each two week treatment period, including measures of anterograde memory, attention, staff ratings of cognitive failures and 'frontal lobe' measures. Clonidine treatment had no significant advantage over placebo on any of the cognitive measures employed. We conclude that acute administration of clonidine activated the thalamic-anterior cingulate system, resulting in an improvement in selective attention in general, and target detection in particular. However, chronic treatment resulted in no cognitive enhancing effect, possibly as a consequence of receptor down-regulation.

394.9

PET ACTIVATION STUDIES COMPARING BLOOD FLOW ACROSS FOUR MEMORY TASKS. T.A. Blaxton, S.Y. Bookheimer, T. Zeffiro, W. Gaillard and W. Theodore*, Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892.

Blood flow was measured following H_2O^{15} injections in 14 human subjects using a Scanditronix II PET scanner. Following study phases in which word lists were presented, subjects performed implicit and explicit versions of word association and word fragment completion memory tasks. Memory-specific activation was assessed against control tasks which were identical to the memory tests except that they contained no previously studied items. Mesial temporal regions showed left hippocampal activation and right hippocampal deactivation for the implicit and explicit versions of fragment completion. The opposite pattern was observed for the word association tasks, however, which produced left hippocampal deactivation and right activation. Deactivation was also observed laterally for implicit and explicit word association in the left middle temporal gyrus. These and other deactivations were interpreted as a possible neurological correlate of "priming" or "transfer" of learning operations from encoding to retrieval.

394.10

INCREASED ATTENTIONAL EFFORT DURING A PITCH DISCRIMINATION TASK ELEVATES REGIONAL BRAIN GLUCOSE UTILIZATION H.H. Holcomb*, B. Gordon, H.L. Loats, E. Gastineau, D. Medoff, C.A. Tamminga University of Maryland, MPRC, Baltimore, MD 21228, Johns Hopkins Medical Institutes, Baltimore, MD, 21205, Loats Associates, Inc., Westminster, MD

The neural mechanisms associated with different aspects of task performance, such as accuracy, skill, practice, attention, and fatigue, are currently the subject of intense investigation. Among the problems complicating these investigations is the likelihood that these different aspects interact in some yet-to-be determined fashion. For example, attention can be expected to vary in the course of a task lasting for 20-30 minutes, yet skill and practice can also be expected to vary.

The patterns of regional cerebral metabolic activity associated with two levels of difficulty in an auditory tone discrimination task were assessed. Four normal subjects were asked to respond with their left hand when they heard a tone of one frequency, and with their right hand when they heard a tone of the other frequency (750 Hz vs 1500 Hz). The two tones were presented at the same loudness. The discrimination was made DIFFICULT by addition of white noise; in the EASY condition, there was little or no white noise. Noise level was varied to maintain two different levels of accuracy (75% versus 95%) and response times for the DIFFICULT and EASY conditions during the course of the task. The DIFFICULT discrimination session promoted greater glucose use in the left middle temporal, left temporo-occipital and right inferior frontal cortices than did the EASY condition. Significant individual differences in the patterns and extent of regional glucose utilization were noted.

394.11

HERITABILITY OF COGNITIVELY-RELATED CEREBRAL FUNCTION: A POSITRON EMISSION TOMOGRAPHY STUDY OF MONOZYGOTIC TWINS. Karen Faith Berman*, Jill L. Ostrem, Daniel Z. Press, Christopher Randolph, Daniel R. Weinberger. Clinical Brain Disorders Branch, NIMH, Washington, D.C. 20032.

To examine the role of genetic determination in the pattern of cerebral response to cognitive challenges, we used the oxygen-15 water method for measuring regional cerebral blood flow (rCBF) with PET to study five pairs of healthy monozygotic twins (two female and three male pairs; mean age 31 years, range 19 to 54). rCBF was measured while subjects performed neuropsychological tests including the Wisconsin Card Sorting Test (WCS), which is a sensitive indicator of the integrity of the dorsolateral prefrontal cortex in man, and Raven's Progressive Matrices (RPM), which is another complex abstract reasoning task that may involve more posterior cortical areas. Sensorimotor control task were designed to be similar to each neuropsychological task in visual characteristics and response mode (verbal for RPM and finger movement for the WCS). rCBF values were normalized (i.e. expressed on a pixel-by-pixel basis as a percent of the whole brain mean). The similarity of regional brain function within twin pairs was assessed by determining the correlation between first- and second-born twins for each of a variety of brain regions.

Significant ($p \leq .05$) correlations were found: for WCS-right inferior frontal gyrus ($r = .98$, $p = .002$), right anterior cingulate ($r = .89$, $p = .04$), and left thalamus ($r = .91$, $p = .03$); for the WCS control-none; for RPM-left inferior frontal gyrus ($r = .89$, $p = .04$), right temporal cortex ($r = .96$, $p = .008$); and for RPM control-right inferior frontal gyrus ($r = .92$, $p = .02$), left anterior cingulate ($r = -.97$, $p = .005$), and right temporal cortex ($r = .87$, $p = .05$). These data may reflect the differential neural systems subserving the various cognitive operations involved in these tasks and may further suggest a high degree of heritablity in cognitively related function in the implicated areas.

394.12

SEX DIFFERENCES IN CEREBRAL BLOOD FLOW DURING SPEECH. S.Y.Bookheimer*, T.A. Zeffiro, W. Gaillard , T. A. Blaxton, and W. Theodore, Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892.

Gender differences in cognitive skills and aphasia patterns have led to the speculation that the sexes differ in cortical language organization. To test this hypothesis, 16 male and female subjects underwent Positron Emission Tomography to measure cerebral blood flow while reading or naming objects. We found both general and task-specific differences in blood flow patterns. On all tasks, females showed greater activity in the posterior cingulate, while males showed stronger activation in motor areas. Females had greater left mid-occipital and angular gyrus activity during reading, while males showed increased mid-temporal activity. Left hemisphere area 44 (Broca's area) appeared more anterior among female subjects. Both groups showed strong left hemisphere dominance on language tasks. Contrary to some theoretical accounts, there was no evidence for relatively greater bilaterality in either group.

DEGENERATIVE DISEASE: PARKINSON'S I

395.1

NEUROTROPHIC ACTIVITY IN HUMAN CEREBROSPINAL FLUID (CSF) IS ELEVATED IN PARKINSON'S DISEASE. S.J. Yu*, L.R. Ptak, E.S. Lo, C.M. Buhrfiend, and P.M. Carvey Rush Medical College, Rush-Presbyterian St. Luke's Medical Center

Human striatal extracts added to rat rostral mesencephalic tegmentum (RMT) cultures stimulates dopamine (DA) neuron growth and neurite extension. This growth promoting activity (GPA) is elevated in Parkinson's disease (PD) striatal extracts, suggesting a compensatory trophic response to DA neuron loss. Initial findings suggest that GPA is soluble and diffusible. Therefore, we postulated that human CSF may also contain GPA. Ventricular CSF from PD and non-PD patients (n=3 for each group) was crudely separated into >10kDa and <10kDa fractions using Centricon 10 microconcentrators. Fractions from each patient were subsequently diluted (1:4) using Hank's Balanced Salt Solution and 50 μl added to freshly plated RMT cultures (8 replicates / patient) growing in 200 μl defined media. 24 hr. later, the number of neurons with processes (dependent measure of GPA) was counted by an individual "blinded" to treatment history. CSF from PD patients consistently exhibited significantly higher GPA than non-PD patients as well as BSA treated controls (PD = 104, non-PD = 27, BSA = 11; $F=31.01$, $p<0.001$). Additionally, post-hoc comparisons revealed a significant difference in GPA between PD and non-PD groups and between non-PD and BSA. GPA was only observed in >10kDa fractions. A >10kD sample from a non-PD patient was separated into 7 fractions using FPLC and added to RMT cultures. GPA was significantly elevated in two of these fractions.These data suggest that 1) the factor(s) responsible for striatal GPA are also present in the CSF of normal patients, 2) CSF-derived GPA is elevated in PD similar to striatal-derived GPA, and 3) this factor(s) is >10kDa and may be amenable to further fractionation and purification. Studies are in progress to ascertain the approximate MW (ion-exchange chromatography) as well as the cellular specificity of CSF-derived GPA. Preliminary observations indicate that the cells with processes are tyrosine hydroxylase positive implying specificity for DA neurons. If confirmed, CSF-derived GPA may be useful in the diagnosis of PD.

395.2

STRIATAL-DERIVED GROWTH PROMOTING ACTIVITY IS DECREASED IN THE AGED RAT: IMPLICATIONS IN PARKINSON'S DISEASE. P.M. Carvey*, L.R. Ptak, D.K. Sierens, S.J. Yu, and Donghui Lin. Rush-Presbyterian St. Luke's MC, Chicago IL 60612

Extracts of adult human and rat striatal tissue stimulate the growth of dopamine (DA) neurons in primary culture. This growth promoting activity (GPA) is elevated in patients with Parkinson's disease (PD). We have proposed that the increased GPA in the PD brain represents a compensatory response to the loss of DA neurons which could slow disease progression by stimulating DA terminal sprouting. However, if striatal GPA is decreased in the aged brain, compensatory increases resulting from DA neuron loss may be inadequate to overcome PD progression. In an effort to determine if GPA varies with age, we evaluated the effects of striatal and cerebellar extracts from 2, 8, 16, and 24 month old rats (n=16) on the growth of low cell density (3,500 cells/well), E15.5 rostral mesencephalic tegmentum, dissociated, primary cultures growing in defined media. Cultures incubated with extracts from 2 month old rats possessed significantly more viable, neuron specific enolase immunoreactive neurons with processes (263%) then cultures incubated with extracts from 24 month old animals ($F=8.06$). Moreover, neuron viability in culture was inversely correlated with age ($r = -0.699$). In contrast, extracts of the cerebellums from these animals possessed very little GPA which was not correlated with age. If DA neurons in vivo are dependent on this trophic activity for continued viability, the age related decline in this GPA may contribute to DA neuron loss. Furthermore, a compensatory increase in GPA brought about by the DA neuron loss of PD may be inadequate to maintain DA neuron viability provided that striatal GPA in humans exhibits a similar relationship to age. An age-related decline in striatal-GPA would therefore predispose older patients to PD.

395.3

MPTP INDUCED CHANGES IN STRIATAL D2-LIKE DOPAMINE RECEPTORS WITHOUT CHANGES IN MESSENGER RNA. R. D. Todd*, J. Colvin, J. Carl, J. S. Perlmutter[1], Departments of Psychiatry and Genetics, and Department of Neurology and Neurosurgery[1], Mallinckrodt Institute of Radiology[1], Washington University School of Medicine, St. Louis, MO 63110.

MPTP induced destruction of dopamine producing cells has been used both as a model for Parkinson's disease and as a denervation agent for studying the regulation of dopamine receptors. In the present study we have determined the temporal pattern of receptor expression in MPTP treated primates.

Baboons were unilaterally lesioned by intracarotid artery injection of MPTP resulting in a hemilateral Parkinsonian syndrome. Striata from injected animals were analyzed for tritiated spiperone binding, messenger RNA levels for dopamine D2, D3, and D4 receptors and dopamine content. In both caudate and putamen there was an increase of two to seven-fold of eticlopride displaceable tritiated spiperone membrane binding which reached peak levels in about 100 days and declined to baseline levels by about 400 days following MPTP injections. These increases and decreases in binding site number occurred in the presence of 98% reductions in dopamine in the ipsilateral caudate and putamen. Putamen messenger RNA levels were quantified by reverse transcription coupled to polymerase chain reaction amplification using receptor specific oligonucleotides. In contrast to results for D2-like binding sites, there were no observable changes in the amounts of messenger RNAs for D2, D3, or D4 receptors. These studies suggest that MPTP induced changes in striatal D2-like receptors are secondary to translational or post-translational effects on receptor number.

395.4

THE RELATIONSHIP BETWEEN ONSET OF SYMPTOMS AND DOPAMINE FIBER LOSS STUDIED BY 11C-CFT AND PET IN A PRIMATE MODEL OF PARKINSON'S DISEASE. P. Hantraye*¶, A.-L. Brownell†, U. Wüllner¶, D. Elmaleh†, R.D. Spealman§, G.L. Brownell†, B.K. Madras§ and O. Isacson¶, †Department of Neurology, Harvard Medical School and Neuroregeneration Laboratory, McLean Hospital, MRC 119, Belmont, MA 02178, ¶Dept. of Nuclear Radiology, Massachusetts General Hospital, Boston, §Dept. of Psychiatry, Harvard Medical School, NERPRC, Southborough, MA 01772-9102

A progressive degeneration of mesencephalic dopamine neurons was induced in Macaca fascicularis monkeys by chronic administration of MPTP (0.5-0.7 mg/kg i.v. at weekly intervals for up to 50 weeks). The progression of striatal dopamine fiber loss was determined in vivo by positron emission tomography (PET) and 11C-CFT (a specific dopamine transporter ligand) as a marker. The behavioral alterations produced by the chronic MPTP treatment was followed in parallel with PET studies.

In the PET experiments, chronic MPTP injections resulted in a progressive decrease of 11C-CFT specific binding in striatal regions. This decrease was characterized by a rapid initial reduction of 11C-CFT specific binding (up to 70 % of the pre-treatment values), in both caudate and putamen, followed by an apparent plateau lasting several months, and concluding with a more severe reduction in the putamen (91%) than the caudate. The animals remained largely asymptomatic during the initial decline and plateau of dopamine fiber degeneration. During the third final phase of 11C-CFT reduction in the putamen, parkinsonian symptoms such as hypokinesia, bradykinesia and tremor progressively appeared.

The present study using 11C-CFT as an index of dopamine terminals, indicates that non-human primates remain asymptomatic despite more than 70% reduction in striatal dopamine terminals as assessed by PET. Parkinsonian symptoms appeared when putaminal 11C-CFT was specifically reduced to less than 10% of control values.

395.5

MODELING CHAOTIC DOPAMINERGIC NEURODYNAMICS. J. Sale, S.H. Price, A.D. Will and J.M. Tosk*. Neurology and Psychiatry Services, Jerry L. Pettis Veterans' Affairs Medical Center, Loma Linda, CA and Depts. of Neurology and Psychiatry, Loma Linda University School of Medicine, Loma Linda, CA 92350.

A nonlinear dynamical model of the nigrostriatal dopaminergic system was proposed by King, Barchas and Huberman (PNAS *81*:1244, 1984) who demonstrated the model's potential for explaining the rapid fluctuations in movement observed among certain Parkinson's patients following chronic treatment with L-dopa. We have explored the complex dynamics of a modification of this model. The modifications may be equated with biological variables involved in the pathophysiology of Parkinson's disease. We show that by varying one or both of two parameters, x, representing mean firing rate of nigrostriatal neurons and s, a variable proportional to postsynaptic dopamine D_2 receptor density, the system exhibited a wide range of dynamics. When the variable x, representing the mean firing rate, was decreased we observed the appearance of a novel family of solutions which exhibited a decreasing incidence of chaotic states. Additionally, we observed the chaotic regime breaking up into a collection of discrete groups of firing rates. When the variable s, which is proportional to dopamine D_2 receptor density, was decreased we observed a shift in dynamics from chaotic to nonchaotic states. On this basis we predict that premorbid nigrostriatal dopamine D_2 receptor densities will influence sensitivity to chaotic dynamics and may influence susceptibility to Parkinson's disease and other movement disorders. We believe these findings may also have important implications in light of recent work being done in the area of experimental control of chaotic dynamics.

395.6

PARKINSONIAN SIGNS IN MPTP-TREATED MONKEYS ARE ALLEVIATED BY THE HIGH EFFICACY D_1 DOPAMINE RECEPTOR AGONIST SKF 81297. B.K. Madras, D. Elmaleh, P. Meltzer, D.E. Lee-Parritz, P. Hantraye, O.Isacson*, G.L. Brownell and A.L. Brownell. Harvard Medical School, New England Regional Primate Research Center, Southborough, MA 01772, Organix, Inc. Woburn, MA, 01801, Massachusetts General Hospital, Boston, 02115.

The therapeutic potential of a high efficacy D_1-selective dopamine receptor agonist was evaluated in two cynomolgus monkeys (*Macaca fascicularis*) treated with the neurotoxin MPTP (3 doses of 0.6 mg/kg) and maintained with a D_2 dopamine receptor agonist quinelorane. Dopamine nerve terminal degeneration was > 90% as monitored by striatal accumulation of a positron emission tomography (PET) marker for dopamine terminals, [^{11}C]CFT (WIN 35,428). The D_1-selective drug SKF 81297 (0.3 or 1.0 mg/kg) reduced postural rigidity and increased mobility in both monkeys. Involvement of D_1 dopamine receptors was implicated as SKF 81297 eliminated striatal accumulation of the D_1 receptor PET ligand [^{11}C]SCH 39166. In a single experiment, the combination of ineffective doses of quinelorane and SKF 81297 also alleviated Parkinsonian signs. High efficacy, selective D_1 dopamine receptor agonists may be useful for reducing Parkinsonian signs when dopamine levels are severely depleted and may allow for dose reduction of D_2 agonists. Supported by Parkinson's Disease Foundation, DA06303, RR000168, ER60519, ER60469.

395.7

ON-OFF EFFECTS OF DIRECT DOPAMINE AGONISTS IN UNILATERAL NIGRAL RATS. P.B. Silverman*, Dept. of Psychiatry, University of Texas Health Sci. Ctr., Houston, TX 77030.

As Parkinson's disease and its treatment progress, the response to pharmacotherapeutic agents becomes increasingly erratic with the development of dyskinesias and "on-off" effects. In the unilaterally 6-hydroxydopamine lesioned rat, with respect to extent of dopamine depletion, a condition of advanced hemi-parkinsonism exists. Here lesioned rats were tested repeatedly with selective direct-acting dopamine agonists and their rotational (circling) response recorded. A remarkable degree of response fluctuation was seen when the selective D2 agonist, bromocriptine, or one of the selective D1 agonists, SKF-82958 or SKF-77434, was administered for a few days. In consecutive daily sessions responses of individual animals varied from zero to hundreds of rotations per hour. When treated daily for 2 weeks with SKF-77434, some rats showed what appeared to be a 2 to 3 day cycle of response fluctuation. In others, as well as in the group as a whole, no consistent pattern was obvious, other than the general appearance of an all-or-none phenomenon. Response fluctuations continued when treatments were spaced at 3 day intervals. This approach may prove useful for the study of "on-off" effects.
Supported by NIDA grant DA06269.

395.8

RESPONSE TRAJECTORIES AND ACCURACY OF NORMAL AND PARKINSONIAN SUBJECTS IN A POINTING TASK. W. A. Hening*[1,2], J. Gordon[3], and M. Rolleri[1], Neurology Depts, VAMC[1], Lyons, NJ & UMDNJ-RWJohnson Med Sch[2], New Brunswick, NJ; and Ctr for Neurobiology & Behavior[3], Columbia Univ, NY, NY

We used a digitizing tablet based pointing task developed by Gordon and Ghez (CSH Symp Quant Biol 55:837-847, 1990) to examine how sensory information and instructions influence basic task performance in normal middle aged adults and patients with Parkinson's disease. With arms hidden, 9 subjects (6 normals, aged 39 to 62, and 3 patients with Parkinson's disease (PD), Hoehn and Yahr stages I or II, aged 61-72) moved a cursor to 6 distances (2.4 to 26.4 cm) in an oblique right or leftward direction to match targets seen on a computer monitor. Without reaction time constraints, subjects were told to make uncorrected, straight movements. In 6 different session types, accuracy, brief movement time or both were stressed while we provided or withheld continous feedback of hand position and/or knowledge of results. All subjects (43 sessions to date) reached different distances by scaling both velocity and duration, although velocity had a quantitatively greater role (ascertained by multiple regression) in every session but one. Not only velocity and duration, but acceleration and time to peak acceleration were scaled to distance. PD patients had trajectory formation and accuracy similar to normals, but one patient (Stage II) had prolonged trajectories which further slowed and undershot distant targets if knowledge of results was unavailable. Therefore, basic performance in the task may be normal in the PD subjects while still revealing evidence of both bradykinesia (slowing) and excessive dependence on visual cues.
This research supported by the Dept of Veterans Affairs Medical Research Council.

395.9

DEMENTIA IN PARKINSON'S DISEASE: CORTICAL INVOLVEMENT WITH A FRONTAL PREDOMINANCE USING THE IMMUNODETECTION OF ABNORMAL TAU PROTEINS . P. Vermersch*[1], A. Delacourte[1], F. Javoy-Agid[2], J.J. Hauw[3], Y. Agid[2]. [1]U 156 INSERM, 59045 Lille, [2]U 289 INSERM and [3]Lab. Neuropathologie R. Escourolle, 75651 Paris, France.

The dementia, frequently associated with Parkinson's Disease (PD), is generally considered to be of the subcortical type but the high frequency of Alzheimer pathology in PD have suggested that dementia may be due to coexisting Alzheimer's Disease (AD). In this study we analyzed the neurobiochemical basis of the cortical lesions from demented PD patients.

Material and methods: Cortical samples from 24 parkinsonian patients not demented and with various degree of dementia were analyzed by western blotting. An anti-Paired Helical Filaments antibody was used for the immunodetection of the abnormally phosphorylated Tau proteins named Tau 55, 64 and 69, known to be specific and reliable biochemical markers of the neurofibrillary degeneration of the Alzheimer type.

Results: The frequency of immunodetection of the abnormal Tau triplet was statistically higher in the demented subgroups than in the non demented subgroup of PD in the prefrontal ($p < 0.05$), the temporal ($p < 0.01$) and the entorhinal cortex ($p < 0.02$) but not in occipital and cingular cortex. A quantification of abnormal Tau triplet by densitometry showed that, in opposition to the results obtained in AD patients, the intensity was higher in the prefrontal than in the temporal cortex of most of the demented PD patients.

Conclusion: This study: (i) gives a biochemical evidence for the presence of AD changes in demented parkinsonian patients; (ii) suggests that lesions of the prefrontal cortex may significatively contribute to the occurence of cognitive changes at least in some PD patients.

395.10

PROBLEM-SOLVING ABILITY IN PARKINSON DISEASE
R.F. Zec*, M. McCool, E. Grames, E. Landreth, A. Hasara, W. Fraizer, S. Fritz, S. Wairman, J. Belman, R. Harris, C. O'Connell, R. Robbs, B. Manyam and R. Elble. Alzheimer Disease and Related Disorders Center, So. Illinois Univ. Sch. of Med., Springfield, IL 62708.
Problem-solving ability in Parkinson disease (PD) patients, elderly control subjects, and young control subjects was studied using the Halstead Category Test. The subjects were 41 PD patients, 40 age-matched elderly controls, and 46 young controls. Errors on the Category Test did not differ significantly (t tests) between the PD group (M=62.6) and their age- and educationally-matched control group (M=65.9). The PD group and the elderly control group did make significantly more errors than the young control group (M+SD: 36.6+20.4). The degree of impairment was -1.3 SD difference from the young control group mean for the elderly control group and -1.4 SD for the PD group. The only indication of greater problem-solving difficulty in the PD group was the somewhat greater percentage of PD patients (43%) as compared with elderly controls (29%) who were impaired by ≥2 SDs from the young control group mean. However, an equal percentage of PD patients and elderly controls (26%) scored above the mean of the young control group. Our findings do not support previous reports of impaired complex problem-solving ability in PD patients.

395.11

DOES LEVODOPA INFLUENCE THE SPEED OF COGNITIVE PROCESSES IN PARKINSON`S DISEASE? G.Ransmayr*, G. Künig, D. Soucek, E. Sofic, P. Riederer. Dept. of Neurology, A-6020 Innsbruck; Dept. of Psychiatry, Neurochemistry Unit, D-7800 Würzburg.
The aim of the study was to determine whether the speed of serial comparisons of sets of 2,3 or 4 memorized digits with a target digit (Sternberg paradigm) is different in "on" and "off" states of Parkinson`s disease and related to the levodopa plasma level. 18 parkinsonian patients (age 61.3±10.1, disease duration 6.8±3.1 years) were tested in drug-free states ("off") and in states of optimal response to oral levodopa ("on"). Central processing time was not different in "on" and "off" (134±129 and 151±148 msec) and significantly slower in "off" than in 13 age-matched normal controls (82±20 msec, Mann-Whitney U test). Moreover, central processing time was not related to the levodopa plasma level and the motor scores (Spearman rank correlation). In 3 patients significant improvement of arousal was observed under levodopa (affect-arousal scale of Bond&Lader) and in parallel marked acceleration of cognition suggesting that levodopa might have an activating effect on cognition in single patients with deficient arousal in "off" states.

395.12

MAGNETIC RECORDING OF RESTING TREMOR RELATED BRAIN ACTIVITY IN PARKINSON'S DISEASE J. Volkmann, F. Lado, A. Ioannides, A. Mogilner, M. Joliot, U. Ribary, E. Fazzini*, R. R. Llinás Center for Neuromagnetism, Dept. of Physiology and Biophysics, New York University Medical Center, New York, NY 10016.

A 14- and a 37-channel magnetoencephalography (MEG) system (BTi) are being used to evaluate the spatial and temporal organization of dynamic brain activity accompanying resting tremor in patients suffering from early stage idiopathic Parkinson's disease. The study is based on the simultanous recording of tremor (acceleration of the middle finger and the rectified-filtered EMG of the superficial flexor and extensor digitorum muscles) and spontanous neuromagnetic activity over 37 to 56 positions of the contralateral hemisphere. Data acquisition is performed at a sample rate of 512 Hz. In a first analytic step, the frequency and phase relationship between tremor and neuromagnetic signals is studied by cross spectral analysis of 200 subsequently recorded epochs of 3 sec duration. In a second analytic approach, approximately 1000 overlapping epochs of 1 sec duration triggered on flexor EMG onset are cut out of the original recordings and averaged. The resulting averaged signals are used for source localization by means of current dipole analysis and recently introduced Magnetic Field Tomography (Ribary et al, Natl Acad Sci 1991 88 11037-11041). Results are displayed on 3-D MRI reconstructions of the patient's brain.

Preliminary analysis of 10 patient recordings indicates the presence of synchronized tremor related neuromagnetic activity over wide areas of the frontal, parietal and temporal cortex. The magnetic field pattern suggests source localizations in premotor, motor and sensory cortex. Magnetic Field Tomography (MFT) obtained in 3 patients shows a rhythmic subsequent activation of frontal areas, precentral and postcentral gyrus with each tremor beat. Analysis of diencephalic magnetic activity using MFT is currently under way .

Our MEG data demonstrates that this technique can be used for a non-invasive monitoring of the functional network underlying resting tremor in Parkinson's disease.

INGESTIVE BEHAVIOR: NPY, GALANIN AND INSULIN

396.1

REFEEDING AFTER STARVATION INCREASES HYPOTHALAMIC NEUROPEPTIDE Y (NPY) MESSENGER RNA. R. Briones-Urbina* and S.R. George. Depts of Medicine and Pharmacology, University of Toronto, Toronto, Ont, CANADA M5S 1A8.

NPY is a potent orexigenic when administered into the CNS. Physiologically, it appears to be involved in promoting eating behavior. Hypothalamic NPY gene regulation in response to starvation and re-feeding was studied in male Sprague Dawley rats to assess a possible role of NPY not only in the initiation of feeding but also in the maintenance of feeding and body weight. Animals weighing 180-200 g at the onset of the study were housed in environmental rooms in 12h dark/light cycles, with free access to chow and water, handled and weighed daily. After an adjustment period of 2 weeks, groups of rats were starved for 12, 24, 48 and 72 h with free access to water. Weight losses of 10, 17, 23 and 30% occurred in each group respectively. Hypothalamic NPY mRNA levels were detected by Northern blotting analysis using a 32P labelled oligodeoxynucleotide probe complementary to bases 1623-1669 of the rat NPY gene. Increased NPY mRNA was detected as early as 12h, peaked at 48h and remained elevated until 72h. Groups of rats were then starved for 24 or 48h followed by free access to chow and water for a further 12, 24 and 48h. After 12 and 24h of refeeding following both 24 and 48h of starvation, there was a further increase in NPY mRNA levels coinciding with weight regained of up to 80%, but no further increase was noted after 48h refeeding. We report time-dependent further increases in hypothalamic NPY mRNA levels in refeeding following starvation coinciding with the period of enhanced food intake, rapid weight gain and reversal of body weight to prestarvation levels. These results suggest a physiological role for NPY in the initiation and maintenance of feeding behavior and likely in the control of body weight as a reflection of energy balance.

396.2

HYPOTHALAMIC EXPRESSION OF NEUROPEPTIDE Y AND GALANIN mRNA IN THE GOLDEN-MANTLED GROUND SQUIRREL, A HIBERNATING MAMMAL
T. Boswell, G.J. Kenagy, A.J. Sipols, D.G. Baskin* and M.W. Schwartz. Depts. of Biological Structure, Medicine and Zoology, Univ. of Washington, Seattle, WA 98195 and V.A. Medical Center, Seattle, WA 98108.

Neuropeptides such as neuropeptide Y (NPY) and galanin may play a role in regulating circannual cycles of feeding in the golden-mantled ground squirrel (Spermophilus saturatus). To investigate this, we analyzed the distribution of NPY and galanin mRNA in ground squirrel brain using in situ hybridization histochemistry. As in rats and mice, NPY mRNA was abundantly expressed in the hypothalamic arcuate nucleus and was also present in the cortex, hippocampus and reticular nucleus of the thalamus. Hypothalamic galanin mRNA was concentrated in the arcuate nucleus and the dorsomedial nuclei. Preliminary findings show that seasonal changes in feeding in ground squirrels are related to changes in the hypothalamic expression of neuropeptide mRNA. Thus, in hyperphagic (food intake = 29.8 ± 0.7 g/day; n=4) and pre-hibernation hypophagic (12.1 ± 1.3 g/day; n=4) animals, the reduction in food intake was paralleled by a decrease in hypothalamic expression of NPY and galanin, although a larger sample size is necessary to confirm this. Thus, golden-mantled ground squirrels express the genes encoding hypothalamic NPY and galanin in a distribution comparable to non-hibernating rodents. Seasonal changes in the expression of these neuropeptides may contribute to circannual cycles of feeding.

396.3

EFFECTS OF FASTING ON GLUTAMIC ACID DECARBOXYLASE (GAD) mRNA LEVELS IN RAT HYPOTHALAMUS. A. J. Sipols*, M. W. Schwartz and D. G. Baskin. Departments of Medicine and Biological Structure, University of Washington and Seattle VA Medical Center, Seattle, WA 98108

Evidence indicates that the inhibitory neurotransmitter γ-aminobutyric acid (GABA) plays a role in the central regulation of feeding behavior. Since food deprivation increases hypothalamic GAD activity, we proposed that fasting may influence GAD gene expression in the hypothalamus. To test this hypothesis, we measured the effect of fasting on hypothalamic mRNA for two GAD isoforms (GAD_{65} and GAD_{67}) using *in situ* hybridization. Following 0, 24, or 48 hr of food deprivation, coronal brain sections from male Wistar rats (initially weighing 275-300g) were hybridized with ^{35}S-labelled oligonucleotide probes complimentary to either GAD_{65} or GAD_{67} mRNA. Northern blot analysis revealed that these probes recognize separate mRNA transcripts. Film autoradiographs were quantified by computer image analysis of hybridization density (μCi/g) in the arcuate nucleus (ARC), dorsomedial nucleus (DMN), and ventromedial nucleus (VMN). The hypothalamic distribution of mRNA encoding the two isoforms was identical. In fed rats, GAD_{65} hybridization was highest in DMN (0.177 ± 0.012) and ARC (0.161 ± 0.011) and lowest in VMN (0.123 ± 0.002). Likewise, in fed rats, GAD_{67} hybridization was highest in the DMN (0.190 ± 0.009) and ARC (0.170 ± 0.007) with lower values in VMN (0.118 ± 0.007). Food deprivation for 24 or 48 hrs did not alter levels of GAD_{65} or GAD_{67} mRNA in any area. However, we observed significant associations between GAD_{65} mRNA expression in ARC and plasma insulin (r=-0.61) and glucose (r=-0.54) levels. We conclude that: 1) GAD_{65} and GAD_{67} mRNA have identical quantitative distributions in hypothalamus; 2) hypothalamic expression of GAD_{65} and GAD_{67} genes is not regulated by food deprivation; and 3) GAD_{65} gene expression in ARC may be influenced by changes in circulating levels of insulin and/or glucose.

396.4

NEUROPEPTIDE Y PROJECTION FROM ARCUATE NUCLEUS (ARC) TO PARVOCELLULAR DIVISION OF PARAVENTRICULAR NUCLEUS (pPVN): SPECIFIC RELATION TO CARBOHYDRATE FEEDING. M. Jhanwar-Uniyal*, B. Beck, Y.S. Jhanwar, C. Burlet and S.F. Leibowitz. The Rockefeller Univ. New York, N.Y. 10021 and Faculty de Medicine, INSERM U.308, Nancy, France.

Neuropeptide Y (NPY) injection into the PVN stimulates food intake, specifically of carbohydrate (CARB). The pPVN is particularly rich in NPY-containing terminals which originate primarily from the ARC. This study examines: a) the relation between endogenous NPY and natural preference for CARB; and b) the specific importance of the ARC-pPVN NPY projection in this relationship. Sprague-Dawley rats were given pure macronutrient diets (CARB, protein and fat), and daily food intake was recorded. Rats were sacrificed 3 wks later, their brains removed, and eight hypothalamic nuclei were micropunched and examined via RIA for endogenous NPY. The results demonstrate that: 1) High CARB eaters, compared to low CARB eaters, have significantly elevated NPY levels specifically in the pPVN ($p<0.01$) but not the magnocellular PVN; in ARC ($p<0.01$); and in dorsomedial nucleus (DMN; $p<0.05$). No such relationship was seen for fat or protein intake; 2) Endogenous NPY content is positively correlated with 24 hr CARB intake, in the pPVN ($r=0.71; p<0.001$), ARC ($r=0.57; p<0.001$) and DMN ($r=0.52; p<0.01$) only and 3) Endogenous NPY levels in ARC, where NPY cell bodies are concentrated, are positively correlated with NPY levels in the pPVN ($r=0.54; p<0.001$) and DMN ($r=0.56; p<0.001$), to which the ARC projects. This demonstrates a close relationship between endogenous NPY, specifically of the ARC-pPVN projection, and natural preference for CARB.

396.5

IN VIVO AND *IN VITRO* EVIDENCE THAT STREPTOZOTOCIN (STZ)-INDUCED DIABETIC HYPERPHAGIA IS DUE TO INCREASED NEUROPEPTIDE Y (NPY) SECRETION IN THE PARAVENTRICULAR NUCLEUS (PVN). A. Sahu*, C.A. Sninsky[a], C.P. Phelps[b], M.G. Dube, P.S. Kalra and S.P. Kalra, Depts. Obstet. & Gynec. and [a]Medicine, Univ. Fla., Gainesville, FL 32610, [b]Dept. Anatomy, Univ. South Fla., Tampa, FL 33612

Since a large body of evidence shows that increased feeding under normal conditions is due to enhanced NPY release in the PVN (PNAS, 88:1093, 1991), we evaluated the levels and pattern of *in vivo* and *in vitro* NPY release from the PVN of STZ-induced diabetic rats displaying hyperphagia. Expt 1: NPY levels were measured by RIA in 7 microdissected brain nuclei of rats 18 days after STZ or vehicle treatment. STZ rats exhibited marked hyperglycemia, hyperphagia and elevated NPY levels in 4 hypothalamic sites including the PVN as compared to controls. Expt 2: NPY release from the microdissected PVN and ventromedial nucleus (VMN) of STZ rats was then studied *in vitro*. Both basal and KCl-induced NPY release was significantly higher from the PVN of STZ-treated than that of control rats. However, NPY release from the VMN of STZ-treated rats was unaffected. Expt 3: NPY release *in vivo* from the PVN of STZ-treated rats was assessed. Push-pull cannulae (PPC) were implanted in the region of the PVN and 1 week later (18-20 days after STZ-treatment) the PVN was perfused via the PPC for 3-4 hours with artificial CSF. Compared to controls, NPY levels in PVN perfusates collected at 10 min intervals from STZ-treated rats were significantly enhanced in association with increased food intake. Cumulatively, these results show that NPY release is augmented in the PVN of STZ-treated rats. Since NPY is a potent, naturally-occurring orexigenic signal, our results support the hypothesis that increased NPY secretion, selectively in the PVN, may be the underlying cause of hyperphagia in diabetes. (Supported by UF DSR KG 717 (AS) and NIH DK37273 (PSK & SPK), VA Merit Review (CAS)).

396.6

NEUROPEPTIDE Y (NPY) CONCENTRATION IS DECREASED IN THE PARAVENTRICULAR NUCLEUS (PVN) OF RATS EXHIBITING EXCESSIVE WEIGHT GAIN PRODUCED BY VENTROMEDIAL HYPOTHALAMIC (VMH) LESIONS. M.G. Dube*, A. Sahu, C.P. Phelps[a], P.S. Kalra and S.P. Kalra, Dept. Obstet. and Gynec. Univ. Fla., Gainesville, FL 32610, [a]Dept. of Anatomy, Univ. South Fla., Tampa, FL 33612

Our observations that central administration of NPY evokes robust feeding and that NPY level and release is upregulated selectively in the PVN in association with increased appetite, implicates NPY as a physiological signal for initiation of feeding in the rat. Since VMH lesions produce profound hyperphagia and obesity, we tested the hypothesis that VMH lesions may increase PVN NPY neurosecretory activity. Adult male rats received either bilateral electrolytic lesions of the VMH, a sham operation or no surgery. Animals were sacrificed either 2 or 21 days later. NPY levels were determined by RIA in several microdissected hypothalamic nuclei. Both short and long-term lesioned rats displayed marked hyperphagia and steady weight gain ($p < 0.01$). Quite unexpectedly, VMH ablation decreased NPY concentrations in the PVN within 2 days (64% of sham levels, $p < 0.05$), a further diminution occurred 21 days post-lesion (28% of sham levels, $p < 0.0001$). Of the other sites investigated, NPY levels also decreased in the dorsal medial nucleus and lateral hypothalamic area of the 21-day post-lesion group. These results show that hyperphagia and body weight gain in the VMH-lesioned rats may be due to a deficiency in PVN NPY which may result either in a compensatory post-synaptic supersensitivity or intervention of other hypothalamic appetite excitatory or satiety signals. (Supported by NIH DK 37273).

396.7

GALANIN IN THE CENTRAL NERVOUS SYSTEM OF LEAN AND OBESE ZUCKER RATS. Beck* B., Burlet A., Nicolas J.P., Burlet C. - INSERM U.308 MRCA , 38 rue Lionnois, 54000 NANCY (France)

Galanin (GAL), a 29 aminoacid peptide, is widely distributed in the central nervous system and especially in the hypothalamus. It strongly stimulates food intake when it is injected in the paraventricular nucleus (PVN). The obese Zucker rat with a well-established hyperphagia is characterized by a general dysregulation of some important neuropeptides involved in the regulation of feeding behavior e.g. neurotensin, NPY or CCK but nothing is known about the central status of galanin in these rats. The aim of this study was therefore to measure GAL in different microdissected brain areas in lean and obese male Zucker rats. As feeding status may modulate the central concentrations of the peptide, it was measured in ad libitum fed rats and in 48h-fasted rats. Bilateral arcuate nuclei (ARC) and parvocellular (PVNp) and magnocellular (PVNm) parts of the PVN as well as the median eminence (ME) were microdissected and sonicated. GAL was measured by a specific radioimmunoassay. The two-way analysis of variance revealed a very significant effect of genotype in the PVNp ($p<0.001$) and in the ME ($p<0.02$). No variations at all were noted in the ARC or in the PVNm. Fasting did not influence GAL concentrations in any areas. GAL concentrations were more than doubled in the ad lib obese rats when compared with the ad lib lean rats ($p<0.005$). On the other hand, in the ME where GAL concentration was about 4-fold greater than in the other nuclei, there was a 20 to 30 % decrease in GAL concentrations in the obese rat ($p<0.05$). Opposite variations of GAL were therefore observed between obese and lean rats in two distinct areas. Increased PVN levels might be related to the hyperphagia of the rats but GAL did not behave exactly like NPY, an other orexigenic peptide, because fasting has no effect on its levels. Its biological action might therefore be fairly different.

396.8

GALANIN-LIKE IMMUNOREACTIVITY (IR) IN HYPOTHALAMIC NUCLEI: RELATION TO FAT INTAKE. *Leibowitz, S.F., Akabayashi, A., Koenig, J.I.[1] and Alexander, J.T. The Rockefeller University, N.Y., N.Y. 10021 and [1]Georgetown Univ. Sch. Med., Washington D.C., 20007

This study examined the relationship between hypothalamic galanin (GAL) levels and macronutrient intake in rats. Albino rats (N=30) were maintained ad lib on diets of protein, carbohydrate and fat. After 3 weeks, the rats were sacrificed by decapitation, 10 hypothalamic nuclei and the posterior pituitary (PP) were microdissected, and GAL-IR was measured by RIA. Among the 10 hypothalamic areas examined, only the magnocellular paraventricular nucleus (mPVN) revealed a significant relationship between endogenous GAL and daily nutrient intake. In this region, where dense GAL cell bodies exist, positive correlations were observed between GAL levels and 24 hr fat ingestion ($r = +0.62$, $p<0.01$), 24 hr fat preference ($r = +0.62$, $p<0.01$), fat intake during the first 90 min of the feeding cycle ($r = +0.68$, $p<0.01$), and body weight gain ($r = +0.37$, $p<0.05$). While a small inverse correlation between mPVN GAL and carbohydrate intake ($r = -0.41$, $p<0.05$) was also detected, there was no apparent relation between GAL and either protein intake, total caloric intake or body weight. GAL in the PP was similarly related to fat intake ($r = +0.40$, $p<0.05$), as well as to GAL levels in the mPVN ($r = +0.40$, $p<0.05$). No such correlations with the other hypothalamic areas were seen. This relationship, between natural fat ingestion and endogenous GAL specifically in the mPVN, agrees with studies of GAL mRNA and also with central injection studies showing the PVN to be the most sensitive brain site to the selective stimulatory effects of exogenous GAL on fat ingestion.

396.9

INSULIN TRANSPORT FROM PLASMA INTO THE CENTRAL NERVOUS SYSTEM IS SATURABLE IN VIVO. G.D. Baura, D.M. Foster, D. Porte Jr., R.N. Bergman and M.W. Schwartz*. Depts. of Bioengineering and Medicine, Univ. of Washington and Seattle VA Medical Center, 98108, and Dept. of Physiology and Biophysics, Univ. of Southern California, Los Angeles, CA 90033.

Circulating insulin enters the central nervous system (CNS) where it acts as a regulator of food intake and body weight. We have previously shown that the kinetics of insulin uptake into cerebrospinal fluid (CSF) from plasma can best be explained by passage through an intermediate compartment, hypothesized to be brain interstitial fluid. To determine if an insulin receptor contributes to this uptake, we subjected anesthetized dogs (n=9) to 90 min euglycemic intravenous insulin infusions to obtain a wide range of plasma insulin levels (69-5064 μU/ml). Plasma and CSF samples were collected over 8 hr for determination of immunoreactive insulin levels, and the kinetics of CSF insulin uptake were analyzed using a mathematical model with three components (plasma→ intermediate compartment→CSF). Frequent sampling during rapid changes of plasma and CSF insulin levels enabled the model to precisely identify rate constants (mean standard deviation=14%) characterizing the uptake of insulin from plasma, through the intermediate compartment and into CSF (k_1k_2), and clearance of insulin from both intermediate compartment (k_3) and CSF (k_4). At physiological plasma insulin levels (83μU/ml), k_1k_2 was determined to be 11.5×10^{-6} min^{-2}. However, with increasing plasma levels, k_1k_2 decreased progressively, being reduced seven-fold at supraphysiologic levels (5064 μU/ml). The apparent Km of this saturation curve was 500-800 μU/ml (~3-5 nM). In contrast, the rates of insulin clearance from both the intermediate compartment and CSF did not vary with plasma insulin ($k_3 = 0.011 \pm 0.0019$ min^{-1} and $k_4 = 0.046 \pm 0.022$ min^{-1}). We conclude that transport of plasma insulin into CNS is saturable. This mechanism is consistent with insulin binding to blood-brain barrier insulin receptors and transcytosis through microvessel endothelial cells.

396.10

INTRAVENOUS INSULIN INFUSION INCREASES DAILY FOOD INTAKE AND ENERGY EXPENDITURE IN RATS. A.E. Willing, H.S. Koopmans* and E.K. Walls. Dept. of Medical Physiology, Univ. of Calgary, Calgary, Alberta, Canada, T2N 4N1.

Chronic insulin treatment in diabetic rats can significantly increase daily food intake, but insulin has previously been found to both increase and decrease energy expenditure (EE). To study the relationship between feeding and EE in diabetic rats, daily food intake, EE and respiratory quotient (RQ) were measured during chronic low dose (2 U/day) or increasing (2 to 6 U/day) insulin infusions into the vena cava of 12 Lewis rats (VC fixed and VC varied). In the VC varied group, daily food intake increased from a Ringer's baseline value of 71.5 ± 1.5 to 93.9 ± 1.3 and 112.2 ± 0.9 kcal/day at the 2 and 3 U/day insulin doses ($p < .01$). EE increased from a baseline value of 54.2 ± 4.1 kcal/day to 63.0 ± 4.9 kcal ($p < .05$) during the 3 U/day insulin infusion and the rats gained weight rapidly. RQ increased from 0.88 ± 0.03 during baseline to 0.95 ± 0.02 at 2 U ($p < .01$) and remained constant. In the VC fixed controls, daily food intake increased from 73.5 ± 0.7 to 98.2 ± 1.4 kcal/day at 2 U/day ($p < .01$) where it remained. There was no significant increase in EE, but RQ increased from 0.87 ± 0.02 during baseline to 0.94 ± 0.02 ($p < .01$) at 2 U/day. Over time, RQ decreased to 0.79 ± 0.03 ($p < .01$). These results suggest that the observed increases in EE that are often obtained when exogenous insulin is administered are a result of the increased food intake that accompanies the insulin infusion. Insulin administration also shifts substrate utilization from fat to carbohydrate. This shift occurs even when there is little change in daily food intake.

396.11

IVT INSULIN DECREASES RESPIRATORY QUOTIENT IN RATS. C.R. Park*, M. Chavez, S.C. Woods. Dept. of Psychology, University of Washington, Seattle, WA 98195

Centrally administered insulin decreases food intake and body weight in several species. In some studies the observed decrease in body weight appeared greater then what would be predicted solely on the basis of decreased food intake. It is therefore possible that central insulin affects metabolic rate independently of its behavioral effect. Male Long-Evans rats (n=6) received either insulin or saline vehicle into the third ventricle. Bolus injections (4 mU in 4 μl) were given twice, 12 hours apart. Water, but not food was available. Respiratory quotient (RQ) was measured by indirect calorimetry. All animals were exposed to both conditions in a counter-balanced design. IVT insulin resulted in a statistically significant decrease of RQ from 0.79 in the vehicle condition to 0.72 in the insulin condition. This change of RQ was accompanied by a significantly greater weight loss during insulin treatment than during saline treatment. Peripheral injections of insulin at the same dosage and time schedule increased RQ. We conclude that central insulin affects metabolic activity and appears to do so by increasing the utilization of fat stores. These results further support the hypothesis that central insulin serves as a negative feedback signal in the central nervous system for body adiposity.

396.12

DIFFERENCES AND SIMILARITIES OF THE FEEDING RESPONSES TO BACTERIAL CELL WALL COMPONENTS AND INTERLEUKIN-1ß. W. Langhans*, S. Weingarten, C. Bauer and M. Senn, Institute of Animal Sciences, Swiss Federal Institute of Technology, 8092-Zurich, Switzerland.

As a primary endogenous mediator of the host's acute phase responses to bacterial infections, interleukin-1ß (IL-1ß) is presumably involved in many pathophysiological effects of bacterial cell wall components, like lipopolysaccharides (LPS) and muramyl dipeptide (MDP). To investigate whether endogenous IL-1ß also mediates the anorectic effects of LPS and MDP, the feeding responses of adult male rats to IL-1ß, LPS nd MDP were compared. All compounds were injected intraperitoneally (IP) at dark onset. Paracetamol (50mg/kg, IP), which preferentially blocks brain cyclo-oxygenase, clearly attenuated the anorectic effect of IL-1ß (50,000 LAF units/kg) more than the anorectic effects of LPS (100μg/kg) and MDP (1.6mg/kg). When injected together, IL-1ß (12,500 LAF units) and MDP (0.4mg/kg) had an additive effect on feeding, whereas the hypophagia after combined injection of IL-1ß (12,500 LAF units/kg) and LPS (25μg/kg) was less than the sum of both compounds' individual effects. Finally, lesion of the area postrema and the adjacent caudal medial region of the nucleus of the solitary tract (AP/NST) did not affect the anorectic effect of IL-1ß, but enhanced the effects of LPS and MDP. The results indicate that stimulation of eicosanoid synthesis in the brain is involved in the hypophagia induced by IP injected IL-1ß. Furthermore, an intact AP/NST is obviously not essential for IL-1ß's anorectic effect, but modulates the effects of LPS and MDP through some as yet unknown mechanism. In summary, endogenous IL-1ß appears to mediate only part of the anorectic effects of LPS and MDP.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CORTEX V

397.1

REGIONAL CEREBRAL BLOOD FLOW CHANGES DURING ACTIVE AND PASSIVE FINGER MOVEMENTS: A PET STUDY.
T. A. Zeffiro* and M. Hallett Medical Neurology Branch, 10-5N226, NINDS, National Institutes of Health, Bethesda, MD, 20892.

Studies in subhuman primates have shown that many cortical neurons that modulate their activity in relation to voluntary limb movement also modulate their activity during passive movement of the same body part, suggesting a relatively tight relationship between a movement and its' sensory consequences. In order to examine this input-output coupling in man, we used $H_2{}^{15}O$ positron emission tomography to record regional cerebral blood flow (rCBF) in 8 subjects during active and passive index finger movements. We studied two different tasks. In the active condition, each subject executed repetitive index finger abduction/adduction movements twice per second. In the passive condition, the index finger was moved with the same rate and range while the subject lay quietly. In the control condition there was no movement. Significant changes in rCBF were detected using covariance analysis and the t statistic.

We found significant contralateral rCBF increases during both active and passive tasks in primary motor cortex, primary somatosensory cortex, supplementary motor area, cingulate motor area and putamen ($p<0.01$). In all these areas the rCBF increase was greatest in the active condition. Significant rCBF increases ($p<0.01$) limited to the active condition were detected in ipsilateral anterior cerebellar cortex, ipsilateral primary motor cortex, contralateral insula and contralateral superior parietal lobule.

In many motor areas, sensorimotor processing of repetitive movement is characterized by tight spatial coupling between activity related to active and passive movement of the same body part.

397.2

FUNCTIONAL MAGNETIC RESONANCE IMAGING OF MOTOR CORTICAL AREAS DURING REPETITIVE FINGER MOVEMENT.
R.Turner, T.A.Zeffiro, P.Jezzard, D.LeBihan and P.Herscovitch* NHLBI, NINDS and CC, National Institutes of Health, Bethesda, MD, 20892.

Focal activation of human sensorimotor cortex during voluntary movement is seen utilizing $H_2{}^{15}O$ positron emission tomography (PET) to record task-related regional cerebral blood flow (rCBF) increases. We have observed similar task-related activation patterns with a rapid, noninvasive MR imaging method that relies on the paramagnetic character of deoxyhemoglobin to measure blood oxygenation changes.

Measurements were made using a gradient-echo version of the echo-planar imaging sequence (EPI) on a 1.5 Tesla whole-body magnet. A gradient set dedicated to head imaging was used to provide a rapidly switching magnetic field gradient in the z direction and a 5" surface coil was used for radiofrequency reception. Gradient echo time was 40ms and image acquisition time was 25ms. Images were collected every 3 seconds for a total of 64 images per run. Five healthy volunteers were studied during alternating 30 second periods of unimanual finger opposition and rest. Task minus rest difference images were computed for each run.

We found focal, task-related, 2-5% increases in signal intensity in the hand representation of the contralateral primary motor and somatosensory cortex. Although these increases are somewhat smaller than the 10-30% rCBF increases seen in the same cortical areas with PET, there is a correspondingly lower image noise that allows clear identification of task-related signal changes. The high spatial and temporal resolution of deoxyhemoglobin echo-planar imaging make it an attractive tool for functional neuroimaging studies of human movement.

397.3

MOTOR SYSTEM CBF RESPONSES DURING FINGER MOVEMENTS ARE RATE INDEPENDENT Scott T. Grafton, Roger P. Woods, Michael Phelps*, and John C. Mazziotta U.C.L.A. School of Medicine, Los Angeles, CA 90024

The goal of this study was to determine the effect of performance rate on the magnitude of regional cerebral blood flow (rCBF) responses in the human brain. Four normal subjects performed a visually guided tracking task with their dominant index finger during serial positron emission tomography (PET) imaging of rCBF. Target speed was set at 0, 8.2, 16.4, 24.6, and 32.8 cm/sec. Tracking at these rates spanned the physical limits of non-ballistic finger movements. Significant changes of rCBF were identified using analysis of covariance and the t statistic after stereotaxic normalization. Movement (versus no movement) was associated with significant ($p<0.05$) rCBF responses in the contralateral primary motor cortex, supplementary motor area, premotor cortex, and ipsilateral anterior cerebellum. The magnitude of the responses in these areas was constant across tracking rates. No other linear or non-linear differences of rCBF associated with a rate effect could be identified in any other cerebral area.

The results are in contrast to previous studies of the visual system that have shown a relationship between photic stimulus rate and the magnitude of rCBF responses in V1. Motor system rCBF responses, representing the integral of local neuronal activity, remain constant across performance rates. These results suggest that the observed longitudinal changes in the magnitude of motor system rCBF responses during skill acquisition and functional recovery are not secondary to a rate effect.

397.4

ROLE OF THE PARIETAL LOBE IN THE GENERATION OF DIRECTED MOTOR RESPONSES. Roger P. Woods, John C. Mazziotta*, Simon R Cherry, Mark J. Morrow, and Robert C .Knowlton. U.C.L.A. School of Medicine, Los Angeles,CA 90024.

Using oxygen-15 labeled water and positron emission tomography (PET), we have found bilateral parietal increases in blood flow in normal humans performing both immediate and delayed visually guided oculomotor saccades to visual targets. These changes are sufficiently robust that they are consistently seen in individual subjects. To determine which components of these tasks are required for parietal responses, we have varied the lateralization of visual input and motor output, dissociated the spatial and temporal information provided by the visual cues, and compared visual to auditory temporal cues. Restriction of targets to a single visual hemifield did not result in lateralized changes, but when subjects maintained central visual fixation and pointed to the targets with one hand, parietal responses were stronger contralaterally. When subjects pointed to locations that they chose at random, a robust contralateral parietal response was still seen. The timing of movements in this task was still cued visually by the disappearance of the central fixation target. Substitution of an auditory temporal cue resulted in a decrease in the parietal response contralaterally, but did not eliminate the response as compared to a motionless control state. A contralateral parietal response was even elicited by pointing to randomly chosen locations using auditory temporal cues in darkness with eyes closed. The contralateral parietal lobe plays an important role in movements to visible external targets and in movements to internally generated targets in the absence of any visual input. Because all of the movements were cued either visually or auditorily, we cannot yet say whether the role of the parietal lobe is more closely related to evaluation of the status of the cue or to the actual initiation of the motor output.

397.5

TASK-SPECIFIC CHANGES OF LOCAL BLOOD FLOW WITHIN THE HUMAN ANTERIOR CINGULATE CORTEX: RELATIONSHIP TO LEVEL OF PERFORMANCE. T. Paus*, M. Petrides, A.C. Evans. Montreal Neurological Institute, McGill University, Montreal, Quebec H3A 2B4, Canada.

Overpractised and Reversal versions of **Speech Oculomotor**, and **Manual** tasks were used to study the role of the anterior cingulate cortex (ACC) in higher-order motor control. In two separate PET experiments, cerebral blood flow was measured in 18 healthy volunteers, using the ^{15}O-water-bolus method . For each subject, one "baseline" and six "task" scans were performed. Accuracy and latency of motor responses were also measured. On the basis of performance in the **Reversal Speech** task, subjects could be classified into two groups: "passed" and "failed". These groups did not differ in performance on the **Overpractised Speech** task. In both experiments, significant CBF changes within the ACC were obtained not only in the reversal minus overpractised speech subtractions (Exp.I:both groups, Expt.II:"passed" group only), but also in the overpractised minus baseline speech subtraction in the "failed" groups. The presence of ACC activation in a well practised speech task represents an atypical pattern of brain activity, the occurrence of which might signal failure in a more challenging task.

397.6

LOCALIZATION OF MOTOR AREAS WITH TRANSCORTICAL MAGNETIC STIMULATION AND MAGNETIC RESONANCE IMAGING IN HUMAN SUBJECTS. M. Daffertshofer, M. Syren, H. Henningsen and M. Zimmermann. Departments of Neurology and Physiology, University of Heidelberg, Germany (Spon: ENA)

Transcortical magnetic stimulation (TMS) and magnetic resonance imaging (MRI) were combined to map motor fields of the human cortex. We used focal "eight-shaped" coils (Novametrix 2 T) to stimulate the cortex as revealed by recording motor potentials by surface EMG electrodes over several muscles of the upper and lower limbs. Focal areas associated with distinct muscles were labelled on the skull surface with a cream rich in water, and numbers were assigned to these labels. Up to 80 sites of stimulation could be discerned. Subjects were then investigated by MRI (Siemens 2 T), and the labelled motor foci were projected to the image of the cortex.

In all 5 subjects studied so far the localizations of the points of motor excitations were consistent with precentral cortical motor fields associated with the limb muscles as were previously established by invasive electrical cortex stimulation. Thus, TMS enables the non-invasive mapping of motor fields with a rather high resolution. This method is valuable to detect changes in the topography of the motor cortex as might occur in amputees.

397.7

Projecting transcranial magnetic stimulation (TMS) maps into brain MRI. E.M. Wassermann*, B. Wang, C. Toro, T.A. Zeffiro, J. Valls-Solé, A. Pascual-Leone and M. Hallett. Human Motor Control Section, NINDS & Biomedical Engineering & Instrumentation Program, NCRR, NIH, Bethesda, MD 20892.

TMS can be used to map motor representations noninvasively, but it has not been possible to correlate these maps with brain anatomy. Ten TMS stimuli were delivered to each of a grid of points 1 cm apart on the left scalp while EMG was recorded and averaged from the right first dorsal interosseous muscle (FDI) in 4 subjects. EMG amplitude maps of the area accessing FDI were roughly circular with a diameter of 1-2 cm for responses more than 60% of maximum response. In each subject the 3-D coordinates of the grid and about 300 points on the head surface were acquired with a magnetic digitizer. A sphere was fitted to the grid to determine the line perpendicular to the scalp at the maximum. The other points were used as a surface for registration with the subject's head surface on MRI. The parameters obtained from the registration were used to map the maxima into the MRI and to compute the intersection of the perpendicular line with each slice. The intersections were within about 1 cm of the precentral gyrus. This technique can also be used to map other types of electrophysiological data into images.

397.8

FAST MODULATION OF HUMAN MOTOR OUTPUTS DURING ISCHEMIC NERVE BLOCK IS MEDIATED BY UNMASKING OF INTRACORTICAL SYNAPTIC CONNECTIONS. J.P. Brasil-Neto, J. Valls-Sole, A. Pascual-Leone, A. Cammarota, V.E. Amassian, M. Hallett and L.G. Cohen*. Human Cortical Physiology Unit, Human Motor Control Section, NINDS, NIH, Bethesda, MD 20892 and Department of Physiology, State University of New York Health Science Center at Brooklyn.

The amplitudes of motor-evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS) in muscles proximal to a transiently ischemic limb segment increase for the duration of ischemia, and return to baseline level afterwards.

To determine the level in the central nervous system at which this fast modulation of human motor outputs takes place, we recorded H reflexes, maximal M responses, MEPs to transcranial electrical stimulation (TES), MEPs to TMS, and MEPs to spinal electrical stimulation (SES) from muscles immediately proximal to an ischemic limb segment.

MEPs induced by TMS were larger during ischemia, whereas those induced by TES or SES were unchanged. Maximal H/M ratios were also unchanged, indicating that alpha-motoneuron excitability to segmental inputs was unaffected by ischemia.

Given the predominantly presynaptic activation of pyramidal tract neurons by TMS, as opposed to a more direct axonal activation by TES and SES, these findings suggest that intracortical unmasking of synaptic connections is the likely mechanism for short-term modulation in the human motor system during ischemic nerve block.

397.9

CEREBRAL NEURAL NETWORK APPROACH SHOWS HOW ANALOGICAL REASONING MAY EMERGE FROM SENSORIMOTOR ACTIONS.
M. Dufossé*S. Allemand, F. Blanc, Inst. Méditeranéen. Technol, 13013 Marseilles, Y. Burnod, Inst. Des Neurosci., Univ. P&M Curie, 75005 Paris, France.

It is not very easy to account for reasoning in terms of connectionist theory. Its distributed mode of parallel processing at a subsymbolic level makes it more suitable for dealing with imprecise reasoning, however. Our aim was to demonstrate the emergence of analogical reasoning within a neural network which mimics the cerebral cortex, using a previously built connectionist model of the human cerebral cortex (Burnod, 1989). This parallel distributed process includes both a network of cerebral areas and a network of cerebral micro-columns.

We studied a robotic task which involves camera-vision, robotic arm manipulation and analogical reasoning ability. The task consisted of first sequentially looking at a list of elementary symbols, secondly finding the next most plausible symbol, and finally drawing that symbol on a vertical board under visual control.

Our algorithm mimicked a network of eight cerebral areas, each simulated on a Transputer microcircuit. These maps were connected through a bus of data and arranged in a ring corresponding to an imaginary circular circuit going through the visual, temporal, infero-temporal (Miyashita, 1988), prefrontal (Fuster, 1989), premotor, motor, somato-sensory, parietal cortical areas and then back to the visual area. Recurrent information exchange between the cortical maps simulated the parallel cortico-cortical interactions. As shown by Caminiti (1991), the columnar activity corresponded to a mathematical bilinear relation between two input vectors.

We demonstrated that analogical reasoning ability may emerge from sensorimotor interactions. As a result, the prefrontal and infero-temporal areas send back to the postcentral associative areas a prediction about a forthcoming sensorimotor event.

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397.10

ANATOMIC ASYMMETRY AND CELL NUMBERS. A.M.Galaburda*, G.F. Sherman, J.M. Richman, and G.D. Rosen. Beth Israel Hospital and Harvard Med. School, Boston, MA 02215.

Previous research has demonstrated that symmetric brain regions are larger than their asymmetric counterparts and that this difference resulted from a greater number of neurons in the symmetric brain (Galaburda, et al., *Cortex* **22**, 151-160, 1986; *Neuropsychologia* **25**, 853-868, 1987). In this study, we tested the hypothesis that this relationship between cell number and asymmetry would be consistent for different cell types as characterized by immunohistochemical labeling.

Nineteen Wistar rats were sacrificed in adulthood by transcardial perfusion with saline followed by 4% paraformaldehyde. The brains were post-fixed for 48 hours before being placed in graded buffered sucrose solutions, sectioned coronally at 30 µm, and one series of every tenth section stained for Nissl substance with Thionin. Adjacent series were immunohistochemically stained for vasoactive intestinal peptide (VIP) or parvalbumin. The somatosensory/somatomotor cortices of the right and left hemispheres were parceled on the Nissl-stained sections, their volumes determined, and the number of labeled neurons within these architectonic regions estimated.

Consistent with previous findings, symmetric brain regions are larger that asymmetric brain regions. In addition, there is a greater number of parvalbumin- and VIP-immunoreactive neurons in the larger of the two sides. There also appeared to be greater cell packing density of parvalbumin-, but not VIP-immunoreactive neurons on the larger of the two sides. These results suggest that there may be asymmetric concentration of neuronal types associated with architectonic asymmetry. Because of the different proportion of parvalbumin-immunoreactive neurons in the large side, the latter may have different functional properties consistent with cerebral dominance.

(This work supported by NIH Grant NS27119).

397.11

CORTICAL NEURONAL PROTEIN SYNTHETIC CAPABILITY IN HYDROCEPHALIC AND DECOMPRESSED ANIMALS. C.L. Wolfgang J.P. McAllister II and J.S. Way*. Dept. of Anatomy and Cell Biology, Temple Univ. School of Medicine, Phialdelphia, PA.

The biochemical etiology of neurological deficits observed in hydrocephalic neonates and the utility of surgical decompression remains obscure because of the lack of a sensitive marker of neuronal function. Therefore, the present study quantified cytoplasmic RNA and nucleolar volume in order to assess the protein synthetic capability (PSC) of neurons in the cerebral cortex of hydrocephalic kittens. The effect of decompression via ventriculoperitoneal (VP) shunt placement was analyzed using the same techniques. Hydrocephalus was induced in cats at 10 days of age by intracisternal injection of kaolin. Some hydrocephalic animals received "early" (7-8 days post-kaolin, moderate ventriculomegaly) and "late" (11-14 days post-kaolin, severe ventriculomegaly) VP shunts; all animals were compared to age-matched controls. Brain sections were stained with azure B for stoichiometric binding to cytoplasmic and nucleolar RNA. Cytophotometric analysis revealed a 19-48% depletion in the PSC from motor, association and visual cortices of both moderately and severely hydrocephalic animals. After shunting, PSC returned to control levels only after early treatment; late shunt produced slight improvement, but PSC remained 26-52% below control levels. Since the normal function of neurons is dependant on the PSC, it follows that cerebrocortical neuronal function is adversely affected by infantile hydrocephaly, and that surgical decompression may restore function, but only if performed at early stages of the disorder.

INVERTEBRATE LEARNING AND BEHAVIOR IV

398.1

MOTOR NEURON CORRELATES OF DISHABITUATION AND SENSITIZATION OF THE GILL-WITHDRAWAL REFLEX IN *APLYSIA*. R.D. Hawkins*, T.E. Cohen, and E. R. Kandel. Ctr. Neuro. & Behav., Columbia Univ., HHMI, NY, NY 10032.

We have developed a simplified preparation, consisting of the isolated mantle organs of *Aplysia*, which undergoes habituation, dishabituation, sensitization, and classical conditioning of the gill-withdrawal reflex (Hawkins et al., 1987, 1990). We previously established that the LE siphon sensory neurons contribute to the reflex in this preparation (Cohen et al., 1991). To investigate the role of different motor neurons, we hyperpolarized them and found that LDG1 contributes approximately 70% of the reflex. We next recorded correlates of habituation, dishabituation, and sensitization in LDG1. There is a decrease in evoked firing of LDG1 during habituation, and an increase 12.5 but not 2.5 min after shock during dishabituation and sensitization. Conversely, there is an increase in spontaneous firing of of LDG1 2.5 but not 12.5 min after shock. To further analyze the mechanisms of these effects, we first hyperpolarized LDG1 and measured the area of the evoked complex PSP. As in evoked firing, there is a decrease in the PSP during habituation, and an increase 12.5 but not 2.5 min after shock during dishabituation and sensitization. We also measured the gill-withdrawal produced by a constant number of spikes in LDG1, and found an increase 2.5 but not 12.5 min after shock. These results suggest that habituation in this preparation is largely due to depression at central synapses, whereas dishabituation and sensitization are due to central and peripheral facilitation with different time courses. 2.5 min after shock there is a large peripheral effect, perhaps due to PTP at the neuromuscular junction, and no central facilitation, presumably because of competing transient inhibition. 12.5 min after shock there is no peripheral effect and significant central facilitation.

398.2

IDENTIFIABLE CNS GILL MOTOR NEURONS OF *APLYSIA*: FINAL COMMON PATH OR HIDDEN LAYER? J. L. Leonard*, J. Edstrom, M. Martinez-Padron, J. I. Goldberg, and K. Lukowiak. Mark O. Hatfield Marine Science Center, Newport, OR 97365; Dept. of Med. Physiol., Univ. of Calgary, and Dept. of Zool., Univ. of Alberta, Edmonton, Canada.

The hypothesis that *Aplysia* gill-withdrawal behaviors could be adequately explained by parallel monosynaptic reflex arcs between six PVG gill motor neurons (GMNs) and the LE sensory cluster (Kupfermann et al. 1974; review in Kandel 1978) made clear, falsifiable predictions that have stimulated experimental work for many years. Results show that the hypothesis is incorrect, both in detail (LE cells fire after motor neurons, Cohen et al. 1991; other cells active, Zecevic et al. 1989; etc.) and in principle (PVG necessary for neither behavior nor learning, Peretz et al. 1976; behavior not reflex, Leonard et al., 1989; behavior not necessarily correlated with MN actifity, Colebrook & Lukowiak 1988; Leonard et al. 1991; in press). Further experimental work requires a new hypothesis consistent with the available data, i.e. that the PNS is sufficient for these behaviors and that the CNS mediates behavioral state and the two interact to produce the behaviors of the intact preparation. We propose that the known CNS GMNs along with a set of inhibitory GMNs, can vary in efficacy, both individually and in concert, with learning and behavioral state and that the parallel CNS pathways act to set the gain of the gill behavior and to phase shift the Int II network. The model suggests that the CNS GMNs are a biological realization of the hidden layer of a neural network. Supported by MRC (Canada), AHFMR, NIMH & NSF.

398.3

A STUDY OF NEURONAL RESPONSE IN APLYSIA ABDOMINAL GANGLION DURING HABITUATION OF THE GILL-WITHDRAWAL REFLEX. Chun X. Falk*, Jian-young Wu, Larry B. Cohen, Akaysha Tang, Yang Tsau. Dept. of Physiology, Yale U. Sch. of Med., New Haven, CT 06510.

Previous studies indicated that habituation results in a decrease in the number of active neurons responding to the siphon touch and demonstrated the diversity of habituation effect on individual neurons. In the present experiments, a longer habituation training (up to 80 stimuli with 30 sec of inter-stimulus interval) were employed. The habituation effects on both the overall neuronal response and the response pattern of individual neurons were examined. The overall neuronal response shows a excellent correlation with the decrease in gill contraction during habituation. They both have two phases: a rapid decease at the beginning of habituation followed with a plateau (zero for the gill contraction). The plateau in the overall neuronal response indicates that the system maintains a constant response level (20-30% of control level) even after complete habituation was achieved. Additional analysis will aim at attributing these two phases to individual neurons or groups.

Supported by NIH grant #NS08437.

398.4

SECOND MESSENGERS CONTRIBUTING TO THE SEROTONIN-INDUCED PRESYNAPTIC FACILITATION IN THE SENSORYMOTOR SYNAPSES OF APLYSIA IN CULTURE. M. Ghirardi, O. Braha, B. Hochner, P.G. Montarolo*, E.R. Kandel, N. Dale. Ctr. Neurobiol. & Behav., Columbia P&S, HHMI, N.Y., N.Y. 10032; Dip. Anat. & Fisiol. Umana, Univ. Torino, 10125 Italy.

Two second messenger pathways, mediated by cAMP-dependent protein kinase A (PKA) and by protein kinase C (PKC), are known to contribute to the presynaptic facilitation of the gill withdrawal reflex in Aplysia (Braha et al., 1986; Sacktor and Schwartz, 1986; Sugita et al. 1992). We studied the relative contribution of each of these on 5HT-induced short term facilitation at nondepressed, partially depressed (reduced to 40% of their initial level) and highly depressed synapses (reduced to 10% of their initial value) and on the spontaneous release. We used Rp-cAMPS, a specific PKA inhibitor, and H7, a general kinase inhibitor that preferentially blocked PKC in intact Aplysia neurons. Our results show that the PKA inhibitor 1) completely prevents the facilitation of nondepressed synapses, 2) blocks 50% of the facilitation of moderately depressed synapses, 3) blocks only a small component of the facilitation of highly depressed synapses and 4) has no effects on the modulation of spontaneous transmitter release by 5HT. By contrast, H7 1) has no effect on the facilitation of nondepressed synaptic connections, 2) blocks only partially the facilitation of moderately depressed synapses, 3) blocks a large component of the facilitation of highly depressed synapses and 4) blocks completely the enhancement of spontaneous release by 5HT. Our results suggest that whereas activation of PKA is sufficient to trigger the facilitation of nondepressed synapses, activation of both PKA and PKC is required to facilitate depressed synapses and that the contribution of PKC becomes progressively more important as synaptic transmission becomes more depressed.

398.5

EARLY STEPS IN LEARNING-RELATED SYNAPTIC GROWTH: cAMP SIMULATES THE HETEROLOGOUS ENDOCYTOSIS OF apCAMS INDUCED BY 5-HT IN SENSORY NEURONS OF *APLYSIA*: C. H. Bailey*, M. Chen, and E. R. Kandel. Ctr. Neurobiol. & Behav., Columbia P&S, NYSPI, & HHMI, NY, NY 10032.

The synaptic growth that accompanies 5-HT-induced long-term facilitation of the sensory-to-motor connection in dissociated cell culture is associated with a down-regulation of cell adhesion molecules (apCAMs) on the surface membrane of the sensory neuron (Mayford et al., Science, 1992). Down-regulation is achieved by a protein synthesis-dependent activation of the endosomal pathway leading to internalization of apCAM (Bailey et al., Science, 1992). Unlike classical receptor-mediated endocytosis, the endocytosis of apCAM is triggered by the binding of ligand to a *heterologous* receptor, the 5-HT receptor, and consequent internalization of apCAM. To explore the mechanisms of this novel form of transmitter-mediated endocytosis in sensory neurons, we examined the effects of cAMP, a second messenger activated by 5-HT, on the distribution of gold-conjugated mAb specific to apCAM. We found that a 1.5-hr incubation in cAMP + IBMX (10^{-4}) simulated the action of 5-HT. It led to a 50% decrease in gold-labeled complexes at the surface membrane (10.8 ± 1.1) when compared to either untreated cells (22.6 ± 1.8; $p < 0.001$) or cells bathed in IBMX alone (22 ± 2.4; $n = 5$; $p < 0.01$). Accompanying the decrease in surface labeling was a sevenfold increase in the percentage of gold within the cell (31.6 ± 1.4 vs 4.3 ± 0.3 or 4.9 ± 0.2, $n = 5$, $p < 0.001$). These findings suggest the endocytic activation triggered by 5-HT can be mediated through the cAMP cascade (either alone or in combination with other second messengers) and now allow us to track the steps within the sensory cells whereby 5-HT binding to its extracellular receptor leads to the internalization and degradation of apCAM.

398.6

LONG-TERM FACILITATION IN *APLYSIA* SENSORY NEURONS IS ASSOCIATED WITH AN INCREASE IN THE LIGHT CHAIN OF CLATHRIN AND COATED VESICLES. Y. Hu*, C. Bailey, M. Chen, and A. Barzilai. Ctr. Neurobiol. & Behav., Columbia P&S, HHMI, NY, NY 10032.

One characteristic feature of long-term memory to the tail-withdrawal reflex is that it requires both RNA and protein synthesis, accompanied by structural changes. In an attempt to identify the proteins involved we have cloned and sequenced one of the serotonin (5-HT)-induced proteins in the sensory neurons of the tail-withdrawal reflex of *Aplysia*. The protein sequence inferred from the cDNA clone demonstrated that it is the light chain of clathrin. S1 mapping analysis showed that the steady-state level of clathrin light chain mRNA was also increased by 5-HT. Whereas in mammalian cells there are light chains A and B, both of which have neuron-specific isoforms in the nervous system, in *Aplysia* we have found only one form of light chain and no neuron-specific clathrin light isoforms. Genomic Southern blot analysis indicates that the *Aplysia* light chain is encoded by a single gene. This one chain has all the important structural and functional domains of both light chain A and B of mammalian clathrin, suggesting that it may represent the original form from which the vertebrate chains developed. Serotonin increases the number of clathrin-coated pits and vesicles in *Aplysia* sensory neurons. These coated vesicles are involved in the internalization of *Aplysia* cell adhesion molecules and probably contribute to the structural change of sensory neurons during long-term facilitation.

398.7

TEMPORAL DISSOCIATION OF 5HT-INDUCED SPIKE BROADENING AND EXCITABILITY IN *APLYSIA* SENSORY NEURONS. L. L. Stark*, N. J. Emptage, and T. J. Carew. Interdept. Neurosci Prog., Depts. of Psych. and Biol., Yale Univ., New Haven, CT 06520.

Tail shock-induced sensitization in *Aplysia* produces spike broadening and increased excitability in tail sensory neurons (SNs). Both of these effects are mimicked by serotonin (5HT) but can be pharmacologically dissociated: cyproheptadine blocks 5HT-induced spike broadening but not increased excitability (Mercer *et al.*, 1991). We report here that these two modulatory effects of 5HT can also be dissociated by their time course.

We recorded from tail SNs and examined both spike broadening and excitability. A range of 5HT concentrations (0.5μM-5μM) all produced significant excitability increases and spike broadening; higher concentrations produced progressively greater effects. In preliminary experiments (N=2), low 5HT concentrations (0.5μM-2.5μM) produced some spike broadening (x increase=6.5%) but no facilitation of the SN monosynaptic EPSP (x= -0.7%), suggesting that spike broadening may have to exceed a threshold level to produce synaptic facilitation. We next measured the time course of both 5HT effects following 5HT washout. At the lowest concentrations (0.5-1μM) only marginal differences were observed. However, at 2.5μM, 5HT-induced spike broadening lasted significantly longer than increased excitability (9.33 min vs. 2.75 min,respectively, $p<.01$, N=4). Increasing 5HT concentration (5μM) increased the duration of the spike broadening effect with no change in the duration of excitability (15.92 min vs. 2.33 min, $p<.005$, N=6). Thus 5HT-induced spike broadening and excitability were dissociated in two ways: (1) spike broadening lasted longer, and (2) the time course of spike broadening (but not excitability) increased at higher 5HT concentrations.

Our data collectively show that the modulatory effects of 5HT in SNs can be both pharmacologically and temporally dissociated.Since 5HT contributes to different forms of plasticity in the SNs,we can now examine the contribution of these modulatory effects of 5HT to different forms of learning and memory.

398.8

LONG-TERM SYNAPTIC FACILITATION DOES NOT REQUIRE SHORT-TERM FACILITATION IN *APLYSIA* SENSORY NEURONS: A PARALLEL PROCESSING HYPOTHESIS FOR SHORT- AND LONG-TERM MEMORY. N.J. Emptage* and T.J. Carew, Yale University, Depts. of Psychol. and Biol., New Haven, CT 06520.

Both short- term (ST) and long-term (LT) memory for sensitization in *Aplysia* involve an increase in synaptic transmission from sensory neurons (SNs) onto their follower cells. ST effects of sensitizing stimuli are mimicked by a single application of serotonin (5HT) while LT effects are mimicked by repeated 5HT applications (Montarolo *et al.* 1986). We recently found that the 5HT antagonist cyproheptadine (CYP) blocks ST 5HT-induced synaptic facilitation in tail SNs (Mercer *et al.* 1991). Here we ask whether the block of ST facilitation by CYP prevents the production of LT facilitation.

Five 5HT (5μM) applications (5 min each at 12 min intervals) produced both ST (<30 min) and LT (>24hrs) synaptic facilitation from tail SNs (ST increase, $\bar{x}$: = 92.2%, $p<0.006$; LT increase, $\bar{x}$: = 76.8%, $p<0.02$; N=4). When CYP (200μM) was present during 5HT applications (N=5), ST increases in synaptic facilitation were blocked ($\bar{x}$ = -8.9%, N.S), but significant LT synaptic facilitation was still observed ($\bar{x}$ increase = 73.1%, $p< 0.006$). Moreover, a between-group analysis showed that, even though ST synaptic facilitation was blocked in CYP+5HT SNs compared to 5HT SNs ($p<0.001$), both groups showed comparable, significant LT facilitation. Finally, Stark *et al.*, (this vol.) showed that low (0.5-2.5μM) concentrations of 5HT produce excitability increases in SNs without producing synaptic facilitation. In preliminary experiments (N=2) we found that these same low concentrations, while producing no ST synaptic facilitation, produced LT facilitation ($\bar{x}$ increase = 65.9%).

Our findings show that preventing ST facilitation either pharmacologically or with low 5HT concentrations does not block expression of LT facilitation, suggesting the hypothesis that ST and LT memory in *Aplysia* SNs are processed in parallel.

398.9

SUBSTRATE TARGETING BY SUBUNITS OF cAMP-DEPENDENT PROTEIN KINASE IN APLYSIA NEURONS. R.G. Panchal, S. Cheley, & H. Bayley*. Worcester Foundation for Experimental Biology, Shrewsbury MA 01545

Protein kinase A (PKA) has been implicated in presynaptic facilitation in Aplysia. Four forms of the catalytic (C) subunit of the holenzyme (R_2C_2) are the products of a single gene: N1A1, N1A2, N2A1, N2A2 (PNAS 89, 1641 (1992)). The A1 and A2 polypeptides arise through alternative splicing of exon cassettes encoding residues near the active site and exhibit different substrate specificities and affinities for a regulatory (R) subunit (Biochemistry 30, 10246 (1991)). The N1 and N2 forms arise from alternative promoters and contain alternative N termini originating at a point distant from the active site. Thus, the N termini may be involved not in the direct modulation of catalysis but in substrate targeting i.e. the binding of free C subunit at cellular locations in the vicinity of substrates. Evidence for this now comes from in vitro phosphorylation of neuronal membranes using recombinant N1A1 or N2A1 subunits. The N1A1 form phosphorylates two polypeptides of ~30 kDa and ~10 kDa significantly more rapidly than does N2A1. In Aplysia neurons, there exist at least five forms of R subunit, four of which are homologs of vertebrate RI (Neuron 8, 387 (1992)). We now provide evidence for RII subunits in Aplysia neurons: (i) high molecular weight RII-binding proteins have been demonstrated by using ^{32}P-labeled bovine RIIα to probe protein blots; (ii) rapid cAMP-independent phosphorylation of two ~50 kDa cAMP-binding polypeptides is stimulated by N1A1 or N2A1 subunits. By contrast, the recombinant Aplysia R_{APL-N4} (type I) subunit is not phosphorylated by C in vitro. RII-binding proteins may anchor PKA holoenzyme near selected substrates. (Supported by the NIH).

398.10

HABITUATION AND DISHABITUATION OF THE PROLEG WITHDRAWAL REFLEX IN LARVAL *MANDUCA SEXTA*. D.E. Wiel* and J.C. Weeks. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

The proleg withdrawal reflex is a simple behavior in which mechanical stimulation of sensory hairs on a larval locomotory appendage, the proleg, causes withdrawal of the proleg toward the body wall. Each sensory hair is innervated by a single sensory neuron that mono- and polysynaptically excites motoneurons innervating proleg retractor muscles (Weeks & Jacobs, 1987, *J. Comp. Physiol. A* **160**: 315-329). Because the monosynaptic component of this circuit exhibits several forms of synaptic plasticity (Trimmer & Weeks, 1991, *J. Comp. Physiol. A* **168**: 27-43), we tested for simple behavioral plasticity in the proleg withdrawal reflex.

Habituation was tested by repetitively deflecting a single hair while measuring proleg retraction using either video analysis or a force transducer. Interstimulus intervals ranging from 30 s to 10 min produced significant response decrement. Dishabituation in response to a tactile stimulus delivered to the body wall of the habituated segment was also significant. These studies indicate that the proleg withdrawal reflex of the tobacco hornworm is capable of behavioral habituation and dishabituation. Studies of the neural correlates of these behavioral plasticities are underway.

Supported by grants from NIH, NSF and Patricia Roberts Harris Foundation.

398.11

HABITUATION OF THE JUMP REFLEX TO OLFACTORY CUES IN NORMAL AND MUTANT *DROSOPHILA*. T. Tully* and S. Koss. Beckman Neuroscience Center, Cold Spring Harbor Laboratory, Cold Spring Harbor NY 11724 and Department of Biology, Brandeis University, Waltham MA 02254.

We are interested to know whether the molecular mechanisms of memory formation after nonassociative and associative learning are similar. Since learning through different sensory modalities may involve different molecular mechanisms and since our associative learning assay is based on odor-shock pairings (Tully & Quinn, 1985, *J. Comp. Physiol. A* **157**: 263), we have developed an assay for nonassociative learning based on olfactory cues. We found that flies would jump up – as if to fly away – when presented with a concentrated, noxious odor stimulus (10% benzaldehyde), as described by McKenna et al. (1989, *PNAS* **86**: 8118). We then semi-automated the olfactory jump procedure to deliver 4-s presentations of an airborne odor stimulus repeatedly to individual flies. With such a procedure, the flies eventually stopped jumping.

This response decrement in wild-type (normal) flies shows many definitive behavioral properties of vertebrate habituation: More stimulus trials are required for a fly to stop jumping if the interval of time between trials (ITI) is longer. Fewer trials are required for a fly to stop jumping if the odor concentration is lower. After habituating, 80% of flies will jump in response to the odor stimulus if they have been dishabituated by a novel, strong stimulus (75 s of vortexing).

Analysis of the (associative) learning/memory mutants has revealed that a) acquisition of habituation is slower than normal, spontaneous recovery (memory retention) is faster than normal and dishabituation is normal in *rutabaga* mutants, b) acquisition can be faster -- or slower -- than normal (depending on ITI), spontaneous recovery is faster than normal and dishabituation is abnormally low in *dunce* mutants and c) acquisition, spontaneous recovery and dishabituation is normal in *amnesiac* and *latheo* mutants. In addition, acquisition of habituation is faster than normal in mutants with abnormal olfaction or locomotion. These results are consistent with the notions that a) the *rutabaga* mutation affects memory, b) the *dunce* mutation affects both memory and sensory or motor processes and c) short-term memory is normal in *amnesiac* and *latheo*.

398.12

ABSORPTION VOLTAGE-SENSITIVE DYES FOR INTRACELLULAR APPLICATION. D.Zecevic# and S. Antic. Sch.Biology. Univ.Belgrade, Yugoslavia.

Absorption optical signals corresponding to membrane potential changes were investigated in neurons filled with voltage-sensitive dyes. We are looking for signals that are large enough to allow the analyses of subthreshold signal integration at the level of individual neurons in invertebrate ganglia. Absorption dyes, as compared to fluorescent molecules, might be more successful since they cause less photodynamic damage. Dyes were tested on individual neurons from the isolated ganglia of young (.3-1 g) snails (Helix). We determined the potential dependent response spectra of several analogs of the most successful potentiometric probes characterized by three different basic chromophores. By comparing signals obtained with most sensitive negatively charged pyrazolone-oxonol probes (RH155, RGA509, RH479, RH482, RGA565A, RGA577, RGA574, JPW1249, JPW1247,JPW1245, JPW1037) with newly synthesized positively charged close analogs (JPW1177, JPW1241) we found that positively charged probes are about 10 times more sensitive in intracellular application. These results indicate that further improvement in signal size might be obtained by synthesizing positive analogs of negatively charged merocyanine and barbituric-acid-oxonol compounds that give relatively large absorption signals in intracellular application. Those are: WW375, JPW1124, NK2367 and WW781.

398.13

MORPHOLOGICAL AND BEHAVIORAL DEVELOPMENT IN THE EMBRYONIC MEDICINAL LEECH. Shirley A. Reynolds, Andreas Baader, and William B. Kristan,Jr.* U.C.S.D. Biology Dept., 0322, La Jolla, CA 92093

As a preliminary to studying the development of neuronal circuits responsible for different behaviors, we have been studying the normal course of behavioral development in the medicinal leech, *Hirudo medicinalis*. Most leech behaviors are first seen during embryogenesis. To establish staging criteria which cover the time of behavioral development, we have been tracking the development of several morphological features as well as the development of both spontaneous and mechanically-evoked behaviors. We have found that behavioral responses to light mechanical stimulation vary among different body regions. At the anterior and posterior ends, the predominant response to mechanical stimulation is shortening. The predominant response at the midbody region early in development is circumferential indentation, which is replaced by local bending. A single mechanical stimulus will sometimes produce a combination of two simple behaviors, which can occur either sequentially or simultaneously. The percentage of mechanical stimuli which produce at least some behavioral response reaches maximum at about one week after the animal first becomes responsive. Swimming develops as progressive refinements of the early swim-like behavior. In contrast, crawling is a complex behavior resulting from the successive integration of simpler behaviors, namely progressive elongation and contraction, rear sucker use, and front sucker use. Both crawling and swimming appear to be fully developed by the end of embryogenesis when the juvenile leech emerges from the cocoon. This work was supported by an NIMH research grant (MH43396) and by Deutsche Forschungsgemeinschaft.

399.1

A RETINA SPECIFIC PROTEIN EXPRESSED TRANSIENTLY ON ZEBRAFISH OPTIC NERVES. H.Chang and W.Gilbert*. Dept. of Cell. and Dev. Biology, Harvard Univ., Cambridge, MA 02138

We are interested in identifying molecules involved in neural development and axonal pathfinding in vertebrates. Zebrafish provide an ideal system because it can be easily studied at early stage and its nervous system is relatively simple.

We have generated a monoclonal antibody, 7A11, which recognized an antigen expressed specifically in zebrafish retina. Immunostain shows that 7A11 MAb first stains retinal ganglion cells around 38 hours after fertilization, later(3 days) it also stains inner plexiform layer(IPL) and inner nuclear layer(INL), at adult retina it only stains IPL and amacrine cells. Most interestingly, 7A11 antigen expressed on the optic nerves during the initial out growth of retina ganglion axons at embryonic stage, but no longer expressed at the adult optic nerve. The expression pattern of 7A11 antigen suggested it may involve in the development and guidance of optic nerve.

Immunoblots showed that 7A11 MAb stained a single band at 28kD , following SDS-PAGE of zebrafish retina sample.

Studies are in progress to clone the gene for 7A11 antigen and to determine its role in retina development.

399.2

A NEW MEMBRANE PROTEIN ON CHICK SENSORY NEURONS. R.G. Perez*, Y.P.L. Yip, W. Halfter, and J. Yip. Dept. of Neurobiology and Dept. of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

A 140 kD cell surface protein was identified by a monoclonal antibody (1A12) obtained from a mouse immunized with dorsal embryonic chick spinal cords. The protein is present on afferent neurons in the CNS and PNS, but absent on motoneurons and their axons. In spinal cord, the protein is present on a subset of primary afferents that project in the dorsal funiculi (DF). During early development, the 1A12 antigen is found in dorsal root ganglia, sensory components of peripheral nerves, and central projections in the DF. Later in development (E16-hatching), 1A12 ceases to be found in the DF and is present only in the lateral dorsal horn of spinal cord. In the visual system, 1A12 is present in the retina, optic nerve and optic tract in early stages of axon outgrowth. It is not present in the optic fiber layer of tectum indicating that the expression of the antigen ceases as optic fibers reach this tissue. By E10, the antigen disappears entirely from the visual system indicating that expression of the antigen is developmentally regulated. The restricted spatiotemporal distribution of the antigen suggests that it is important for the development of afferent projections.

399.3

Mab E1 (SG III-1) delineates neuronal cell classes in neuron-glial cell interaction and affects neurite outgrowth.
A. Zimmermann* and A. Sutter, Institute of Zoology, Technical University, Schnittspahnstr. 3, 61 Darmstadt and Dept. of Immunopharmacology-Neuroimmunology Group, E. MERCK, 61 Darmstadt FRG.

Axonal growth processes are controlled by neurotrophic factors as well as neuron-glial contacts. We describe a neural cell surface antigen E1, which appears to be involved in axonal growth regulation.

Mab E1 defines an early glial antigen of chick embryonic dorsal root ganglia (drg). In the drg E1 antigen is exclusively represented on glial cells until embryonic day 7 (E7). At later times it also appears on a fraction of drg neurons. When antigen expression was analyzed in indirect immunofluorescence staining in single cell neuron-glial cultures of chick drg or in drg explant cultures grown in the presence of nerve growth factor (NGF), it became apparent that E1 positive neurons displayed a growth behaviour different from E1 negative neurons. E1 negative neurons grew associated and became ensheathed with E1 positive glial cells while E1 positive neurons did not. When Mab E1 was added to neuron-glial cultures of chick drg (E8), the NGF induced neurite outgrowth was blocked completely, suggesting a role of E1 antigen in neurite outgrowth which would appear to be glia mediated in the case of the E1 negative neurons. Also in the CNS the E1 epitope is widely distributed. Here, motoneurons of the spinal cord constitute one example for an E1 negative neuronal cell class growing out into the periphery in close association with E1 positive glial cells.

399.4

CLATHRIN IS POSSIBLY INVOLVED IN NEURITE FORMATION INDUCED BY A PROTEASE INHIBITOR IN PC12 CELLS. Y.SAITO, T.KADOTA[1], S.TSUBUKI, T.HAMA[2]* AND S.KAWASHIMA. Dept. Molec. Biol. Tokyo Metropol. Inst. Med. Sci. Tokyo 113. [1]Dept. Anat. Chiba Univ. School Med., Chiba 280. [2]Dept. Neurosci. Mitsubishi Kasei Life Sci. Inst., Tokyo 194 Japan.

We have shown that a protease inhibitor of leupeptin analogue, acetyl-Leu-Leu-Nle-aldehyde (ALLNal) or benzyloxycarbonyl-Leu-Leu-Leu-aldehyde (ZLLLal) induces neuronal differentiation in PC12 cells. In an attempt to identify a target molecule(s), Leu-Leu-Leu-al was immobilized and used as a ligand for affinity chromatography. Proteins of 33K, 35K and 180K were isolated from the membrane and cytoplasmic fraction of PC12 cells. ZLLL-COOH had no ability to induce neurite outgrowth in PC12 cells, and 33K, 35K and 180K did not bind to the LLL-COOH affinity column. Several lines of evidence suggest that these proteins are clathrin light chains (33K and 35K) and clathrin heavy chain (180K), components of clathrin which is well known for its role in endocytosis. Separation of clathrin into its heavy and light chains showed the clathrin heavy chain had ability to bind to the LLLal affinity column directly. Furthermore, ZLLLal but not ZLLL-COOH enhanced the rate of polymerization of clathrin triskelion to the coat structure. These results suggest that the role of clathrin should be incorporated into a model describing pathways during the initiation of neurite outgrowth.

399.5

NEURITE OUTGROWTH FROM CULTURED EMBRYONIC MOUSE RETINAL CELLS IS SUBSTRATE DEPENDENT. *C.E. Lagenaur and †M.H. Hankin. *Univ. Pittsburgh, Pittsburgh, PA and †Medical College of Ohio, Toledo, OH.

Is neurite outgrowth from mouse retinal ganglion cells (RGCs) of different development ages substrate-dependent? Previous studies indicate that RGC neurite outgrowth may be influenced by specific substrate molecules, but it is not clear whether the early developing RGCs show a preference for outgrowth on any particular substrate.

To address this issue, we have monitored neurite outgrowth *in vitro* from dissociated fetal (E12-16) or early postnatal (P0-6) CD-1 mouse retinal cells on purified substrate molecules (L1, NCAM & laminin). RGCs from fetal retinae were identified by staining with an antibody to neuron-specific β-tubulin (TuJ1), a specific marker for early differentiating RGCs (Watanabe et al., *J Neurobiol* 22:85). RGCs from neonatal animals were identified in vitro after retrograde labelling *in vivo*. The ability for given substrates to support neurite outgrowth was also compared with their expression *in vivo*.

Cultured embryonic cells expressed a variety of morphologies (unipolar, bipolar and multipolar), and all cells were TuJ1$^+$. Neurite outgrowth on L1 was apparent shortly after cell attachment, and was vigorous after 18-24h (many cell with neurites extending 500 μm). After 48-56h, neurite length in many cells exceeded 1500 μm (indicating a growth rate of about 1mm/day). Few neonatal RGCs survived beyond 24h, although other retinal neurons survived on L1.

Outgrowth from embryonic retinal neurons on non-L1 substrates was detectable only from a few cells per coverslip. The majority of cells on NCAM had growth cone-like membrane expansions, but elongation was not seen. Neurite outgrowth from RGCs on laminin was seen only rarely. In contrast, cortical cells from the same embryos showed vigorous outgrowth on L1 and NCAM substrates.

These data show that developing (E12-16) RGCs prefer to grow on L1 substrates; at some point between E16 and birth, RGCs lose their ability to survive and extend neurites on L1.

399.6

RETINAL AXON EXTENSION IS ENHANCED ON L CELL MONOLAYERS EXPRESSING TRANSFECTED NCAMS Li Liu and Richard A. Akeson*, Division of Basic Research, Children's Hospital, Cincinnati Ohio, 45229

The neural cell adhesion molecule NCAM has been reported to enhance axon outgrowth from several neural sources including retina. To investigate the full extent of NCAM function, we examined the biologic activities of distinct NCAM isoforms particularly those which vary in the fourth immunoglobulin-like domain by the presence or absence of a ten amino acid exon termed VASE. Axon outgrowth from chick retinal neurons was tested on control fibroblast L cells and L cells transfected to express rat 140 kD NCAM± VASE. Axon outgrowth from retinal strips cultured from 6 day embryos was analyzed as a function of the density of substrate cells upon which they were explanted. No changes in axon outgrowth compared to control laminin layers were observed when substrate cell monolayers were 10-20% confluent. Monolayers of control L cells which were more than half confluent inhibited axon outgrowth by >60%. Axon outgrowth on NCAM expressing L cells at this confluency was 2-3 times greater than that on control L cells but less than that on laminin. Thus NCAM expression by the transfected cells partially, but not totally, overcame the inhibitory capability of the L cell itself. At greater cell confluencies, axon outgrowth was further diminished but at all densities tested NCAM expressing cells supported greater axon outgrowth and L cells expressing NCAM without the VASE exon supported consistently greatest axon outgrowth. To further test the effects of NCAM on axon outgrowth, dissociated retinal cells were plated on monolayers of L cell±NCAM, B35 neuronal tumor cells which express NCAM, and B35 derivatives that had been selected to lack NCAM. B35 cells both with and without NCAM supported much more extensive axon outgrowth than any of the L cell derivatives. The combined results confirm that while NCAM is one substrate factor which supports axon outgrowth, other substrate components are also active. They further indicate that NCAM+VASE which is present in mature neural tissue is less supportive of axon outgrowth than NCAM lacking VASE which is found in embryonic tissues. Supported by grants EY08490 and HD21065.

399.7

MODULATION OF LAMININ'S NEURITE-PROMOTING ACTIVITY.
D. Muir.* Developmental Neuro-Oncology Lab., Departments of Pediatrics and Neuroscience, University of Florida College of Medicine, Gainesville, FL 32610

Purified laminin (LN) promotes neurite outgrowth by regenerating neurons and NGF-treated PC12 cells. Schwann cells secrete LN and other extracellular matrix molecules including proteoglycans (PGs) that bind to LN but do not affect its activity. In contrast, neurite-promoting activity is completely inhibited when LN is complexed with a Schwann cell-derived, high-density, chondroitin sulfate/heparan sulfate PG. The inhibitory PG appears to specifically address LN since it did not substantially inhibit the neuritic response of PC12 cells to other matrix molecules. Initially I observed that, in contrast to purified LN, LN activity in crude extracellular matrix extracts was not vulnerable to inhibition by the high-density PG. Recombination experiments demonstrated that LN in the extract was bound to low-density PG(s) which protected its activity by preventing the inhibitory PG from complexing with LN. Other LN-binding proteins including entactin and collagen type IV were not protective. In addition, when purified LN was first complexed with the inhibitory PG, inhibition was not reversed by the addition of LN-binding molecules including other PGs. Neurite-promoting activity was, however, restored (de-inhibited) by treating inhibited LN-PG complex with the metalloprotease stromelysin. Apparently, stromelysin selectively abolishes the inhibitory activity allowing LN to express its neurite-promoting activity. This mechanism for de-inhibition may be particularly relevant since PC12 cells secrete stromelysin at the onset of neurite-outgrowth.

These findings suggest the neurite-promoting activity of LN is modulated by forming complexes with either protective or inhibitory PGs and this modulation is counteracted by protease(s) secreted during neurite outgrowth. (Supported by Telios Pharmaceuticals, Inc.)

399.8

DEGRADATION FRAGMENTS OF L1 ANTIGEN ENHANCE TYROSINE HYDROXYLASE-POSITIVE NEURITE OUTGROWTH IN MESENCEPHALIC CELL CULTURE.
M. Poltorak*, J.R. Williams and W.J. Freed. NIMH Neuroscience Center at St. Elizabeths, Washington, DC 20032.

The L1 antigen is implicated in axonal elongation during formation of major fiber tracts and promotes neurite outgrowth in culture. It is possible that during injury of brain tissue, neuronal surface molecules such as L1 antigen are shed, and degradation fragments may therefore be present adjacent to the damage. These L1 fragments might then influence regeneration and injury-induced growth. We have evaluated neurite outgrowth from tyrosine hydroxylase-positive (TH+) E13 mesencephalic neurons grown *in vitro* on intact mouse L1 antigen and three degradation fragments separated by molecular weight. Mouse MAG, laminin, fibronectin, poly-D-lysine, and fetal calf serum served as control substrates. L1 antibodies were added to one set of cultures (experimental), and compared to control cultures containing normal rabbit serum. After 3 days *in vitro*, the cultures were stained using an antibody against TH, and the length of the TH+ neurites was measured by computer assisted image analysis in a double blind fashion. TH+ neurites were significant longer when grown on intact L1 antigen, as well as on each of the three degradation fragments, as compared to the control substrates. As compared to control normal rabbit serum, L1 antibodies blocked fasciculation and eliminated the neurite-promoting effect of the L1 substrate and of the L1 degradation products. These data suggest that the presence of L1 fragments *in vivo* might influence regeneration or synaptic restructuring.

399.9

NCAM-POLYSIALIC ACID AND L1 EXPRESSION ON GROWING AXONS OF ISOLATED NEURONS.
W. T. Kim, W. F. Collins*, and A. N. van den Pol. Sect. Neurosurgery, Yale Univ. School Med., New Haven, CT 06510.

Although NCAM has been considered to be a homogeneously distributed neuron adhesion molecule with a general role in development, NCAM expression at the cellular level may vary in molecular density and the amount of polysialic acid (PSA), thereby influencing adhesion and axonal growth. To study the relative densities of NCAM and PSA on the different surfaces of single isolated rat hippocampal neurons, we used immunogold cytochemistry and digitally-processed backscatter electron imaging in an Amray 1810 scanning electron microscope. Based on the gold particle density per membrane area from thousands of images representing all surface membrane domains of isolated cells, we found NCAM immunoreactivity distributed evenly throughout the plasmalemma of single cells. NCAM labeling among different cells in the same culture dish varied as much as 225%. PSA, which may reduce the homophilic binding of NCAM, was strongly expressed on axonal growth cones and their filopodia. PSA immunoreactivity was found at higher densities (37% $\pm$ 12% S.E.) on axons than on dendrites of the same cell in 6 out of 7 neurons, and at higher densities (35% $\pm$ 11%) on the distal axon near the growth cone than on the proximal part of the same axon in all 7 cells. Since NCAM may influence the homophilic binding of the L1 molecule, we undertook parallel examinations of L1. Neurons after one day in vitro showed L1 immunoreactivity on all processes; as soon as one process, the putative axon, extended farther than the others, it showed a higher level of expression. L1 immunoreactivity was 417% greater on axons than on dendrites at five days in vitro. Axonal growth cones and their filopodia were strongly immunoreactive for L1. The presence of NCAM-PSA and L1 on the growth cone of axons, together with evidence of a strikingly heterogeneous distribution of these molecules in the cellular microenvironment, support the hypothesis that NCAM-PSA and L1 may participate in some aspects of axonal extension and guidance.

399.10

DIFFERENTIAL MODULATION OF NEURONAL POLARITY BY EXTRACELLULAR MATRIX AND CELL ADHESION MOLECULES.
A. Lochter* and M. Schachner. Department of Neurobiology, Swiss Federal Institute of Technology, CH-8093 Zurich, Switzerland.

The effects of extracellular matrix glycoproteins and cell adhesion molecules on neurite growth have been discussed mainly in the context of promotion of neurite initiation and axonal elongation. Only recently have polarity and inhibition of neurite growth been investigated. To gain insight into the mechanism of establishment of neuronal polarity, we have studied the kinetics of the morphological differentiation of hippocampal neurons in vitro on polyornithine-substrates coated with different proteins. On the extracellular matrix substrates tenascin, laminin and fibronectin an initial phase of increased neurite outgrowth was followed by an increased growth rate of the axon-like, major neurite and a cessation of growth of dendrite-like, minor neurites, when compared with neurons on control substrates without coated proteins. In contrast, neurite growth on the cell adhesion molecule L1 and the lectin concanavalin A showed an initial burst of neurite outgrowth and continuous growth of major neurites but no decreased growth of minor neurites. Therefore polarity was not increased.

The pronounced polarity of neurons grown on tenascin substrates could be reduced strongly with inhibitors of protein kinases, suggesting that specific intracellular signalling mechanisms participate in the establishment of different neuronal compartments.

399.11

GROWTH CONE COLLAPSE INDUCED BY MEMBRANE-BOUND MOLECULES MAY BE MEDIATED BY PERTUSSIS TOXIN (PTX)-SENSITIVE G PROTEINS.
M. Igarashi*, T. Vartanian, S.M. Strittmatter, and M.C. Fishman, Developmental Biology Lab. and Cardiovascular Res. Ctr., Massachusetts General Hospital-East, Charlestown, MA 02129.

Inhibitory signals to growth cones are crucial for the regulation of axonal pathfinding. The transduction mechanisms that mediate such "stop signals" are poorly understood. The collapse of growth cones in culture has been used to partially isolate inhibitory substances associated with brain membranes (Neuron 4: 21-9) and myelin (J. Cell Biol. 106: 2181-8). We have previously demonstrated that G_o is one of the major proteins in growth cone membranes. In the present study, we examined the potential roles of G proteins in growth inhibition, using the growth cone collapse assay. CHAPS-solubilized collapsing activity of chick brain membranes causes growth cone collapse of both DRG and retinal explants. PTX-pretreatment blocks growth cone collapse completely in DRG, and partially (about half of the newly induced collapse) in retinal neurons. Mastoparan (MP), a direct activator of G_i/G_o, mimics the collapsing activity of brain membranes, such that 1 μM MP collapses 70% of DRG growth cones, an activity blocked by PTX-pretreatment. The collapsing activity of rat CNS myelin solubilized by octylglucoside induces 65% growth cone collapse in DRG neurons, and this effect is also inhibited by PTX. These results suggest that some of the membrane-bound inhibitory signals to growth cones may act by stimulating certain receptors coupled to PTX-sensitive G proteins. Blockade of such G proteins appears to abrogate the inhibitory signals, thereby permitting continued growth cone advance.

399.12

PURIFICATION OF GROWTH CONE COLLAPSE-INDUCING GLYCOPROTEIN FROM ADULT CHICKEN GREY MATTER.
Geoffrey M.W. Cook, Alan R. Johnson, Marina S. Gordon, Scott A. Akker & Roger J. Keynes*.
Department of Anatomy, University of Cambridge, Cambridge, U.K.

Adult avian grey matter contains a glycoprotein fraction that causes growth cone collapse *in vitro* (Abstr. Soc. Neurosci. 16, 77.6, 1990). We have also found collapse activity in mammalian (human, sheep, rat and mouse) grey matter. The avian activity is removed by immobilized peanut agglutinin (PNA), and may be related to a somite-derived, PNA-binding glycoprotein that is involved in the generation of a segmented pattern of spinal nerves. As a further test of similarity between brain and somite activities, chick CNS (E7 retinal) neurons have been grafted into the spinal region of E3 chick embryos; like outgrowing spinal nerves, retinal axons avoid posterior half-somite and grow exclusively through anterior half-somite. A purification schedule for the isolation of the grey matter-derived molecule(s) has been devised. On ultrafiltration, activity is retained with a membrane of 100,000 M.W. cutoff. The macrosolute is then fractionated by chromatography on immobilized reactive dyes in combination with nondenaturing gel electrophoresis. Material electroeluted from a single band, running just behind a BSA marker on a 10% gel, induces growth cone collapse. We hypothesize that this component may inhibit growth of normal and regenerating axon terminals in the adult brain.

399.13

PURIFICATION AND CHARACTERIZATION OF INHIBITORY PROTEINS FROM RAT CNS MYELIN. **F. Keller*, B. Rubin, C. Bandtlow and M.E. Schwab. Brain Research Institute of the University of Zürich, CH-8029 Zürich, Switzerland.**

CNS myelin and mature oligodendrocytes are inhibitory for neurite outgrowth. When myelin proteins from rat CNS are separated by SDS-PAGE, inhibitory activity can be eluted from the 35 kD and 250 kD region. Two monoclonal antibodies (IN-1 and IN-2) were raised against the gel-purified inhibitory fractions and were found to neutralize the inhibitory properties of CNS myelin and oligodendrocytes (Caroni and Schwab, 1988). In addition, IN-1 was found to promote regeneration of transected corticospinal and septo-hippocampal axons in young and adult rats (Schnell and Schwab, 1990; Cadelli and Schwab, 1991). In Western blots of rat CNS myelin, IN-1 and IN-2 bind to a partially overlapping set of bands of approx. 35, 45, 55 and >200 kD. These bands appear to be specific for CNS myelin, since they are not found in PNS myelin. Interestingly, and in contrast to several of the major myelin proteins, some of these bands are resistant to extraction with nonionic detergents, and can only be solubilized with SDS. An immunoaffinity column was prepared by covalently crosslinking the IN-1 antibody to an anti-IgM matrix. After adsorption of SDS-extracted, CHAPS-diluted CNS myelin to the column, part of the inhibitory activity was retained by the column and could be eluted by moderately increasing the salt concentration. This suggests that the activity binds to the column by means of weak interactions. The inhibitory fraction eluted from the column contained several protein bands between 35 kD and 250 kD that are recognized by the IN-1 antibody. Other proteins eluted by lowering the pH did not show inhibitory activity, nor did they crossreact with the IN-1 mAb.

399.14

THE IDENTIFICATION OF A CANDIDATE GLYCOPROTEIN FROM ADULT CHICK BRAIN THAT INDUCES COLLAPSE OF DORSAL ROOT GANGLIA GROWTH CONES. Yuling Luo* and Jonathan A. Raper. Department of Neuroscience, University of Pennsylvania, School of Medicine, Philadelphia, PA 19104.

Factors that inhibit growth cone extension are likely to play a role in both axon pathfinding and regeneration. We previously reported that a growth cone collapsing activity can be recovered from embryonic chick brain membranes and enriched by column chromatography (Raper, J. A. and Kapfhammer, J. P. (1990) Neuron 2, 21-29). Here we report the further enrichment of this collapsing factor and the identification of a protein that is likely to comprise the activity. Detergent-solubilized collapsing activity was enriched over 2,500 fold with a yield of approximately 5% by chromatography on a combination of Q-Sepharose, S-Sepharose, Wheat Germ Agglutinin, and Mono S FPLC columns. The enriched material was highly active, inducing collapse of over 50% of DRG growth cones at a concentration of about 6 ng/ml. After further separation of this highly enriched material by Mono S FPLC, active fractions, in contrast with other fractions, were found to contain one prominent protein band of approximately 100 kDa on Coomassie Blue stained SDS-PAGE gels. This protein band was estimated to comprise 10-20% of the total staining intensity. When highly enriched material was run on a Superose-12 FPLC gel filtration column, the collapsing activity was found to be eluted between apparent molecular weights of 80-160 kDa. Together our results indicate that the 100 kDa protein is likely to possess collapsing activity. The very high specific activity of our most enriched preparation is consistent with the hypothesis that collapse is mediated by a signal transduction event.

399.15

IDENTIFICATION OF A NONPERMISSIVE SUBSTRATE PROTEIN IN THE PLASMA MEMBRANE OF MAMMALIAN BRAIN AND SPINAL CORD. L. Quan, E. Ling, L.M. Jordan and K.W. Cheng*. Spinal Cord Res. Ctr., Dept of Physiology, Univ. of Manitoba, Winnipeg, Manitoba, Canada, R3E 0W3.

In higher vertebrates, lesions in the central nervous system (CNS) are irreversible due to the lack of regenerative growth from the injured axons. Recent studies have identified neurite outgrowth inhibitory proteins from rat CNS myelin, and glycoproteins of growth cone collapsing activity from embryonic chick brain, posterior somite and optic tectum. Using cultures of neuroblastoma X glioma hybrid NG108-15 cells as an *in vitro* neurite outgrowth assay we have identified a nonpermissive substrate protein from cell-surface plasma membranes, isolated from adult rat brain and spinal cord. The enriched plasma membrane fraction was prepared by homogenizing tissues in isotonic buffer, followed by ultracentrifugation on a sucrose step gradient. Upon extraction under alkaline conditions of NH_4OH, a solubilized protein fraction was obtained for coating dishes as substrate for cell cultures. Precoating with the solubilized membrane proteins from rat CNS not only prevented cell adhesion and differentiation of NG108-15 cells, which remained undifferentiated as clusters of round cells even after 24 hrs, but was also nonpermissive for primary cultures of fetal rat brain cortical neurons, which formed clumps and adhered only loosely upon further culture. This solubilized inhibitory protein was partially purified by gel filtration on Sepharose CL-4B, and by adsorbing onto DEAE-cellulose, followed by elution with 0.2 M NaCl. Upon basic polyacrylamide gel electrophoresis (PAGE) and SDS-PAGE, the inhibitory activity appeared to be an acidic protein of Mr 50-70 KDa. A similar acidic protein of nonpermissive substrate activity was also observed in extracts of rabbit brain and spinal cord. These results indicate that cell-surface plasma membranes of the mammalian CNS is a nonpermissive substrate for neuritogenesis, and an acidic membrane-protein is responsible, totally or partly, for the inhibitory effect.

399.16

ARE INDUCED ACTION POTENTIALS THE TRIGGER FOR GROWTH CONE COLLAPSE IN SYMPATHETIC PREGANGLIONIC NEURONS?. C.B.McCullum, J.J.Walker, R.I.Hume*, Department of Biology, University of Michigan, Ann Arbor MI 48109.

When the growth cones of chick sympathetic preganglionic neurons (SPNs) contact dorsal root ganglion neurons (DRGs) *in vitro*, the growth cones exhibit rapid, oscillatory increases in calcium and collapse. One possible mechanism for this pattern of calcium change is that contact with DRGs induces the generation of action potentials in the SPN. The depolarizing phase of the action potential might create a brief, rapid influx of calcium that would oscillate with the frequency of induced action potentials. To test this hypothesis, SPNs were retrogradely labelled with DiI *in vivo*, dissociated into single cells, and plated at low density on a laminin substrate. After 16 to 32 hours, the culture medium was replaced with a standard recording medium and whole cell current-clamp recordings were made from SPNs. These neurons had stable resting membrane potentials and did not spike spontaneously. Once a stable resting potential was established, a DRG was manipulated onto the SPN growth cone and the SPN membrane voltage was monitored for the occurrence of action potentials. Our major finding was that in recordings made at either room temperature or at 33° to 37°C, we never detected voltage changes from baseline levels during the ten to twenty minutes of contact with a DRG. This failure to observe action potentials was not due to compromised cell integrity, since action potentials could be evoked in all cells studied. These results suggest that the previously observed calcium oscillations were not due to DRG induction of action potentials in the SPN and indicate that other mechanisms for increasing intracellular calcium should be explored.

399.17

PRODUCTION OF ANTIPROLIFERATIVE AND NEURITE-MODULATING FACTORS BY THE CG4 CELL LINE OF O-2A PROGENITORS. S. Takayama, S. Varon* and J.C. Louis. Department of Biology 0601, University of California, San Diego, La Jolla CA 92093.

CG4 is a permanent cell line of oligodendrocyte-type 2-astrocyte progenitor cells (O-2A) that can be continuously maintained in the proliferative stage or differentiated to oligodendrocytes (OC) (Louis et al., *J. Neurosci. Res.* 1992, 30:193-204). The availability of the CG4 line offers a unique opportunity to analyze which factors are produced by O-2A progenitors and their derivatives. Here, we report evidence that culture media conditioned by CG4 cells contain i) an activity that inhibits their own proliferation and ii) an activity that modulates the neuritic outgrowth of CNS and PNS neurons. The antiproliferative activity is present in the media conditioned by CG4 cells that are expanded as undifferentiated O-2A progenitors, as well as by CG4 cells induced to differentiate to OC. The inhibition of mitotic activity is nearly complete and fully reversible, and is accompanied by the conversion of the CG4 cells' phenotype from $A2B5^+/O4^-$ progenitors to $A2B5^-/O4^+$ pro-OC. The production of the growth inhibitory activity does not depend on the nature of the mitogen used to propagate the source CG4 cells (medium conditioned by the B104 neuroblastoma cells, PDGF or FGF) and similarly, is not dependent on which mitogen is used in the test CG4 proliferation assay. These observations indicate that O-2A cells constitutively secrete and remain responsive to an autocrine antiproliferative factor. Cultures of newborn rat hippocampal neurons and E8 chick ciliary ganglion neurons, were used to determine that after conversion to OC, CG4 cells also release into the medium a polyornithine/laminin-binding neurite-promoting activity for CNS as well as PNS neurons. A preliminary characterization by gel filtration and cation-exchange chromatography, indicates that the neurite-modulating activity resides in a heat-stable, strongly acidic fraction of Mr > 50 kDa.

Supported by NINDS grant NS-16349.

400.1

SIMULTANEOUS MULTI-SITE RECORDING OF ELECTRICAL ACTIVITY AND INTRACELLULAR CALCIUM IN PATTERNED NEURONAL NETWORKS. A. Kawana*, H.P.C. Robinson, Y. Jimbo, E. Maeda and K. Torimitsu., NTT Basic Res. Labs., Musashino, Tokyo, 180 Japan

To study signal transmission and processing in large assemblies of neurons, patterned networks in which connections may be localized or even specified would be advantageous. We have already developed an effective method for the formation of organized neuronal networks in culture (A. Kawana et al; Soc. for Neurosci.,1991). Here, we describe simultaneous multichannel recording of the extracellular electrical activity and intracellular calcium concentration in such simplified neuronal networks.

A silica glass substrate was employed with 16 or 60 transparent electrodes in wells which were connected by microgrooves. A metal mask with holes at positions corresponding to the substrate wells was used to locate rat hippocampal or cerebral cortex neurons selectively in the wells. Intracellular calcium concentration was measured using fluo 3 and an Ar laser(488 nm) for excitation whose light was fed to the optical microscope by an optical fiber. Under low magnesium conditions, intermittent bursts of action potentials were detected from multiple substrate electrodes. Intracellular calcium concentration transients were synchronized to the electrical activity. The period of bursting could be controlled by single-site electrical stimulation through individual substrate electrodes. By plating different types of neurons in different wells, specific types of synapses were reconstituted. This method offers a promising approach for the study of information processing in multi-neuron networks.

400.2

OPTICAL MAPPING OF NEURAL RESPONSE PATTERNS TO GLOSSOPHARYNGEAL NERVE STIMULATION IN THE EMBRYONIC CHICK BRAIN STEM. T. Sakai, Y. Momose-Sato, K. Sato, A. Hirota and K. Kamino*. Department of Physiology, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113, Japan.

In an effort to understand the functional organization/architecture of the brain stem, we have used multiple-site optical system and a voltage-sensitive merocyanine-rhodanine dye (NK2761) to record neural activity in the embryonic chick brain stem. The intact and slice preparations including the brain stem and glossopharyngeal nerve were dissected from 7- and 8-day old chick embryos and stained with the dye. When a brief electrical stimulation was applied to the glossopharyngeal nerve, optical signals were evoked in the brain stem. They were recorded simultaneously from 127 contiguous sites using a 12 x 12-element photodiode array. In the evoked optical signals, two components, a fast spike-like signal and a slow signal were identified. The fast signal reflected action potentials, and the slow signal revealed glutaminergic excitatory postsynaptic potentials. Based on these results, we have constructed maps representing the spatial pattern of the neural response, and we have suggested a possible embryonic origin of the functional organization of the nucleus of solitary tract in the embryonic chick medulla oblongata. Supported by The MESC of Japan and The Mitsubishi Foundation.

400.3

EXPRESSION OF NITRIC OXIDE SYNTHASE IN THE DEVELOPING VISUAL SYSTEM OF THE CHICK. C.V. Williams*, D. Nordquist and S.C. McLoon. Dept. Cell Bio,Univ. of Minnesota, Minneapolis, MN 55455.

Normal function of the mature visual system requires precise patterns of axonal connections between the retina and visual nuclei. Development of this precise pattern involves formation of a rough prepattern followed by refinement in the pattern. Evidence suggests this refinement process involves communication from the postsynaptic cell to presynaptic elements. The neurotransmitter nitric oxide (NO) has characteristics that would allow it to participate in this retrograde communication. This study examined the expression of NO in the developing chick tectum to determine whether it might be present in cells upon which retinal axons terminate. Diaphorase histochemistry was used to reveal the presence of nitric oxide synthase (NOS), the enzyme responsible for NO production. The expression of NOS by cells in the developing tectum coincided spatially and temporally with the arrival of retinal axons in the tectum. NOS expression reached a peak in the tectum at the time that refinement of the initial pattern of connections is occurring. Anterograde labeling of retinal axons with WGA/HRP showed that the processes of NOS positive cells in the tectum were coincident with the region of retinal axon termination. Diaphorase histochemistry with anophthalmic chick embryos showed that NOS expression is dependent on the presence of retinal axons. Northern blot analysis using a cDNA probe for NOS from rat brain (Bredt et al., 1991) verified the histochemical results. These results in the developing chick suggest that retinal axons interact with tectal cells that express NO which is consistent with the possibility that NO has a role in the refinement of the pattern of retinotectal connections.

400.4

THE FORMATION OF THE TOPOGRAPHICAL RETINO-TECTAL PROJECTION: A TWO-DIMENSIONAL NEURAL NETWORK MODEL S.E. Hua, L.L. Massone*, and J.C. Houk Dept of Physiology, Northwestern Univ., Chicago, IL 60611.

The topographical projection from the retina to the tectum is thought to form by two mechanisms. First, a gross topographical map is formed by axon guidance and position cues, and later a refined map is formed by correlated activity in the retina. We have simulated the formation of a topographical retino-tectal map by constructing a two-dimensional neural network based on correlated activity, Hebbian modifiable synapses, and adhesive marker gradients. We first show that in a two-dimensional model of the retina and the tectum, two gradients of adhesive markers are necessary to establish the correct orientation of the map in both axes. The model also shows specificity as well as plasticity in the connections when retinal rotation and hemi retinal ablation experiments are simulated.

Recent evidence has shown that temporal retinal fibers are repelled by posterior tectal membranes while nasal retina shows no preference. To test whether a hemi-retinal adhesion difference is enough to generate a topographical map, we constructed a neural network without gradients which specified that the temporal retina is less attracted to the posterior tectum and the dorsal retina is less attracted to the medial tectum while the nasal and ventral retinas show no preference. We show that a topographical map is formed, and under certain conditions, the map can form more rapidly than the map formed using the two dimensional gradient model. Thus the retina and tectum need not be specifically labeled by two gradients. Instead, hemi-retinal and hemi-tectal differences in adhesion or repulsion that separate each surface into quadrants of differential adhesion/repulsion provide enough positional information to allow proper map formation.

400.5

THE CHOLINERGIC SYSTEM IN GOLDFISH TECTUM MAY BE NECESSARY FOR RETINOTOPIC SHARPENING **W.M. King* and J.T. Schmidt, Dept. Biol. Sci., SUNY Albany NY 12222**

The cholinergic feedback loop in tectum, which mediates a presynaptic augmentation of retinotectal transmitter release, was either removed using the cholinergic neurotoxin AF64A or blocked using nicotinic antagonists. Intracranial injection (IC) of AF64A (30-130 nmoles) resulted at one week in the selective elimination of the cholinergic-mediated polysynaptic field potential following optic nerve shock with monosynaptic transmission unaffected, and in the loss of virtually all immunostaining for choline acetyltransferase (ChAT). ChAT staining later recovered in some deep cell bodies but not in the fibers of SFGS, the retinal terminal layer. IC injection of AF64A at 18 days postcrush, when regenerating retinal fibers are beginning to form synapses, resulted in unsharpened projections when mapped at 60-80 days. There was a rough retinotopic map but the multiunit receptive fields (MURFs) recorded at each tectal point averaged 34° vs 11° in vehicle-injected control regenerates. AF64A treatment before nerve crush also blocked sharpening, ruling out a direct effect on retinal growth cones or fibers, as AF64A rapidly decomposes.

IC infusion of α-Bungarotoxin (αBTX) during regeneration also prevented sharpening (MURFs of 29.4°). Pure αBTX does not, however, substantially block the polysynaptic component, the carbachol augmentation of the monosynaptic field potentials or the carbachol depolarization of the optic nerve terminals, as curare (10μM) does. Neuronal Bungarotoxin (nBTX), a possible contaminant of αBGT, blocked the polysynaptic component, but did not fully block the carbachol augmentation of field potentials. We are still determining if and to what degree αBTX or nBTX prevent sharpening by blocking the cholinergic system. Supported by NEI grant EY-03736.

400.6

FUNCTIONAL MAP FORMS BEFORE ANATOMICAL MAP IN THE CORTICORUBRAL PATHWAY OF KITTENS. W.-J. Song*, K. Okawa, H. Oikawa+, M. Kanda+, M. Kanda+, K. Mikatsuki+, T. Ohno and F. Murakami, Dept. Biophys. Engn., Fac. Engn. Sci., Osaka Univ., Toyonaka 560, Japan. + Abrahi Labs., Shionogi & Co., Ltd., Shiga, Japan.

The development of topographic maps has been studied in many regions of the CNS. For technical reasons, however, the development of maps of functional synaptic connection has rarely been an object of study. We studied this issue using the corticorubral (CR) system of the cat as a model. CR axons elaborate their terminal arbors during the first postnatal month, and adult-like topographic distribution of the axons within the red nucleus is observed only after postnatal day (P)13 (Higashi et al., 1990). Here we carried out intracellular recording from rubrospinal neurons of P1-P28 kittens and examined monosynaptic EPSPs evoked from different sites of the sensorimotor cortex, under Nembutal anesthesia. As expected from the anatomical results, most (26/28) rubrospinal cells in kittens aged P13-P28 showed responses indicating the presence of adult-like functional maps: cells antidromically activated only from C1 and those both from C1 and L1 receive inputs mainly from the forelimb and hindlimb cortical regions, respectively. However, against what is expected from the anatomical results, cells in kittens aged P1-P10 showed similar results to the older animals' (50/60). These findings suggest that the basic feature of excitatory connectional map in the CR system is established at birth, before the maturation of CR axons and before clear topography of the axons is observable.

400.7

SEGREGATION OF SPINOCEREBELLAR MOSSY FIBER TERMINALS IN +/*LC* MUTANT MICE. M.W. Vogel* and J. Prittie.
MD Psychiatric Research Center, Baltimore, MD 21228.

Spinocerebellar mossy fiber afferents segregate during early postnatal development into sagittal columns. Studies of other mouse mutants and x-irradiated rats (Nunes et al, 1988. J. Comp. Neurol. 273:120-136) suggest that Purkinje cells provide cues for the parasagittal sorting of these afferents. We have examined the distribution of spinocerebellar mossy fiber terminals in the +/*Lc* mutant to determine if mossy fiber terminal segregation is affected by the death of +/*Lc* Purkinje cells during the early weeks of postnatal development.

The distribution of spinocerebellar mossy fibers in 1 juvenile (P38) and 4 adult (P>90) +/*Lc* mutants and 1 juvenile (P44) and 2 adult wild type mice (P>90) was visualized by injecting 2% WGA-HRP into the anterior lumbar region of the spinal cord (T13-L2). Cerebella were subsequently processed for TMB histochemistry.

Although the granule cell layer is reduced in size in +/*Lc* mutants due to increased granule cell death, the distribution of spinocerebellar mossy fiber terminals is similar to that seen in wild type mice. Lumbar mossy fiber terminals predominate in anterior and posterior vermis. While mossy fiber terminal columns are present, they are less distinct than in the wild type. In some areas of the granule cell layer there are clear gaps between regions of dense terminal fields. In other areas, terminal fields are separated only by regions with less dense staining. The poorly defined columns in the +/*Lc* mutant may be explained either by a failure to form well segregated columns due to +/*Lc* Purkinje cell death, or by the merging of well formed columns subsequent to granule cell death. (Supported by NARSAD and a NIH Shannon Award.)

400.8

SYNAPTIC REPLACEMENT IN THE INTERPEDUNCULAR NUCLEUS. T. Graessle and M. Murray.* Department of Anatomy and Neurobiology. Med. Coll. PA., Philadelphia, PA 19129.

The interpeduncular nucleus (IPN) receives its major projections from the medial habenulae via the fasciculi retroflexi (RF). Substance P (SP) neurons project to the lateral subnuclei and the cholinergic neurons to the intermediate and central subnuclei. The SP projection is present at birth; the cholinergic projection develops in the second postnatal week. We compared the synaptic populations in these subnuclei in control IPN and in IPN at least 8 weeks after bilateral FR lesions made in adult or 10 day old rats. The lateral subnuclei contain simple synaptic contacts, about 1/3 of which have dense core vesicles (DCV). The number of terminals with DCVs is decreased after adult but not after neonatal lesions. There are few changes in the terminal population in the central subnucleus after FR lesion at either age. The intermediate subnuclei contain large numbers of crest synapses, normally formed by one terminal from the left and one from the right habenula. Similar numbers of crest synapses are present after bilateral FR lesions in adults but fewer develop when FR is lesioned neonatally and they are likely to be morphologically aberrant. Our results are consistent with reactive reinnervation, but the pattern of synaptic replacement differs depending on whether the lesion is made in neonates or adults. Supported by NS-16556.

400.9

DEVELOPMENTAL REGULATION OF LOW AFFINITY NGF RECEPTORS IN THE HABENULO-INTERPEDUNCULAR SYSTEM IN THE RAT. **K.S. Bozek, F. Haun, A.F. Pimenta* and M. Murray. Dept. of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129.**

Part of the habenular projection to the interpeduncular nucleus (IPN) is nicotinic cholinergic and therefore may be subject to regulation by NGF internalized via membrane bound NGF receptors. Immunocytochemical studies indicate that cholinergic habenular neurons innervate their target subnuclei in the IPN in the second postnatal week, the only period during which lesion induced sprouting can be demonstrated. This suggests that either NGF production or NGF receptors might be developmentally regulated in this system. We therefore used immunohistochemistry to examine the distribution and expression of low affinity NGF receptors (antibody 192, a gift from Dr. Eugene Johnson) during the development of the IPN in the rat. At postnatal day 4, NGF receptor staining is present on axons in the pathway from the habenula that innervates the IPN but is absent in the IPN. By 10 days, recognizable immunoreactivity appears in the intermediate subnuclei of the IPN, the subnuclei receiving densest cholinergic innervation from the habenula. This staining reaches its maximum by 14 days. Thereafter, the receptor staining decreases and is no longer detected by day 28. These observations indicate that habenular axons express the low affinity NGF receptor transiently during the time that they innervate their targets.
Supported by NIH Grants NS16556 (MM) and NS28856 (FH).

400.10

DEVELOPMENT OF AXONAL AND DENDRITIC ARBORIZATIONS IN THE DORSAL HORN OF THE EMBRYONIC RAT SPINAL CORD. I Silos-Santiago* and W.D. Snider. Dept. Neurology, Washington University Medical School, St. Louis, MO, 63110.

Both the axonal and dendritc arborizations that comprise the circuits of the mammalian dorsal horn have been carefully studied with classical neuroanatomical techniques and intracellular staining. However, little is known about how this complex circuitry develops. We have approached this problem using lipid-soluble tracers to label both afferent and efferent components of dorsal horn circuits during embryonic development in the rat.

Using focal applications of DiI we labeled single primary dorsal root afferents and single interneurons in laminae I-V. The major classes of primary afferent fibers had entered the thoracic spinal cord by E17. We could clearly distinguish between fibers entering medially and projecting to laminae III-V and short fibers in lamina I and II which enter more laterally and project rostrocaudally. By E19, several classes of afferents could be recognized on the basis of characteristic morphology. These afferents were in their target fields by E19 and no evidence of rearrangement was seen through the first postnatal week. We also studied the morphology of the target neurons in the dorsal horn. By E17, we could distinguish 2 different cell types in lamina I , 4 types in lamina II, 4 types in lamina III, and 6 types in lamina IV on morphological grounds. Many of these have been observed in the adult, but we found several classes of dorsal horn neurons not previously described. In most cases, the dendritic arbors of these cells were not limited to the lamina where the soma was located.

We have shown that most of the classes of primary afferents and dorsal horn neurons described in adult animals can be recognized on the basis of characteristic morphology in embryonic rats. In contrast to other areas in the CNS, we find no morphological evidence of rearrangement during development in this system.

400.11

SPECIFICITY OF SYNAPTIC CONNECTIONS BETWEEN MUSCLE AFFERENTS AND MOTONEURONS IN THE DEVELOPING RAT SPINAL CORD. B.S. Seebach* and L. Ziskind-Conhaim. Dept. Physiol. and Ctr. Neurosci., Univ. of Wisconsin, Madison WI 53706.

Mature vertebrates have specific synaptic connections between muscle afferent fibers and motoneurons that innervate limb muscles. Motoneurons receive monosynaptic excitatory inputs from homonymous and synergistic muscles, and polysynaptic inhibitory inputs from antagonistic muscles. To determine whether specific sensory-motoneuron contacts are established during early synaptogenesis in the mammalian spinal cord, we have studied the pattern of these synaptic connections in embryonic (E20-22) and newborn rats (P0-4). Nine hindlimb nerves were stimulated and the evoked potentials were recorded intracellularly from motoneurons of isolated spinal cords. Monosynaptic potentials were distinguished from polysynaptic potentials by their shorter latency and resistance to high-frequency stimulation. Depolarizing IPSPs reversed at -50mV membrane potential. In the embryonic spinal cord, 20% of motoneurons received monosynaptic excitatory inputs from antagonistic muscles. These inappropriate connections persisted until at least 3-4 days after birth, suggesting that some reorganization of synaptic connectivity occurs in the rat during postnatal development.
Supported by RCDA (NS01314) and NS23808.

401.1

TRKAn: AN ALTERNATIVELY SPLICED FORM OF TRKA EXPRESSED BY NEURONS. P.A Barker*, C. Lomen-Hoerth, S.O. Meakin, E.M Gensch, E. M. Shooter, Department of Neurobiology. Stanford University School of Medicine, Stanford, California. 94305.

The product of the Trk proto-oncogene has been identified as a signal-transducing receptor for nerve growth factor. This tyrosine kinase receptor was originally cloned from human K562 cells, a human leukemic cell line. We have identified a variant of the TrkA receptor, TrkAn, which differs from originally cloned form of TrkA by virtue of a small insertion within the extracellular domain. Both forms of Trk receptors are found within rats and humans and the amino acid sequence of the insertion is strictly conserved between species. The inserted domain is encoded by a distinct exon whose presence appears to be regulated by tissue-specific alternative splicing. Several nonneuronal tissues contain both forms of TrkA mRNA. However, only TrkAn is expressed within CNS and PNS neurons of rat and humans.

Rat TrkA and TrkAn have been expressed in Cos cells and shown to specifically bind NGF and display slow dissociation kinetics and trypsin sensitivity characteristic of the slow NGF receptor. Although the functional role subserved by these distinct forms of the TrkA protein remain unknown, the evolutionary conservation of the inserted domain across species together with the tissue-specific expression of the alternative transcripts indicates some functional role.

401.2

COLOCALIZATION OF NGF BINDING SITES TO *TRK* AND P75-EXPRESSING CELLS IN BASAL FOREBRAIN CULTURES. J.L. Tassone, M. Yokoyama, I.B. Black, and C.F. Dreyfus.* Department of Neuroscience and Cell Biology. Robert Wood Johnson Medical School/UMDNJ. Piscataway, NJ 08854 and Rutgers College, New Brunswick, NJ 08903

A recent controversy in the neurotrophin field concerns the identity of the biologically active NGF receptor. The *trk* proto-oncogene product and the p75 molecule have been tentatively identified as NGF receptors, but the exact role of each molecule remains unclear. To approach this problem, we have localized either *trk* or p75, and NGF binding sites on cultured basal forebrain neurons dissociated from embryonic day 17 (E17) rat. *Trk* was visualized using 43-4 IgG from a polyclonal antiserum corresponding to the 14 carboxy terminal amino acid residues of gp140^{trk} (a gift of R. Klein, F. Lamballe, and M. Barbacid, Bristol Myers-Squibb Pharmaceutical Research Institute, Princeton, NJ). These antibodies can react with all *trk* receptors. p75 was visualized using 192 IgG (a gift of M. Chao, Cornell Medical College, New York, NY and E. Johnson, Washington University Medical School, St. Louis, MO). Cultures were grown for seven days in serum-containing media to allow the growth of both neurons and glia. To visualize low and high-affinity NGF binding sites, cultures were incubated in 125I-labeled NGF (.2nM). Autoradiographic and immunocytochemical techniques were employed to visualize the expression of NGF binding sites and *trk* or p75. Our results indicate that the great majority of cells which bind NGF express both *trk* and p75. We conclude that in cultured normal primary basal forebrain neurons, NGF binding is associated with expression of both *trk* and p75. (Support: NIH:HD23315, NS10259-Javits Award, Henry Rutgers Scholars Program, Rutgers College)

401.3

A QUANTITATIVE REVERSE TRANSCRIPTION - PCR ASSAY TO STUDY p75 NERVE GROWTH FACTOR RECEPTOR GENE EXPRESSION IN DEVELOPING RAT BRAIN. Chu-Kuang Chen*, Stephen Kinsman, and Michael Johnston. The Kennedy-Krieger Research Institute and Dept. of Neurology, Johns Hopkins Medical Institutions, Baltimore, MD

The action nerve growth factor (NGF) as well as other neurotrophins BDNF and NT-3 are mediated through the activation of specific receptors. Expression of these receptors occurs not only in the adult brain but also during important developmental periods. Recent investigations suggest that p75 is one component of the high affinity NGF receptor complex. Transient elevation in levels of p75 mRNA in perinatal rat cerebellum have been demonstrated using RNAse protection assay. However, a more sensitive assay will allow a more detailed examination of the ontogeny of p75 gene expression.

We developed a sensitive quantitative RT-PCR assay to study p75 message in regions of low abundance expression or where tissue availability is limited. Our assay is based on using a shortened p75 cRNA an an internal RNA standard to control for variability in reverse transcription and polymerase chain amplification of the endogenous p75 assayed. To make our method quantitative, we have examined the kinetics of the PCR reaction for two p75 templates (endogenous and shortened standard p75 cRNA). p75 mRNA levels are determined in total RNA samples by comparison of co-amplified endogenous p75 mRNA with a known amount of shortened p75 standard cRNA. We have used this assay to examine the expression of p75 during ontogeny of the cerebellum and striatum.

Our results demonstrate this assay to be roughly 1,000-fold more sensitive than current methods for assaying a low abundance message such as p75. In Sprague-Dawley rat cerebellum, p75 mRNA level was most abundant at PND 2 (2.7 pg/µg total RNA), and then declined to a lower level throughout development and in adult (<1 pg/µg total RNA). Levels of p75 mRNA are over 7-fold higher in immature P10 striatum than they are in adult striatum. This extremely sensitive assay will facilitate studies of the role of neurotrophic factors during development.

401.4

THE TRK PROTO-ONCOGENE PRODUCT p140 IS EXPRESSED BY GANGLION CELLS OF THE HUMAN RETINA. A. Merighi', G. Carmignoto, S. Ghidella', M. Lipartiti*, A Zanellato and C. Comelli. 'Dip. Morfofisiologia Veterinaria, 10100 Torino, and Fidia Research Laboratories, 35031 Abano Terme, Italy.

In the retina of different animal species including non-human primates a significant number of ganglion cells (RGCs) has been reported to express the low affinity NGF receptor (p75-NGFR). The finding that following axotomy in the adult rat the same neurons can be rescued from degeneration by intraocular administrations of NGF led to the hypothesis that RGCs represent a neuronal population of the central nervous system sensitive to the action of NGF. Since the biological activity of NGF is mediated by a high affinity membrane-bound receptor, we have studied the immunocytochemical distribution of both the trk proto-oncogene product p140, which likely corresponds to the high affinity NGFR, and the p75-NGFR in tranverse sections of human and rat retina. We found that in the human retina p75-NGFR immunoreactivity is restricted to Müller cells while a high number of neurons in the GC layer is immunopositive for the p140-trk. On the basis of morphological criteria at least part of these neurons can be classified as RGCs. Conversely, in the rat retina a sub-population of RGCs and Müller cells are immunoreactive for both antigens. Binding studies and affinity cross linking/immunoprecipitation experiments performed in purified *in vitro* preparations of RGCs from neonatal rat retinae confirm the presence of the high affinity NGF receptor. These results suggest that RGCs in humans could be sensitive to the action of NGF and raise the possibility that NGF, or a closely related molecule, could represent a new therapeutic approach for the treatment of retinal neurodegenerative pathology.

401.5

NGF GENE EXPRESSION IN MOUSE AND MAN: ROLE OF RETINOIC ACID RECEPTORS(RARs). C. Jiang*, M. Cartwright and G. Heinrich. Biomolecular Medicine, Boston University Hospital, BOSTON, MA 02118.

Retinoic Acid(RA) induces NGF mRNA in mouse L cells and high affinity NGF binding in chicken embryonic sympathetic neurons, suggesting an important role in coordinating induction of neuronal NGF dependence, responsiveness and NGF production by targets. We previously cloned the mouse, rat and recently the human NGF gene promoter regions. An intronic AP-1 element is conserved in the NGF promoter region of all species, but an upstream AP-1 consensus sequence is human specific. Gel shift analysis showed that the RAR-alpha can bind to the upstream AP-1 consensus sequence in the human NGF but not to the conserved intronic AP-1 elements in either human or mouse NGF promoters. Furthermore, the RAR-alpha suppressed binding of AP-1 complexs to all AP-1 sites. Several RA response element(RARE) like motifs are found within 1,000bp upstream of both mouse and human NGF promoters, suggesting direct binding of RARs and transcriptional regulation of NGF by RA in vivo. We are analyzing the differential binding of additional RARs(beta and gamma) to mouse and human NGF promoters and their functional significance, also beginning to address the relationship between the structurally and functionally defined RAREs and the AP-1 consensus sequences.

401.6

NGF INDUCES *C*-FOS IN THE BASAL FOREBRAIN NEURONS EXPRESSING NGF RECEPTOR (NGFR): A DOUBLE IMMUNOCYTOCHEMICAL STUDY IN THE RAT. Z.-C. Peng, S. Chen, M. Fusco°, M.G. Nunzi°* and M. Bentivoglio, Institute of Anatomy, University of Verona; °Fidia Research Laboratories, Abano Terme, Padova; Italy.

NGF (10 µg in 10 µL) was administered into the lateral cerebral ventricle in adult male Wistar rats through a previously implanted cannula. Cytochrome c was injected with the same procedure in control animals. All cases were sacrificed 3 hrs later and the brains were processed with a double immunocytochemical protocol for the visualization of *c*-Fos (using antibodies raised in sheep) and the low affinity NGFr (using monoclonal antibodies raised in mouse), exploiting two different chromogens. A considerable number of *c*-Fos-immunoreactive (ir) neurons was evident after NGF treatment throughout the basal forebrain, in contrast to the control cases in which this region was devoid of *c*-Fos-ir cells. In the areas that contain NGFr-ir neurons - including the medial septal and basal nuclei, and the horizontal and vertical limbs of the diagonal band - almost all the *c*-Fos-ir cells were also NGFr-ir. Double immunostained (NGFr-ir and *c*-Fos-ir) neurons ranged from 5% to 12% of the total NGFr-ir cell population. Single and double immunopositive cells were intermingled, and the latter were slightly more numerous on the side ipsilateral to NGF administration. After the injection of a higher concentration of NGF (20 µg in 10 µL) a striking increase of *c*-Fos-ir neurons, up to at least 30% of the NGFr-ir cell population, was observed in the basal forebrain. Altogether these data demonstrate that the administration of NGF *in vivo* elicits a selective, specific and dose-dependent activation of the basal forebrain neurons which express NGFr.

401.7

LAR TYROSINE PHOSPHATASE RECEPTOR: EXPRESSION IN THE MAMMALIAN NERVOUS SYSTEM. F.M. Longo[1] and J.M. Le Beau*[2]. Depts of Neurology, UCSF Sch. of Med[1]., S.F., CA 94143 & the Diabetes Institutes, Eastern Virginia Med. Sch[2]., Norfolk, VA 23510

The Leukocyte Common Antigen-Related (LAR) gene expresses a tyrosine-phosphatase receptor with extracellular domain sequence similarity to N-CAM. Tyrosine phosphatase receptors may influence cell growth or differentiation by modulating effects of tyrosine kinase receptors. In a previous study we demonstrated LAR expression by northern blot analysis of poly (A) RNA obtained from various brain regions during development, cultured neurons and astrocytes and PC-12 cells (Longo et al, Soc Neurosci Abst 17:762, 1991). The expression of LAR mRNA *in vivo* in the mammalian nervous system has not been reported and it is not known if neurons *in vivo* express LAR. We used sense and antisense riboprobe derived from a 2.2 kb cDNA insert corresponding to the 3' cytoplasmic domain of rat LAR to perform in-situ hybridization of adult rat brain and dorsal root ganglia. Tissue was fixed and frozen sections were probed as described by Le Beau et al (J Neurosci Res 28:299, 1991). Tissue incubated with LAR anti-sense probe showed significant hybridization compared to tissue treated with sense probe. Significant hybridization signal was associated with neurons in the following regions: cortex, cerebellum, hippocampus, striatum and olfactory tubercle. Hybridization was also associated with non-neurons such as glia. In dorsal root ganglia, significant mRNA was detected in sensory neurons and little if any mRNA was found in their corresponding satellite cells. These findings suggest that the LAR tyrosine phosphatase receptor is expressed by neurons *in vivo* in the mammalian central and peripheral nervous system. Supported by United Cerebral Palsy (1) and the Diabetes Institutes (2).

401.8

LAR TYROSINE PHOSPHATASE RECEPTOR: A NOVEL GC-RICH TRANSCRIPT EXPRESSED IN RAT CNS. J.S. Zhang and F.M. Longo* Dept of Neurology, UCSF Sch. of Med., San Francisco, CA 94143

The Leukocyte Common Antigen-Related (LAR) gene codes for a tyrosine-phosphatase receptor with extracellular domain sequence similarity to N-CAM. Tyrosine phosphatase receptors may influence cell growth or differentiation by modulating effects of growth factors acting through tyrosine kinase receptors. LAR is expressed during neural development and in rat PC-12 cells (Longo et al, Soc Neurosci Abst 17:762, 1991). While screening a rat brain cDNA library for additional LAR transcripts, we found an approx 4 kb insert suggestive of an alternative LAR transcript: rat LAR-2. Sequencing of LAR-2 revealed a 33 bp-insert just upstream from the first tyrosine phosphatase domain which maintained the open reading frame. PCR with primers flanking the potential splice junctions using genomic DNA template was consistent with 2.3 kb and 0.7 kb introns upstream and downstream of a 33 bp cassette exon. In addition, the 3' untranslated region of LAR-2 contains GC-rich repeats similar to those found in genes associated with myotonic dystrophy and fragile X syndrome in which the repeat becomes unstable. The discovery of GC-rich repeats in an LAR transcript is especially intriguing in light of recent mapping of human LAR to 1p32-33 (Streuli et al, EMBO 11:897, 1992) and deletions observed at 1p32 in neuroblastoma. In preliminary experiments, antisense (but not sense) oligonucleotides directed to LAR splice junctions inhibited NGF-induced PC-12 cell differentiation. Functional implications of alternative sequences in LAR-2 include: i) the 33 bp insert could modify activity of the nearby tyrosine phosphatase domain and ii) the alternative 3' untranslated region could result in a LAR transcript with altered stability. (Supported by United Cerebral Palsy).

401.9

DISTRIBUTION AND REGULATION OF MEMBERS OF THE *TRK* FAMILY IN THE RAT BRAIN. J.P. Merlio[1,3], P. Ernfors[1], J. Bengzon[2], M. Jaber[1], Z. Kokaia[2], B. Dufy[3]*, O. Lindvall[2] and H. Persson[1]. [1]Laboratory of Molecular Neurobiology, Karolinska Institute, S-104 01 Stockholm. [2]Restorative Neurology Unit, University Hospital, S-221 85 Lund. [3]URA CNRS 1200-Laboratoire d'Histologie-Embryologie. Université de Bordeaux II. F-33076 Bordeaux.

Tyrosine protein kinases *trk*, *trk*B and *trk*C are signal transducing receptors for the neurotrophins Nerve Growth Factor, Brain-Derived Nerve Growth Factor, neurotrophin-3 and neurotrophin-4. We have isolated cDNA fragments encoding a part of rat *trk* and *trk*B proteins respectively and characterized a full-length cDNA clone encoding rat *trk*C. Cells expressing mRNAs for the different members of the *trk* family were identified in the rat central nervous system by in situ hybridization using oligonucleotide probes designed from the isolated cDNA sequences. The expression of *trk* mRNA was found to be restricted to neurons of the basal forebrain, caudate putamen with features of cholinergic cells and magnocellular neurons of several brainstem nuclei. In contrast, cells expressing *trk*B and *trk*C mRNAs were widely distributed in many areas of the brain, including olfactory formations, neocortex, thalamic and hypothalamic nuclei, brainstem nuclei, cerebellum and spinal cord motoneurons.Comparison between our data and previous analyses of cells expressing mRNAs for neurotrophins and the low-affinity NGF receptor suggests that different mode of action and different combination of receptors mediate biological responses to neurotrophins in the adult rat brain. Moreover, seizures induced by hippocampal kindling lead to a rapid and transient increase of mRNA in the hippocampus for *trk*B, a functional receptor for BDNF. No change was seen in mRNAs for *trk* or *trk*C, components of the high-affinity NGF or NT-3 receptors, respectively. The increase of *trk*B mRNA was blocked by the AMPA receptor antagonist NBQX. The transient increase of *trk*B mRNA showed the same time course and distribution as previously reported increases of BDNF mRNA. This suggests that BDNF and its receptor could play a local role within the hippocampus in kindling-associated neural plasticity.

401.10

NEUROTROPHIN RECEPTORS IN THE ADULT RAT BRAIN: RELATIVE DISTRIBUTIONS OF THE LOW-AFFINITY NGF RECEPTOR (LNGFR) AND MEMBERS OF THE *TRK* FAMILY OF TYROSINE KINASE RECEPTORS. S.J. Wiegand,* P. Wright, C. Alexander, L. Pan, R.M. Lindsay, G.D. Yancopoulos and N.Y. Ip. Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591.

The neurotrophins, NGF, brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), appear to bind with similar affinities to the LNGFR, but with markedly different affinities to the *trk* family of tyrosine kinase receptors. NGF binds to the product of the *trk* proto-oncogene (trkA), while BDNF and NT-3 are ligands for the related trkB and trkC receptors. While the relative contributions of the trks and the LNGFR to binding affinity and specificity and to signal transduction are now being elucidated *in vitro*, little is known regarding the co-distribution of LNGFR and trk receptors *in vivo*. We have examined the relative distribution of cells expressing these different neurotrophin receptors in the adult rat brain using *in situ* hybridization and immunohistochemistry. LNGFR message and protein are present in relatively restricted sets of neural and non-neural elements. trkA mRNA appears to be expressed predominantly if not exclusively in neurons, and shows a close correspondence in its neuronal distribution to that of LNGFR. However, non-correspondence of expression of trkA ,mRNA and LNGFR is apparent in some brain areas. In agreement with retrograde transport and binding studies, cells expressing trkB mRNA are much more numerous and widely distributed than those which express either trkA or LNGFR. Most neurons which express trkB did not contain detectable levels of LNGFR mRNA or protein.

401.11

DISSECTION OF NGF SIGNALLING PATHWAYS BY PERTURBATIONS OF LOW-AFFINITY RECEPTOR ($gp75^{NGFR}$) INTERACTIONS. S.M. Dostaler, R.A. Murphy*, and R.J. Riopelle*. Queen's University, Kingston, Ontario, Canada K7L 3N6, and *University of Alberta, Calgary, Alberta, Canada, T2N 2T2.

Growth factors share many signalling events but different biological effects indicate that signalling routes diverge. NGF signal transduction selectivity likely begins at the receptor level because this growth factor interacts with two receptors. To address this suggestion, a peptide (R3) identical to a cytoplasmic domain of $gp75^{NGFR}$ with predicted amphiphilic properties (Myers et al., Soc. Neurosci. Abstr. 17, 1498, 1991) was coincubated with NGF using a PC12 pheochromocytoma target. R3 accelerated NGF-mediated neurite growth in a time frame consistent with its uptake into cells. The wasp venom, mastoparan (MP), a cationic amphiphilic peptide, did not influence NGF-mediated neurite growth, but both R3 and MP accelerated NGF-mediated $cfos^{mRNA}$ induction in these cells. R3 did not influence the effects of NGF on PC12 cell mitochondrial succinate MTT reductase. These observations indicate that the R3-like domain of $gp75^{NGFR}$ is involved in neurite growth but not in mitochondrial respiratory activity induced by NGF. Further, while $cfos^{mRNA}$ induction involves an interaction with $gp75^{NGFR}$, this event is not essential for neurite growth. The present studies suggest that at least some of the specificity of NGF signalling subserving neurite growth occurs at the level of one of its receptor monomers, and that further downstream signal divergence occurs. This signalling may involve interactions with both or either of two proteins (70 and 90 kDa) that can be detected in extracts of PC12 cells using R3 as an affinity probe, and thus, likely interact with the amphiphilic domain of $gp75^{NGFR}$. Supported by the Canadian Federal Networks of Centres of Excellence Program in Neural Regeneration and Functional Recovery.

401.12

OVEREXPRESSION OF THE CYTOPLASMIC DOMAIN OF $p75^{NGFR}$ RESULTS IN DOWN-REGULATION OF $p140^{trk}$ TYROSINE KINASE ACTIVITY. M. BENEDETTI[+], A. LEVI[+], and M.V. CHAO*. [+]Istituto di Neurobiologia, CNR, Rome, Italy 00157; Dept. of Cell Biology & Anatomy, Cornell University Medical College, New York, New York 10021.

The low affinity p75 NGF receptor participates in the formation of high affinity NGF binding sites with the product of the trk proto-oncogene. To further investigate the function of $p75^{NGFR}$ in the NGF signal transduction pathway, a mutant $p75^{NGFR}$ was engineered, containing the transmembrane and cytoplasmic domain of $p75^{NGFR}$, 3' to an exogenous signal peptide and a myc tagging epitope. This truncated receptor lacked the ligand binding domain. When stably transfected in PC12 cells, several independently selected clones displayed altered responses to NGF. Most notably, in cells overexpressing this construct, trk tyrosine kinase activity was markedly lower. Also, the products of the VGF8a, tyrosine hydroxylase, and $p75^{NGFR}$ genes were lower than parental cells. Although the transfected cells were still capable of differentiation by NGF, there were no detectable high affinity binding sites. These results suggest that the truncated receptor may disrupt an interaction between $p75^{NGFR}$ and $p140^{trk}$ that prevented correct formation of high affinity binding sites, and resulted in down-regulation of the trk tyrosine kinase.

401.13

EVIDENCE FOR KINASELESS AND ALTERNATE 5' TERMINAL FORMS OF TRKB AND TRKC IN CHICK. A. S. Garner* and T. H. Large, Department of Neuroscience, Case Western Reserve University, Cleveland, OH 44106.

The *trk* family of tyrosine kinase receptors mediate neurotrophin-induced responses as diverse as proliferation, survival, and process outgrowth. Alternative splicing is one potential mechanism for generating diversity at the level of ligand binding and/or signal transduction. To examine possible splice variants and as a prelude to investigating the role of *trk* receptors in the developing chick visual system, we have isolated cDNA clones of chick *trk*B (ch*trk*B) and *trk*C (ch*trk*C).

An E13 chick brain cDNA library, screened initially with a mouse *trk*B cRNA probe, yielded a total of 23 ch*trk*B and 16 ch*trk*C clones. Amino acid alignment of a full length ch*trk*B clone with mouse *trk*B identifies conserved domains that are likely to be functionally important. Within the extracellular domain, the second immunoglobulin-like domain is strongly conserved (85%). The *trk*B juxtamembrane domain (between the transmembrane and kinase domains) is also highly conserved (98%) and may be the principal regulator of kinase activity and/or specificity, especially given the small size of the C-terminus (15 aa) and kinase insert (11 aa) domains.

Alternate 5' and 3' splice variants of ch*trk*B have also been identified. Similar to those reported for rat and mouse, a kinaseless ch*trk*B clone is truncated just after the transmembrane domain and the 11 alternatively spliced C-terminal amino acids are completely conserved. In alternate 5' terminal clones (ch*trk*B-Δ5'), the start ATG, signal sequence and first cysteine cluster are replaced with a sequence that contains an upstream stop signal. This sequence is also found in the 5' untranslated region of the full length clone and is flanked by a splice donor site, indicating ch*trk*B-Δ5' results from the splicing of 5' untranslated sequence into the extracellular domain.

We have also identified full length, kinaseless, and alternate 5' terminal clones of ch*trk*C. As with ch*trk*B, ch*trk*C-Δ5' clones replace the start ATG, signal sequence, and first cysteine cluster with a novel sequence encoding an upstream, in-frame stop. Alternate 5' transcripts lacking a start ATG may represent an unusual method of regulating expression. Alternatively, translation may be initiated at a downstream ATG or non-ATG codon, resulting in a *trk* receptor with a truncated extracellular domain.

401.14

TRANSDUCTION OF THE trkA GENE BY A DEFECTIVE HSV-1 VECTOR INTO CULTURED NODOSE GANGLION NEURONS RENDERS THEM NGF RESPONSIVE. H.Xu*, L.Parada#, H.J.Federoff and J.A.Kessler, Albert Einstein College of Medicine, Bronx, NY 10461, #NCI-FCR, Frederick, MD 21702.

The gp140^{trk} (trk) gene encodes the signaling tyrosine kinase component of the NGF receptor (Kaplan et al., Science 252:554,1991) that upon stimulation phosphorylates neuronal substrates that transduce the NGF signal. As a first step in exploring the generality of the neurotrophin transduction mechanism, we have examined whether the expression of trkA could convert NGF unresponsive nodose ganglion neurons into NGF responsive neurons. A full length rat trk cDNA was cloned into a defective HSV-1 vector (pHSVtrkA), packaged into virions and used to infect primary cultures of neonatal nodose neurons. Infections were carried out for 8 hours in the presence of BDNF (10 ng/ml), after which time the virus and BDNF were removed and replaced with NGF (10 ng/ml)-containing media. Neuron survival was determined on day 1 through 6 after infection with pHSVtrkA virus. Survival in cultures infected with pHSVtrkA virus and treated with NGF was 56.1% ± 5.3 on day 6, whereas survival in uninfected cultures was only 10.8% ± 3.0. Survival of neurons cultured continuously in BDNF was 61.1%± 6.9. Each percentage represents the mean of 7 experiments. These data indicate that the transduction of the trkA gene into nodose neurons renders them NGF responsive. Moreover, the ability of trkA to confer NGF responsiveness suggests that the neuronal substrates required for transduction of the NGF signal are found in neurons that are not normally NGF responsive.

401.15

DORSAL ROOT GANGLION NEURONS EXPRESSING TRK ARE SELECTIVELY SENSITIVE TO NGF DEPRIVATION IN UTERO. S.L. Carroll, K. Ruit, S.E. Frese, J. Milbrandt and W.D.Snider*, Departments of Pathology, Laboratory Medicine, and Neurology, Washington University School of Medicine, St. Louis, Mo. 63110.

In utero immune deprivation of the neurotrophic molecule nerve growth factor (NGF) results in the death of most, but not all, mammalian dorsal root ganglion (DRG) neurons. The recent identification of trk, trkB, and trkC as the putative high affinity receptors for NGF, brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT3), respectively, has allowed an examination of whether their expression by DRG neurons correlates with differential sensitivity to immune deprivation of NGF.

NGF deprivation in fetal rats was produced by injecting a specific, high titer anti-NGF antibody into embryonic rats in utero (Ruit et al, Neuron 8:573-587). We then performed in situ hybridization on DRG sections from control and experimental animals with probes for trk, trkB, trkC, and p75NGFR, the low affinity NGF receptor. We have found that more than 90% of DRG neurons expressing trk are lost when deprived of NGF in utero. In contrast, most, if not all, neurons expressing trkB and trkC survive this treatment. The low affinity NGF receptor, p75NGFR, is expressed in both NGF deprivation-resistant and sensitive neurons.

These experiments show that DRG neurons expressing trk require NGF for survival and that DRG neurons which do not require NGF express the high affinity receptor for another neurotrophin. Furthermore, they provide evidence that trk, and not p75NGFR, is the primary effector of NGF action in vivo.

401.16

EFFECTS OF NGF ON RAT PERITONEAL MAST CELLS—SURVIVAL PROMOTION AND IMMEDIATE EARLY GENE INDUCTION. K. Horigome, P.A. Lampe,* and E.M. Johnson, Jr. Department of Molecular Biology & Pharmacology, Washington University School of Medicine, St. Louis, MO 63110

Rat peritoneal mast cells (RPMC) express only one of the NGF receptor molecules, p140*trk* (see Pryor *et al.*, accompanying abstract). In analogy to the effects of NGF on neuronal cells or PC12 cells, we determined whether NGF or other neurotrophins were able to promote the survival of RPMC *in vitro*.

When RPMC were maintained in 10% FCS/MEM, all the cells died within 6 days. NGF prevented the death of the vast majority of RPMC in a dose-dependent manner (EC_{50} = 1 nM). The antimitotic drug, FUdR, had no effect on cell numbers remaining after 6 days in culture and [^{3}H]-thymidine was not significantly incorporated into RPMC; therefore, NGF promoted the survival but not the growth of RPMC. The activity of conditioned medium prepared after 3-day cultivation was neutralized by anti-NGF antiserum. The immediate early genes, c-*fos* and NGFI-A, were also induced in RPMC with a timecourse similar to that seen in PC12 cells. Other neurotrophins, BDNF and NT-3, had no effect on RPMC survival or gene expression.

The secretory response of RPMC to NGF was completely dependent on a specific phospholipid, lysophosphatidylserine (lysoPS) *in vitro*. Although the dose dependency and ligand specificity of the secretory, survival-promoting, and gene-expression responses to NGF were very similar, only the secretory response required lysoPS. This suggests a difference in the signal transduction pathways leading to the secretory response and the survival-related responses, although they were all triggered by the same tyrosine kinase receptor, *trk*. (Supported by NIH grant NS24679 and the Sumitomo Chemical Company.)

401.17

MAST CELLS EXPRESS *TRK* BUT NOT LOW AFFINITY NERVE GROWTH FACTOR RECEPTOR. J.C. Pryor, K. Horigome, and E.M. Johnson, Jr.* Departments of Molecular Biology & Pharmacology and Neurological Surgery, Washington University School of Medicine, St. Louis, MO 63110.

The physiologic response to nerve growth factor (NGF) is best understood in sympathetic and sensory neurons and PC12 cells. Rat peritoneal mast cells (RPMC) release histamine in response to NGF when lysophosphotidylserine is present. We have studied the expression of the LANGFR and *trk* in mast cells to understand the receptor(s) mediating the physiologic response to NGF in these cells.

Total RNA was isolated from RPMC, non-mast peritoneal cells (non-RPMC) and PC12 cells; Northern blots were done with probes to *trk* and LANGFR. These blots showed a strong signal at the appropriate length for *trk* in mast cells and PC12 cells, but a very low signal in the non-RPMC fraction. A strong message was detected for LANGFR in PC12 cells, but no signal was detected in mast cells or non-RPMC. RNA from mast cells, PC12 cells, and superior cervical ganglion sympathetic neuron culture (SCG) were reverse transcribed and PCR was performed by using oligonucleotides specific for *trk* and LANGFR. Message for *trk* was stronger in the mast cell fraction than in PC12 cells; the signal in the non-RPMC was weak. A strong signal for LANGFR was detected in PC12 cells and SCG cultures, but none was seen in mast or non-RPMC fractions. Radioiodinated NGF was cross-linked to mast cells and PC12 cells; the solubilized receptor NGF complex was immunoprecipitated with polyclonal anti-NGF or monoclonal anti-LANGFR. Bands consistent with LANGFR were demonstrated with PC12 cells, but were not observed in mast cells. Our results strongly suggest mast cells have only one NGF receptor species, *trk*, yet are capable of responding to NGF with a physiologic response. This immunoregulatory function of NGF does not appear to require interaction between the *trk* proto-oncogene and LANGFR in mast cells. (Supported by NIH grants NS24679 and NINDS 5 T32 NF07205-10, and the Sumitomo Chemical Company.)

401.18

HIGH-AFFINITY NGF RECEPTORS ON C6 ASTROCYTOMA CELLS: GROWTH-DEPENDENT EXPRESSION. A. Zanellato, L. Facci*, R. Dal Toso and S.D. Skaper. Fidia Research Labs, Abano Terme, Italy

Both neurons and glia present receptors for nerve growth factor (NGF). Rat C6 astrocytoma cells, which contain low-affinity NGF receptors, were used to study the possible expression of high-affinity NGF receptors. NGF receptors crosslinked with ^{125}I-NGF revealed both molecular forms of the receptor, upon immunoprecipitation using anti-NGF antibodies. Phosphotyrosine antibodies labeled the high-affinity receptor on immunoblots after a 5-min treatment of cells with NGF. Antibodies against low (IgG 192) and high (gp 140trk) affinity NGF receptors showed a largely overlapping, albeit heterogeneous, distribution of immunoreactivity for the two antigens in non-synchronized C6 cells. Exposure of growth-arrested C6 cells to serum demonstrated anti-trk immunoreactivity that was localized to immediate nuclear, plasma membrane or cytoplasmic regions as a function of cellular growth state, suggesting the presence of different maturational (glycosylated) forms of the trk protein. This in vitro model may help in delineating regulatory elements of NGF receptor expression.

401.19

EVIDENCE FOR THE EXPRESSION OF FUNCTIONAL LOW AFFINITY AND trkA NGF RECEPTORS IN CULTURED HIPPOCAMPAL NEURONS. V. L. Smith-Swintosky* and M. P. Mattson. Sanders-Brown Research Center on Aging and Department of Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536.

There is considerable evidence that nerve growth factor (NGF) promotes the survival and maintenance of specific populations of sympathetic, sensory and CNS neurons. Recent data suggest that NGF can protect/rescue CNS neurons from insults such as hypoglycemia and ischemia, including neuronal populations (e.g. hippocampus) previously believed to be unresponsive to NGF (*Neuron* 7:1031, 1991). Previous studies identified both a low affinity 75 kDa NGF receptor and a high affinity receptor which is identical to the trkA proto-oncogene product. Both types of NGF receptors have been proposed to be important for the biological actions of NGF. Immunocytochemical and Western blot studies using polyclonal antibodies to the 75 kDa NGF receptor (REX) and trkA (generous gifts from G. Westkamp, F. Lefcort, and L. Reichardt) demonstrated the presence of these proteins in neurons in embryonic rat hippocampal cell cultures. In order to determine which NGF receptors might mediate actions of NGF in hippocampal neurons, we employed antisense oligonucleotides (AO) directed against rat 75 kDa NGF receptor or trkA (sequence provided by D. Clary). Levels of the 75 kDa receptor and trkA were reduced in neurons exposed to the respective AOs. Administration of AOs for either NGF receptor resulted in neuronal death. However, neuronal death was more rapid in cultures exposed to trkA AO (< 24 hr) as compared to 75 kDa NGF receptor AO (2-3 days). The NGF receptor AOs also eliminated the neuroprotective effect of NGF in glucose-deprived cultures. Taken together, our data suggest that hippocampal neurons in culture possess both the low affinity NGF receptor and trkA. Both NGF receptors may play a trophic role and protect hippocampal neurons against environmental insults. (NIH and Alzheimer's Association support).

401.20

Purine analogs inhibit a trk-associated kinase activity.

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Previous studies showed that purine analogs block with varying potency and specificity certain of the effects of NGF on PC12 cells. These analogs inhibit protein kinase activities with varying specificity; for example, 2-aminopurine (2-AP) blocks several kinases whereas 6-thioguanine (6-TG) specifically inhibits protein kinase N (PKN), an NGF-activated protein kinase. In the present work, immunoprecipitates of trk-NGF receptors from PC12 cells (+/- NGF treatment) were assayed for kinase activity using myelin basic protein (MBP) and histone HF1, under conditions optimized for PKN and in the presence or absence of purine analogs. Activity was detected and approximately 50-80% was inhibited by 2-AP and 6-TG. The purine analog-sensitive activity was maximally stimulated by NGF within 5 min, was partially decreased by 30 min and still remained over basal levels after 15 hours of NGF treatment. Neither 2-AP nor 6-TG inhibits the NGF-dependent induction of the purine analog-inhibited trk-associated kinase activity. Analysis of MBP phosphorylated by the anti-trk immunoprecipitates revealed mainly phosphothreonine. Phosphorylation on threonine, but not phosphorylation on tyrosine, was inhibited by 6-TG, indicating that this analog does not inhibit the tyrosine kinase activity associated with the NGF receptor. The purine analog-sensitive, trk-associated kinase activity is in many aspects similar to cytosolic PKN and therefore suggests interaction between PKN and functional NGF receptors.

401.21

INFECTION OF DISSOCIATED COCHLEAR GANGLION CELL CULTURES WITH pHSVtrkA CHANGES THE SURVIVAL RESPONSE OF AUDITORY NEURONS TO EXOGENOUS NGF. T.R. Van De Water*, P.P. Lefebvre, W. Liu, H. Xu, G. Moonen, J.A. Kessler, and H. Federoff. Depts. of Otolaryngology, Neuroscience, Medicine & Neurology, Albert Einstein College of Medicine, Bronx, NY 10461, Dept. of Physiology, University of Liege, B 4020 Liege, Belgium.

Auditory neurons in cell cultures of dissociated cochlear ganglia of the mouse after embryonic day 13 are not responsive to the survival promoting effects of nerve growth factor (Lefebvre et al., 1991, Acta Otolaryng. 111:301-311). Transfection of $PC12_{nnr}$ cell mutants deficient in high affinity NGF binding with a full length rat trk cDNA restored the responsiveness of this $PC12_{nnr}$ cell line to NGF (Loeb et al. Cell 66:961-966, 1991).

A defective herpes simplex virus-1 vector (HSV-1) containing a gp 140^{trk} construct (i.e. pHSVtrkA) was used to infect cultures of dissociated cochlear ganglia that contained auditory neurons that were unresponsive to NGF. The expression of gp 140^{trk} was driven by the HSV IE 4/5 promoter so that all infected cells of the culture would express the gp 140^{trk} construct. When infected cultures were compared to uninfected ones, a significant enhancement of the survival of auditory neurons in the pHSVtrkA infected cochlear ganglion cell cultures treated with exogenous human recombinant NGF (i.e. 100 ng/ml) was evident in contrast to the uninfected control cultures treated with hrNGF.

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OTHER FACTORS AND TROPHIC AGENTS: IGF

402.1

EXPRESSION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-4 AND -5 mRNAs IN ADULT RAT BRAIN. Kaye L. Stenvers[1]*, Michela Gallagher[1], Ellen M. Zimmermann[2], and P.Kay Lund[1]. [1]Curriculum in Neurobiology and [2]Dept. of Medicine, University of North Carolina, Chapel Hill, NC 27599.

Insulin-like growth factors (IGFs) are believed to serve as neurotrophic factors in developing and adult rat brain. The IGFs are associated with a family of at least six IGF binding proteins (IGFBPs) which prolong the half-life of plasma IGFs and modulate IGF action. Although IGFBP-4 and -5 are widely expressed in adult rat tissues, their cellular sites of synthesis are still poorly characterized. To localize sites of IGFBP-4 and -5 mRNA expression, we performed *in situ* hybridization histochemistry using ^{35}S-labeled antisense riboprobes specific for either rat IGFBP-4 or IGFBP-5 mRNA (provided by Drs. Nicholas Ling and A.J. D'Ercole, respectively). We examined fresh-frozen, post-fixed sections of adult rat brain. Specificity of hybridization was confirmed by negative results in sections hybridized with sense strand riboprobes or pre-treated with RNase A prior to hybridization. The two IGFBP mRNAs were abundantly expressed within discrete regions of brain. Generally, the expression patterns of the two genes were non-overlapping. Notably, IGFBP-4 mRNA was highly expressed within hippocampal and cortical areas, whereas IGFBP-5 mRNA was not detected above background in these areas. Within hippocampus, strong hybridization of the IGFBP-4 probe was detected in pyramidal neurons of a medial segment of the CA1 subfield and throughout the CA2 subfield of Ammon's horn. Strong hybridization was also detected within the indusium griseum and subiculum. In cortex, IGFBP-4 mRNA was widely expressed in most cortical areas and layers. In contrast, IGFBP-5, but not IGFBP-4, mRNA was detected within thalamic nuclei. The distinct expression patterns of IGFBP-4 and -5 mRNAs within brain suggest that these IGFBPs may modulate compartmentalization of the IGFs or paracrine/autocrine actions of the IGFs in discrete brain regions. Furthermore, expression of IGFBP-4 mRNA by hippocampal pyramidal neurons suggests a role for IGFBP-4 in modulating neuronal effects of the IGFs.

402.2

ASSOCIATION OF INSULIN-LIKE-GROWTH FACTOR 1 RECEPTORS (IGF1r) ON BASAL FOREBRAIN CHOLINERGIC NEURONS.

A. Beaudet*, C. Desjardins and M-P Faure. Neuroanatomy Lab, Montreal Neurological Institute, McGill University, Mtl, Que, Canada H3A 2B4.

IGF1 was previsouly shown to stimulate the release of acetylcholine in adult rat brain slices. It remains unknown whether IGF1 exerts its effects directly on central cholinergic neurons. To test the hypothesis that IGF1 receptors may be associated with basal forebrain cholinergic cells, we examined by double immunofluorescence, the labeling of IGF1r and ChAT in rat serial sections. Primary antibodies were revealed with secondary antibodies conjugated with FITC and Texas Red, respectively. In the medial septal nucleus, diagonal band of Broca and substantia innominata a large proportion of ChAT positive neurons exhibited IGFr immunoreactivity. Reconstruction of serially sectioned images using confocal laser microscopy (CLSM) substantiated that IGF1r and ChAT immunoreactivity were present in the same neurons. The cellular staining pattern of IGF1 was mostly membranous and took the form of highly fluorescent punctate dots. Hybrid cells derived from septal cholinergic neurons, produced by the fusion of embryonic murine septal cells with murine neuroblastoma cells, were used to characterize the biological effect of IGF1. The hybrid cells were treated for different times and concentrations with nerve growth factor, basic fibroblast growth factor, platelet-derived growth factor and IGF1. Stimulation with IGF1 (10 ng/ml) for 48 hours produced a change in cell shape and significant neurite outgrowth (15-2μm) whereas none of the other factors produced any effect under these conditions. Moreover, fluorescence ChAT immunoreactivity was evaluated by CLSM and expressed a 10 fold increase following IGF1 stimulation. These results suggest that IGF1 may play an important role in the growth and transmitter regulation of basal forebrain cholinergic neurons and, consequently, that the impairment of its interaction with IGF1r may be involved in the degeneration of cholinergic systems such as occurs in Alzheimer's disease.

402.3

A RAPID INCREASE IN ACTION POTENTIAL FIRING RATE IN RESPONSE TO GROWTH FACTORS. R.H. Selinfreund* and L.A.C. Blair. Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

The role of growth factors in the adult mammalian central nervous system is unknown. We have examined the rapid modulation of action potential frequency and ionic currents by growth factors known to be present in the central nervous system. Patch clamp recording techniques were used to indentify spontaneous electrical activity and ion channel events in GH_4C_1 pituitary cells. It was found that culturing in serum-depleted conditions blocked the firing of spontaneous action potentials. Brief exposure to serum or to a pool of 14 growth factors normally contained in serum fully reconstituted electrical activity. Subsequent experiments demonstrated that addition of insulin, IGF-I and IGF-II are sufficient to restore normal action potential behavior. Specifically, using on-cell single channel recordings or whole-cell recordings in the current clamp configuration, the reconstitution of electrical activity was rapid (requiring 30 seconds to 5 minutes) and stable. Furthermore, preliminary whole-cell voltage clamp analysis suggests that both outward potassium currents and inward calcium currents are altered by the presence of growth factors. One of these growth factor-modulated currents is tetraethylammonium-sensisitive but insensitive to 4-aminopyridine, indicating that Kv1 might be one of the potassium channels regulated by growth factors. Together, these experiments suggest that the insulin family of growth factors may be capable of inducing rapid changes in calcium channels and one or more potassium channels.

402.4

NEUROTROPHIC EFFECTS OF INSULIN-LIKE GROWTH FACTORS ON PC12 CELLS. Margarita L. Contreras*, Julia M. Pearson, and Alethea Gordon. Dept. of Pharmacology/Toxicology and Neuroscience Program, Michigan State Univ., East Lansing, MI 48824.

Since receptors for insulin-like growth factor I and II (IGF-I and IGF-II) are present on PC12 cells (Biochem. Biophys. Res. Commun. 154:1018, 1988), the effect of insulin-like growth factors on neuronal differentiation in these cells was examined. PC12 cells were treated with or without IGF-I or IGF-II (10 nM) for 2 days. Neither IGF-I nor IGF-II stimulated neurite outgrowth. However, in the presence of 50 ng/ml nerve growth factor (NGF), IGF-I and IGF-II potentiated the NGF-stimulated neurite outgrowth. Compared to the percent of neurite-bearing cells seen in the presence of NGF alone, there was approximately a 50% increase in the percent of cells with neuronal processes when either IGF-II or IGF-I was included in the media. The IGF-mediated potentiation of neurite outgrowth was dose-dependent, with the maximal effect apparent at approximately 3 nM of either IGF-I or IGF-II.

To determine whether the mannose-6-phosphate/IGF-II receptor was involved in the potentiating effects of IGFs on neuronal differentiation, the effect of mannose-6-phosphate (M6P) on NGF-stimulated neurite outgrowth was assessed. M6P did not potentiate the neurite promoting effect of NGF. Also, the percent of cells bearing neurite outgrowths seen in the presence of IGF-II and NGF was unaffected by the inclusion of M6P in the growth medium, suggesting that the M6P/IGF-II receptor was not involved in mediating the potentiating effects of IGF-II on neuronal differentiation in PC12 cells. The IGF-I receptor may therefore mediate the potentiating effect of IGFs on neuronal differentiation in the PC12 cells. NSF supported.

402.5

INSULIN LIKE GROWTH FACTOR I AND ITS RECEPTOR ARE PRESENT IN NEURON CELL CULTURES. R.Schechter, J.Whitmire, D.Beju, R.Harlow, K.Jackson, J.R.Gavin III and M.E.O'Connor*. St. Francis Med. Res. Inst., Univ. of Okla. Hlth. Sci. Ctr., Univ. of Tulsa, Tulsa and OK City, OK 74136 and 73190.

We have studied the presence of insulin like growth factor I (IGFI) and its receptors in 22 day and 18 day gestation neuron cell cultures (NCC) from fetal rabbit brains. The 22 day NCC were incubated in IGFI free/serum free medium (ISFM) and the 18 day NCC in serum medium. The 18 day NCC died in an ISFM. The peroxidase anti-peroxidase method using rabbit anti-IGFI antibody (RIGF) (1/100)(E.Lilly) showed IGFI present. Antibody absorbed with IGFI (E.Lilly) and rabbit serum lacked immunoreaction. In situ hybridization using a 36 base biotinylated oligonucleotide revealed IGFI mRNA. Electron microscopy using RIGF (1/10000) and anti-neuronfilament (1/10000) showed IGFI in the neurons' endoplasmic reticulum, Golgi, cytoplasm and prolongations. RIA of ISFM revealed IGFI. IGFI receptors were detected by using ^{125}I IGFI binding of NCC in both NCCs. The 18 day NCC survived when IGFI (100ng/ml) was added to ISFM. We conclude: A) fetal NCC produce IGFI, B) IGFI promotes cell survival, and C) exogenous IGFI may be needed in early brain development.

402.6

EFFECTS OF INSULIN, INSULIN-LIKE GROWTH FACTOR I, AND BASIC FIBROBLAST GROWTH FACTOR ON RETINAL CELL PRODUCTION. Andreas F. Mack* and Russell D. Fernald. Institute of Neuroscience, University of Oregon, Eugene, OR 97403 and Neuroscience Program, Stanford University, Stanford, CA 94305.

Rod photoreceptors are added to the fish retina from unique progenitor cells in the outer nuclear layer continuously throughout life. To investigate the regulation of cell production in the teleost retina we tested the effects of several growth factors on proliferation and differentiation of rod photoreceptor cells. Using organotypic slice cultures for the differentiated fish retina, we labelled dividing cells with ^3H-thymidine and revealed specific phenotype expression with an antibody staining rod photoreceptors exclusively. We found that insulin and insulin-like growth factor I (IGF-I) increased the number of neuronal progenitor cells undergoing cell division in culture significantly. Basic fibroblast growth factor (bFGF) did not have a significant effect on the number of dividing cells. In the presence of bFGF, however, a greater proportion of the cells that had divided in culture expressed a rod photoreceptor-specific phenotype compared to control slices. This suggests that bFGF promotes the differentiation of neuronal progenitor cells whereas insulin and/or the related IGF-I are involved in the regulation of neuronal cell division in retinal slice culture. Supported by NIH grant GM 07257.

402.7

INSULIN LIKE GROWTH FACTOR-1 HAS A ROLE IN THE DEVELOPING RAT OLFACTORY BULB: EVIDENCE FROM INTRAOCULAR GRAFTING

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Recent studies have indicated that insulin like growth factor-1 (IGF-1) and IGF-1 receptor mRNA are abundant in both the developing and in the mature rat brain olfactory bulbs (Ayer-Le Lievre et. al. 1991, Werther et. al. 1990, Bondy et.al. 1992). By using the method of intraocular transplantation we were able to study effects of truncated IGF-1 (tIGF-1), antibodies against IGF-1 and IGF-1 binding protein-1 on developing olfactory bulbs. E16 olfactory bulbs were grafted to the anterior chamber of adult host rats. Grafts were treated with either 20 µg/ml tIGF-1, a polyclonal antibody against IGF-1, 1 mg/ml IGF binding protein or vehicle solution alone prior to grafting. Similar solutions were administered by intraocular injections 5, 10 and 15 days postgrafting. Growth of grafts was monitored by direct observation and measurement through the cornea of the living animals. Grafts treated with IGF-1 antibody grew significantly larger than grafts receiving any other treatment. When similar experiments were carried out on E16 and E17 parietal cortex grafts, no enhancement in graft size was seen after IGF-1 antibody treatment. However tIGF-1 has in previous studies (Giacobini et. al. 1990) been shown to enhance growth of intraocularly transplanted fetal parietal cortex. Thus, IGF-1 appears to influence brain development in a regionally specific manner. Immunohistochemical studies of the olfactory bulb grafts are currently under investigation.

402.8

INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) INCREASES RATE OF FUNCTIONAL RECOVERY FROM SCIATIC NERVE CRUSH IN MICE. P.C. Contreras*, C. Steffler and J. L. Vaught. Pharmacology, Cephalon, Inc. West Chester, PA 19380.

IGF-1 has been shown to stimulate several growth associated processes and to support the survival of α-motor neurons in vitro. IGF-1 has also been shown to enhance neuronal sprouting after sciatic crush in rats. The purpose of this study was to assess whether IGF-1 also increases rate of functional recovery from sciatic crush in mice. After Swiss-Webster mice (25-35 g) were anesthetized, both sciatic nerves were exposed and crushed for 10 sec with a hemostat, covered with plastic tubing. Mice were injected with vehicle (1%BSA) or IGF-1 (1mg/kg) s.c. after recovery from the anesthetic and for the next 17 days. Functional recovery from the sciatic crush was measured by 1) determining the number of times/5 trials the mice were able to grasp an inverted screen with both hindpaws; and 2) assessing changes in gait. Mice treated with IGF-1 were able to grasp the inverted screen and returned to normal responses sooner than vehicle-treated mice. There were also parallel improvements in several parameters used to measure gait, such as toe spread, in IGF-1-treated mice compared to vehicle-treated mice. These results indicate that IGF-1, which enhances survival of α-motor neurons, also enhances functional recovery. These data support the utility of rhIGF-1 for the treatment of ALS

403.1

BASIC FGF AND FGF RECEPTOR-4 OCCUR IN CHICKEN. P. C. Evers and M. Bothwell*. Dept of Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195.

The fibroblast growth factor (FGF) family presently consists of 7 members: aFGF, bFGF, int-2, k-FGF, FGF-5, FGF-6, and KGF. These ligands bind, with specificity which is not fully defined, to receptors encoded by 4 genes: FGF receptor-1 (FGFR1), FGFR2, FGFR3, and FGFR4. Our previous results show FGFR1 expressed in the developing chick neuroepithelium, where it is likely to have a role in proliferation and differentiation. To further explore the role of FGFs in neural development, we plan to describe the expression of the FGFs and other FGFRs in neural tube. Hence, we have sought to clone segments of each FGF and FGFR gene for use as in-situ hybridization probes. We have cloned portions of 6 genes: aFGF, bFGF, FGFR1, FGFR2, FGFR3, and FGFR4 from chicken using PCR and degenerate oligonucleotides. The sequences for two of these genes, bFGF and FGFR4, have not been reported previously. Sequence identity between the putative chicken bFGF and the mouse bFGF is 83% at the nucleotide level and 94% at the amino acid level. The nucleotide sequence identity between the putative chicken FGFR4 and the three known chicken FGFR genes is 72-79%, whereas the nucleotide sequence identity between the putative chicken FGFR4 and the human FGFR4 is 79%. Sequence comparisons suggest that the two cDNAs are portions of the bFGF and FGFR4 genes in chicken. The bFGF and FGFR4 cDNAs along with the cDNAs for aFGF, FGFR1, FGFR2, and FGFR3, will be useful in determining the roles of FGF in neural development.

403.2

FGF-5 mRNA DISTRIBUTION IN THE RAT BRAIN: AN IN SITU HYBRIDIZATION STUDY. F. Gómez-Pinilla* and C.W. Cotman. Dept. of Psychobiology, U. California Irvine, CA 92717

Fibroblast growth factors (FGFs) are potent growth factors with roles ranging from development to adult plasticity in the brain. FGF-5 is a new member of the FGF family with a potential role in brain function and pathology. In order to evaluate a possible action of FGF-5 in the brain, we have examined the locus of synthesis of FGF-5 in the rat brain using *in situ* hybridization of S^{35}-labelled cRNA probe complementary to FGF-5 mRNA. FGF-5 mRNA was expressed in neurons in selected regions of the rat brain. FGF-5 mRNA *in situ* hybridization labelling was particularly intense in the olfactory bulb within periglomerular elements and mitral cell layer. The primary olfactory cortex also showed strong FGF-5 mRNA labelling and mostly within cell layer II, throughout its rostro-caudal extent. In the hippocampal formation, the greatest intensity of FGF-5 mRNA labelling was shown in hippocampal pyramidal cells within subfields CA3 and CA2, and granular cells within the dentate gyrus. The cerebral cortex (neocortex) showed a modest labelling throughout its rostro-caudal extent, mostly within external layers. The entorhinal cortex showed a slightly higher labelling intensity as compared to the neocortex. The cerebellum, thalamus and striatum displayed light labelling. Glial-like cells scattered throughout the brain appeared to express low levels of FGF-5 mRNA. In general FGF-5 mRNA was mostly shown by limbic structures, suggesting the possibility that FGF-5 may play a role in limbic system function or pathology.

403.3

BASIC FIBROBLAST GROWTH FACTOR (bFGF) GENE EXPRESSION IN RAT BRAIN: AGE AND REGIONAL COMPARISON BY QUANTITATIVE RT-PCR. A. El-Husseini[1], J.A. Paterson[2]* and R.P.C. Shiu[1], Departments of Physiology[1] and Anatomy[2], Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3.

Investigations *in vitro* have shown the importance of bFGF as a mitogen for astrocytes and as a neurotrophic factor for many neuronal types. This study seeks to compare bFGF gene expression in selected regions of the brain of rats at different postnatal ages. The reverse transcription-polymerase chain reaction (RT-PCR) was used to measure the levels of bFGF mRNA. These levels were quantitated relative to the levels of mRNA for glyceraldehyde-3-phosphate dehydrogenase, the latter being constant at the ages being studied. We first compared the levels of bFGF mRNA in the cerebrum from male rats of ages 1, 3, 7, 14, 21 and 28 days and one year. Our results showed that by the end of the first month, the cerebral bFGF mRNA was about 10-fold that of the 1 day old rat, with the greatest increase occurring between the first and the second postnatal weeks. One year old cerebrum showed high levels of bFGF mRNA similar to those of 28-day old. Analysis of different regions of the 28-day old brains revealed that the lowest levels of bFGF mRNA occur in the cerebellum, and that the highest levels occur in hippocampus (7 times the levels in cerebellum, 7X), followed closely by cingulate cortex, occipital cortex, and inferior colliculus (4-5X). The hypothalamus and combined pons-medulla showed intermediate levels (2X). Preliminary studies of these regions from 1 day old rats rats showed there is a different pattern of regional expression at this age. Our results are consistent with the hypothesis of a role of bFGF in brain development.
(Supported by Manitoba Health Research Council and MRC of Canada).

403.4

CO-LOCALIZATION OF bFGF WITH NEUROPEPTIDES AND FGF-RECEPTOR IN THE RAT BRAIN: EFFECTS OF COLCHICINE. C. Grothe*. Dept. of Anatomy and Cell Biology, University of Marburg, D-3550 Marburg, Germany.

Recently, it was shown that basic fibroblast growth factor (bFGF) is present in distinct neuronal subpopulations of the brainstem (Grothe et al., J. Comp. Neurol. 305, 1991). Immunocytochemical analysis revealed a partial overlap of bFGF and neuropeptides (SOM, SP, CGRP) or tyrosine hydroxylase in some brainstem nuclei but no strict co-localization of bFGF with one of the neuropeptides or tyrosine hydroxylase.

To identify bFGF-responsive cells in the brain, immunocytochemistry and binding studies were performed. FGF-receptor could be localized in the cortex and, like bFGF, in neuronal subpopulations of several motor and sensory brainstem nuclei.

After colchicine treatment bFGF-IR disappeared in brainstem nuclei expressing the FGF-receptor and appeared or increased in nuclei lacking the FGF-receptor. Whether this change in the bFGF-IR is exclusively due to transported bFGF has to be clarified by in situ hybridization.

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403.5

EXPRESSION OF bFGF mRNA AND IDENTIFICATION OF FGF-RECEPTOR-POSITIVE CELLS IN RAT SENSORY GANGLIA. T. Janet, K. Unsicker*, C.Grothe. Dept. Anat. Cell Biol., Univ. Marburg, Germany.

We have shown that bFGF-immunoreactivity (IR) is present in a neuronal subpopulation of postnatal and adult crest- and placode-derived sensory ganglia (Weise et al., Cell and Tissue Res.,267, 125-130, 1992). In dorsal root ganglia (DRG), bFGF was strictly co-localized with the somatostatin-IR subpopulation. Northern blots of total RNA from DRG using a rat cDNA probe revealed 7.0 and 3.7 Kb mRNA. In situ hybridization showed that the bFGF mRNA is present in nearly all DRG neurons. To identify neurons with putative bFGF responsiveness, FGF-receptor immunocytochemistry and binding studies were performed. FGF-receptor IR was present in all bFGF-immunoreactive neurons suggesting that the protein mediates its effects in an autocrine and/or paracrine manner. The possible explanations of the discrepancy in the distribution of bFGF-IR and bFGF mRNA may include i) different translational regulation in distinct neuronal subpopulations, ii) different stabilities of mRNA and proteins.

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403.6

DEVELOPMENTAL EXPRESSION OF ACIDIC AND BASIC FIBROBLAST GROWTH FACTORS IN SUBSTANTIA NIGRA AND SENSORY NEURONS.
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Fibroblast growth factors (FGFs) are a family of related peptides which have been characterized by their ability to induce cellular differentiation and their mitogenic/angiogenic properties. We have observed the acidic and basic FGF (aFGF, bFGF) mRNA and protein in sensory and motor neurons of the rat including the substantia nigra (SN). In contrast to the widespread distribution of bFGF, aFGF expression is quite restricted. We examined the expression of aFGF and bFGF in the rat SN and dorsal root ganglia (DRG) from E16-18 to PN90.

In DRGs, aFGF mRNA and protein were present at E18 and were observed through adulthood. bFGF mRNA was present in the SN at all ages tested. aFGF mRNA was first detectable in the SN at PN20 and was expressed through adulthood. The differential developmental expression of aFGF and bFGF in the SN may indicate different functional roles in this brain region.

403.7

BASIC FIBROBLAST GROWTH FACTOR (bFGF) PROMOTES THE SURVIVAL AND PROLIFERATION OF MESENCEPHALIC NEURONAL PRECURSORS IN VITRO. M.M. Bouvier* and C. Mytilineou, Dept. Neurology, Mt. Sinai Sch. of Medicine, New York, N.Y., 10029.

We recently demonstrated that treatment with epidermal growth factor (EGF) results in the survival and proliferation of neuronal and glial stem cells from embryonic day 16 (E16) rat mesencephalon (Mytilineou et al., 1992). To determine whether dopaminergic neuronal precursors could also be induced to proliferate in vitro, we used ventral mesencephalic cultures from E12 rat embryos, a developmental stage that coincides with the beginning of the birth of mesencephalic dopamine neurons. Mesencephalic cells were treated with EGF (10ng/ml), bFGF (10ng/ml) or a combination of EGF and bFGF at the day of plating. Control and trophic factor-treated cultures were observed with phase contrast microscopy and analyzed after 7 days in vitro (DIV) by immunocytochemistry with antibodies to neuron specific enolase (NSE), tyrosine hydroxylase (TH) and glial fibrillary acidic protein (GFAP) to identify differentiated neurons, dopaminergic neurons and astrocytes, respectively. Some cell division, resulting in colony formation, occurred in the first 24-48 hrs after plating in all trophic factor-treated cultures, as well as in untreated controls. However, treatment with bFGF resulted in greater cell number and the formation of larger colonies. EGF had no apparent effect in these cultures. Cell loss was prominent in control and EGF-treated cultures after 7DIV, but it was less apparent in cultures treated with bFGF. At 7DIV the majority of surviving cells were positive for NSE while <1% stained with GFAP. TH immunocytochemistry revealed clusters of dopamine neurons in control and EGF-treated cultures, but their numbers were higher after treatment with bFGF. (Supported by NIH grant NS-23017 and the United Parkinson Foundation).

403.8

PROTECTIVE ACTIONS OF INTRAVENTRICULAR HUMAN RECOMBINANT BASIC FIBROBLAST GROWTH FACTOR INFUSION ON MPTP-INDUCED DEGENERATION OF THE NIGROSTRIATAL DOPAMINE SYSTEM IN THE BLACK MOUSE. A STEREOLOGICAL, IMAGE AND BEHAVIOURAL ANALYSIS.

G. Chadi[1], K. Fuxe[1], A Møller[2], A.M. Janson*[1], L. Rosén[1], A. Cintra[1], Y. Cao[3], M. Goldstein[4], S.O. Ögren[1] and R. Petterson[3]. [1]Dept. of Histology and Neurobiology, Karolinska Inst., Stockholm, Sweden; [2]NeuroSearch, Glostrup, Denmark; [3]Ludwig Inst. for Cancer Research-Stockholm Branch, Karolinska Inst., Stockholm, Sweden; [4] Dept. of Psychiatry, New York Medical Center, N.Y., USA.

Basic fibroblast growth factor (bFGF) immunoreactivity (IR) has recently been demonstrated in the vast majority of dopamine (DA) neurons of the ventral midbrain of the rat and neuroprotective actions of the bFGF in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model have been reported. The actions of bFGF have now been further analyzed in a combined morphological and behavioural analysis in the MPTP model of the adult black mouse. Four hours after the injection of MPTP (40 mg/kg, s.c.) a 7 day continuous intraventricular (i.v.t.) infusion of human recombinant bFGF (1 µg/24 h) or of mock cerebral spinal fluid (CSF) was started using Alzet osmotic minipumps. On day 6 the locomotion of the MPTP+mock-CSF group was significantly reduced versus the saline+mock-CSF treated group and the locomotor activity was restored by the bFGF treatment. Tyrosine hydroxylase (TH) immunocytochemistry in combination with computer assisted microdensitometry demonstrated significant counteractions of the MPTP-induced disappearance of neostriatal nerve terminal TH IR following bFGF treatment. This counteraction was present also 1 week after cessation of treatment. Unbiased estimates of the total number of nigral TH IR neurons, using the optical disector (Olympus) and stereological methods, showed that the MPTP treatment reduced the total number of DA nerve cells from 13,700±1,000 (n=5) to 6,000±1,000 (n=4). bFGF significantly increased this number of DA nerve cells to 10,000±700 (n=4), an action which was maintained also 1 week after cessation of treatment. These results demonstrate that human recombinant basic FGF upon i.v.t. infusion exerts protective actions on nigrostriatal DA neurons exposed to the MPTP neurotoxin.

Supported by FAPESP (91/2989-9) and Wenner-Gren Center Foundation.

403.9

EFFECTS OF BASIC AND ACIDIC FGF ON GROWTH AND DOPAMINE NERVE FIBER PRODUCTION OF INTRAOCULARLY TRANSPLANTED FETAL MESENCEPHALIC GRAFTS

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Basic FGF has been shown to increase dopamine neuron survival in mesencephalic cultures (Ferrari et. al. 1988) as well as inducing regrowth of damaged DA neurons in vivo (Otto, Unsicker, 1990). We have followed the growth and survival of developing E14-E16 mesencephalic grafts under chronic intermittent treatments with either aFGF or bFGF in the anterior eye chamber of adult rat hosts. Pieces to be grafted were incubated in either 25 µg/ml aFGF, 25 µg/ml bFGF or vehicle solution alone prior to grafting and 5 µl of similar solutions were injected intraocularly on day 5, 10 and 15. Host animals were sympathetically denervated 2 weeks prior to grafting enabling evaluation of catecholaminergic fiber outgrowth onto the host iris in whole-mount preparations by use of the Falck-Hillarp technique. Both aFGF and bFGF significantly increased the volume of transplanted mesencephalic grafts when compared to grafts treated with the vehicle alone, bFGF being a more potent growth stimulator than aFGF. Both bFGF and aFGF increased the area of the host iris innervated by graft-derived catecholamine containing fibers. Immunohistochemical evaluations of grafts are currently under study as well as studies of treatment of mesencephalic grafts using the described model with other growth factors.

403.10

INTRACEREBRAL GRAFTING OF CELLS GENETICALLY MODIFIED TO EXPRESS BASIC FIBROBLAST GROWTH FACTOR.

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Basic fibroblast growth factor (bFGF) has been recognized as a neurotrophic factor and affects survival and differentiation of a variety of central neurons as well as other cells. We have previously reported the expression and characterization of bFGF producing primary fibroblasts (Soc. Neurosci. Abst. 17:43,1991). This study was undertaken to assess the biological effects of bFGF producing fibroblasts grafted into the adult rat nervous system.

Primary skin fibroblasts were infected with a retroviral vector containing a cDNA for rat bFGF. Those cells revealed positive immunoreactivity for bFGF protein in vitro. Radioimmunoassay showed the amount of bFGF in the cells lysate was between 5-18 times higher than non-infected control fibroblasts. bFGF producing primary fibroblasts and those infected with a retroviral vector containing the cDNA for *E. coli* ß galactosidase (ß gal) were grafted into Fischer rat striatum. At 8 weeks after grafting, the rats were perfused and sections of the brain were processed for immunohistochemistry. The bFGF immunoreactive cells were more prominent in the bFGF grafts than in the ß gal grafts, though some endothelial cells and astrocytes-like cells were also stained within both grafts. Staining with neurofilament(200kd) or tyrosine hydroxylase antibodies revealed immunoreactive axonal profiles within both grafts. The density of axonal growth was higher in bFGF grafts, compared to ß gal ones. No significant differences were seen following staining with NGF receptor and choline acetyl transferase antibodies between two grafts. Vascularization revealed by laminin staining was greater in bFGF grafts, whereas glial fibrillary acidic protein staining showed no noticeable difference. These results suggest that implanted bFGF producing fibroblasts have some biological effects in the adult rat nervous system.

403.11

THE COMBINED ACTION OF BASIC FGF AND SUBSTRATA PROMOTES DIFFERENT CHROMAFFIN CELL FATE

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Basic fibroblast growth factor (bFGF),like NGF,induces cell division and neurite outgrowth from adrenal chromaffin cells . In vitro , we found that human recombinant bFGF , unlike NGF , promotes different neurite outgrowth responses on neonatal rat chromaffin cells with different substrata.To determine the extent of chromaffin cell transformation into neurons , we counted the number of cells with tyrosine hydroxylase positive staining and neurites and cell division was assayed by [3H] thymidine incorporation. A marked increase of transformed neurons, up to 80% was seen when chromaffin cells were cultured on laminin , whilst cell survival and bFGF-stimulated cell division were significantly reduced on laminin compared with collagen Type I. By contrast,in cultures grown on collagen Type I , bFGF elicited little neurite outgrowth , and promoted the proliferation and survival of chromaffin cells.In addition, dexamethasone inhibited neurite outgrowth in response to bFGF on laminin and increased the proportion of surviving and proliferative cells .These results show that bFGF and laminin act synergisticly to promote sympathetic neuronal transformation , whilst on collagen Type I or in the environment of glucocorticoids, bFGF acts as a mitogen and survival factor of chromaffin cells.

403.12

REGULATION OF BASIC FIBROBLAST GROWTH FACTOR (bFGF) EXPRESSION IN CULTURED ADRENAL MEDULLARY CELLS.

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Basic FGF is expressed in catecholaminergic cells of substantia nigra and in sympatho-adrenal system. We have recently shown that in adrenal medullary (AM) cells exogenous bFGF modulates expression of tyrosine hydroxylase and proenkephalin genes, suggesting that it may serve as an autocrine or paracrine regulator in catecholamine and enkephalins biosynthesis. The present study was undertaken to determine subcellular localization of bFGF in AM cells and to examine whether bFGF expression is regulated by stimuli that affect synthesis of AM hormones. In cultured bovine AM cells stained with different antibodies against recombinant bFGF, granular cytoplasmic and nuclear bFGF-immunoreactivity (bFGF-IR) was observed. In majority of cells bFGF staining was more intense in the nuclei than in the cytoplasm. Incubation of cells with exogenous 18 kDa bFGF ($5x10^{-10}$ M) led to an increase in nuclear bFGF-IR within 10 min, which reached a maximum between 1-3 hrs. Incubation with forskolin produced a dramatic increase in nuclear bFGF-IR, which attained a maximum after 12 hours. Less pronounced increases in nuclear staining, were observed in cells treated with angiotensin II or with the depolarizing agent, veratridine. Western blot analysis revealed the presence of at least 3 bFGF isoforms (18,23 and 24 kDa). Their levels were upregulated by forskolin and angiotensin II. Those changes were associated with induction of bFGF mRNA. Regulation of bFGF gene expression, protein levels and nuclear translocation in AM cells by hormonal (angiotensin) and neural (depolarization) stimuli, and by 2nd messenger (cAMP), supports the hypothesis that bFGF plays an active role in the plasticity of the adult nervous system. It also suggests that the long-term genomic effects of bFGF may be mediated directly in the nucleus.

403.13

MITOGENIC EFFECTS OF ACIDIC FIBROBLAST GROWTH FACTOR AND TRANSFORMING GROWTH FACTOR BETA ON BOVINE AND PORCINE ENDOTHELIUM IN VITRO. T.C. Ryken and V.C. Traynelis*. Department of Surgery, Division of Neurosurgery, University of Iowa College of Medicine, Iowa City, Iowa 52242.

The mitogenic effects of acidic fibroblast growth factor (aFGF) and transforming growth factor beta (TGF-beta) were assayed alone and in combination on cultured bovine and porcine endothelium. Triplicate growth-arrested cultures of each cell type were grown in the presence of aFGF (300 ng/ml) and/or TGF-beta (5 ng/ml) for 96 hours, renewing the media each 24 hours. The mitogenic effects were assayed by cell counting at the conclusion of the 96-hour period. Results are reported as a stimulation index (ratio of final cell number to initial cell number).

Both bovine and porcine endothelial cell cultures incubated with aFGF underwent a marked increase in mitogenic activity, increasing in stimulation index from 1.60 (± .05) for control to 4.51 (± .27) and 3.81 (± .06), respectively. Endothelium incubated in the presence of TGF-beta underwent a decrease in mitotic activity with a stimulation index of 1.16 (± .10) and 1.17 (± .05) in the bovine and porcine cultures. Cells incubated in a combination of aFGF and TGF-beta demonstrated an attenuation of the mitogenic effects observed in the presence of aFGF alone, decreasing the stimulation index to 3.25 (± 11) in bovine endothelium and 1.80 (± .30) in porcine endothelium.

These results suggest that the mitogenic effect of aFGF in cultured endothelium can be antagonized by TGF-beta. The interaction of growth factors on endothelium is of interest to elucidate the mechanisms involved in angiogenesis and transformation.

403.14

EFFECTS OF ACIDIC FIBROBLAST GROWTH FACTOR ON RAT PRIMARY CULTURED NEURONS AND HIPPOCAMPAL LTP. H.Hisajima, K.Abe, N.Nishiyama, H.Saito*. Dept. of Chem. Pharmacol., Faculty of Pharmaceut. Sci., Univ. of Tokyo 113, Japan.

Effects of human recombinant acidic fibroblast growth factor (haFGF) on brain neurons of various regions in primary culture and hippocampal long-term potentiation (LTP) in rats were investigated. Dissociated cells from 8 regions of embryonic day 16 rats were cultured in medium containing 10% serum for 1 day and chemically-defined serum-free medium with haFGF for 3 days. 10-100 ng/ml haFGF enhanced neuronal survival in the cortex, hippocampus and substantia nigra, while 10 ng/ml CS23 (modified human basic FGF) was effective in all regions tested. Next, effects of haFGF and CS23 to the increases of the spike amplitude induced by tetanic stimulation were measured in the dentate gyrus of 24 hr fasted and non-fasted rats. Ten ul of drug was i.c.v. injected before the application of tetanic stimulation. haFGF (400 ng) didn't influence the LTP induced by the tetanus of 100 pulses at 100 Hz in both fasted and non-fasted rats, but significantly facilitated the generation of LTP by tetanus of 20 pulses at 60 Hz only in fasted rats. However, 400 ng CS23 induced LTP when the tetanus of 20 pulses at 60 Hz was applied in both fasted and non-fasted states. Protein kinase C activities of hippocampal cytosol fraction was decreased with the i.c.v. injection of 400 ng haFGF in 24 hr fasted rats but not in non-fasted rats. These results suggest that haFGF might be one of regulating factors of feeding and memory and there might be different regulational mechanisms on FGF receptors from those of basic FGF.

403.15

Gene Expression of bFGF, NGF and c-fos by Glutamate Receptor Activation in Astrocytes and Hippocampal Neurons.

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Basic fibroblast growth factor (bFGF) and nerve growth factor (NGF) are multifunctional proteins for neurons and astrocytes in culture. The mechanisms regulating the expression of bFGF and NGF in neurons and astrocytes of developing or injured rat brain are not well understood. Previous work demonstrated that hydrogen peroxide induces bFGF, NGF and c-fos in astrocytes[1]. It has been shown that excitatory amino acids (EAA) induce calcium influx in hippocampal neurons.

In this study we demonstrate that EAA treatment increases c-fos and growth factor mRNA in a time dependent manner. When neurons and astrocytes were incubated in the presence of DNQX and APV the expression of the mRNA was reduced. These findings suggest a possible interaction between glu-receptor activation and growth factor expression as an important aspect of neuronal-glial communication.

1.Pechan et al. 1992, Neuroreport submitted.

403.16

SUPPRESSIVE EFFECT OF bFGF ON NEURONAL DIFFERENTIATION OF NEURAL TUBE CELLS IS DIRECTED TO POST-MITOTIC MOTONEURON PRECURSOR CELLS. Y. Kinoshita*, C. Kinoshita, H. Tanaka[1] and M. Bothwell. Dept. of Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195 and [1]Inst. for Medical Genetics, Kumamoto University Medical School, Kumamoto 862, Japan.

bFGF does not always act as a neurotrophic factor. We previously showed an adverse effect of bFGF on dissociated brachio-thoracic neural tube cells from E2.5 chick embryos, resulting in decreased number of neurons. (Soc. Neurosci., Abst. p. 44, 1991). Here we characterized these bFGF-sensitive neurons after 1.5-2 days in culture. Only about 10% of the neurons identified with anti-neurofilament antibody incorporated BrdU as visualized by double immunostaining with anti-BrdU antibody both in control and bFGF-treated cultures. Most neurons differentiating in our culture system appeared to be motoneurons: SC1 monoclonal antibody, which specifically labels motoneurons in the neural tube at this stage, stained about 90% of neurons in control cultures. With bFGF this percentage dropped to 70-80%. When neurons were stained with SC1 antibody, the bFGF effect of reducing the number of neurons was more profound as compared to that determined with anti-neurofilament antibody. Adverse effects of bFGF on neuronal differentiation were also detected in slice cultures of neural tube and whole embryo in collagen gel, although the effect was less noticeable with increasing size of explants.

These results suggest that post-mitotic motoneuron precursor cells are susceptible to the bFGF action. bFGF may alter the developmental fates of those post-mitotic cells or simply force them to remain undifferentiated or stem cell-like.

PATTERN FORMATION, COMPARTMENTS AND BOUNDARIES II

404.1

In vivo imaging of gene expression in zebrafish embryos: an insertional mutagenesis approach to neural development. C. Fulwiler*, Pamela Yelick & Walter Gilbert. Prog. in Neuroscience, Harvard Med. Sch. & Biological Labs, Harvard University.

We are interested in the genetic control of pattern formation in the nervous system. For a mutational analysis of this problem in the zebrafish, we have developed methods for insertional mutagenesis that will lead directly to the molecular characterization of the genetic loci involved.

A gene trap construct containing the LacZ reporter gene (a gift from J. Rossant & A. Joyner) allows us to identify insertions into zebrafish genes. After microinjection of DNA into eggs, expression of the reporter during development of the animal is visualized with a vital stain for ß-galactosidase. This assay allows us to pre-screen transgenic lines for insertions likely to produce phenotypes. Since the precise timing and localization of the endogenous gene's expression is revealed by the reporter, we can also screen transformants for insertions into specific genes of interest.

In our initial experiments, 599 animals were tested. We saw 19 examples of expression: 5 localized and 14 diffuse. These were pooled and 14 tested for germline integration. 6 are positive (43%), compared with 2/20 (10%) animals injected with the same construct without pre-screening.

The first of these six pre-screened lines has now been tested for a phenotype by incrossing transgenic F1's. About 1/4 of the F2's share a characteristic abnormality in their circulation which can be identified as early as 1.5 days. When the same parents are crossed to non-transgenic F1 siblings, all the F2's develop normally. We are currently attempting to determine if the trangene insertion is always associated with the phenotype by testing the individual F2 offspring.

404.2

STAGE AND CONCENTRATION SPECIFIC EFFECTS OF RETINOIC ACID ON THE DIFFERENTIATION OF *XENOPUS* HINDBRAIN AND EAR. T. J. Neary* and B. Fritzsch. Anatomy Div., Creighton University, Omaha, NE 68178.

Retinoic acid (RA) causes developmental defects of certain brain areas (Papalopulo et al., Development **113** (1991) 1145) that could result either from abnormal inductive interactions between mesoderm and neuroectoderm or from direct effects of RA on the developing CNS. To discriminate between these possibilities we exposed *Xenopus laevis* embryos (stages 15 - 21; from completion of gastrulation to completion of neurulation) to 30 min. RA pulses (5×10^{-7} - 5×10^{-6} M RA). Reticulospinal projections, known to be altered by RA (Manns and Fritzsch, Neurosci. Lett. **136** (1992) 1) and connections of the inner ear were examined in later stages with fluorescent dextran amines. Effects on the reticular formation, (supernumerary Mauthner-like cells) were found with increasing concentrations of RA as late as st 21. In the ear, the separation of the utricular and saccular macula and the formation of the horizontal canal is suppressed with exposure to increasing concentrations of RA as late as st. 21. Reductions in the numbers of sensory ganglia and efferent cells were concomittant with this effect. These data suggest that RA has a direct effect on the CNS and inner ear. Supported by Health Future Foundation.

404.3

A MONOCLONAL ANTIBODY THAT RECOGNIZES A NOVEL POSITIONALLY- REGULATED EPITOPE IN THE RAT CNS

S. Tole,* Z. Kaprielian, S. Ou, and P. H. Patterson 216-76, Division of Biology, Caltech, Pasadena, CA 91125

In an attempt to generate mAbs against downstream products of homeobox genes expressed on neuronal surfaces, we employed NT2D1 embryonic carcinoma cells. Retinoic acid induces homeobox gene expression and a neuronal phenotype in these cells. After the mouse's immune response to normal NT2D1 cell membranes was suppressed with cyclophosphamide, membranes from the same cells treated with retinoic acid were injected. Hybridoma supernatants from this fusion were screened on sagittal sections of whole E14.5 and E18.5 rat embryos.

mAb OTO (olfactory-telencephalic-otic) staining is detected from E10.5 to E14.5 in the procencephalon and part of the diencephalon. The only other areas positive for OTO are the nasal pits and the otic placodes. The rest of the nervous system and all non-neural tissues are negative. At E15, staining also appears in the glomerular layer of the olfactory bulb, the olfactory epithelium, and the lateral nasal gland. From E18 through P1, limited staining is also detected in some regions of the cortex and thalamus. In the adult, the entire brain is positive.

Immunoblotting identifies high molecular weight, proteoglycan-like antigens with a tissue distribution consistent with the immunohistochemical localization at E14.5.

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404.4

THE MURINE *HOX-1.2* GENE CONTAINS A CNS REGION SPECIFIC REGULATORY DOMAIN. M. Hunter-Ensor, A.-Q. Yu, S. Scherer, A. Messing† and J. Y. Garbern*. Dept. of Neurology, Univ. Penn., Philadelphia, PA 19104 and †Dept. Pathobiol., Univ. Wisc. Sch. Vet. Med., Madison, WI 53706.

The patterns of *Hox* gene expression during mammalian central nervous system development are remarkable for their spatial restriction. In general, within a cluster of *Hox* genes, each gene has a more rostral extent than does its immediate 5' neighbor. We have begun a study of the murine *Hox-1.2* gene to investigate mechanisms of this intriguing spatial control of gene expression.

We have examined two lines of transgenic mice bearing 3.7 kB of the *Hox-1.2* gene upstream of the translational start site fused to the E. coli β-galactosidase reporter gene. At embryonic day 12.5 there are high levels of reporter protein staining in the dorsal medulla, conforming with *in situ* hybridization studies. Staining appears to be localized to a small group of neuroblasts, which we have provisionally identified as the nucleus tractus solitarius (NTS), which is supported by labelling of this region by the carbocyanine dye dil applied to the glossopharyngeal/vagal ganglia. Interestingly, the vagus, trigeminal and facial nerves and ganglia are also stained. These latter nerves also have projections to the NTS, suggesting that *Hox* genes may participate in the specification of functionally integrated neural pathways.

The reporter gene is also expressed in the ventral horns of the caudal spinal cord and in a few lateral cells of the thoracic dorsal root ganglia. In one transgenic strain, at embryonic days 13 to 15, virtually the entire peripheral nervous system is stained, including the sympathetic chain and ganglia. In this same strain, reporter expression persists into adulthood, with prominent staining in the granular layer of the cerebellum, prepontine nuclei and the dorsal horns but not the ventral horns of the spinal cord. Our results demonstrate that one *Hox* gene has temporally regulated expression in several distinct and noncontiguous regions of the CNS. Therefore, expression of these genes is not confined to a single timepoint or single region of the neuraxis, although they may participate in positional or functional specification within those areas.

Supported by NIH K08 NS01464-01 and March of Dimes #5-FY91-0702 to JG.

404.5

EVIDENCE FOR LINKAGE OF *TES-1* AND *DLX-1*, TWO HOMEOBOX GENES EXPRESSED IN THE DEVELOPING MAMMALIAN FOREBRAIN. T. L. McGuinness, G. P. MacDonald, T. K. Koch*, and J. L. R. Rubenstein. U.C.S.F., San Francisco CA 94143.

Tes-1 and *Dlx-1* are members of the *Distal-less* family of homeobox genes and are likely candidates for regulating positional identity or cell differentiation in the developing forebrain. *Tes-1* and *Dlx-1* are both transiently expressed in the cells of the embryonic ventral forebrain that give rise to the basal ganglia, ventral thalamic nuclei, and olfactory bulb. The present studies used pulsed-field gel electrophoresis (PFGE) to determine if *Tes-1* and *Dlx-1* are physically linked to each other, as is the case for the *Hox* genes. Mouse fibroblast (A9) genomic DNA aliquots were digested with nine rare-cutting restriction enzymes, subjected to PFGE, transferred to nylon filters, and sequentially hybridized to ^{32}P-labeled *Tes-1* and *Dlx-1* cDNA probes. The probes co-hybridized to fragments generated by the four restriction enzymes: *Nru* I, *Not* I, *Sal* I, and *Sfi* I. The physical linkage of these two genes was further demonstrated by co-hybridization of the probes to DNA fragments generated by digestion with combinations of restriction enzymes. The smallest DNA fragment (generated by digestion with *Sfi* I and *Not* 1) that was recognized by both probes was approximately 45 kb. These results indicate that *Tes-1* and *Dlx-1* are physically linked within approximately 45 kb on the genome. These two genes may exist as part of a larger gene complex that acts in a coordinated fashion to regulate forebrain development.

404.6

GENETIC CONTROL OF SEGMENT-LIKE PATTERNS OF GENE EXPRESSION IN THE MOUSE CEREBELLUM. J.D. Oberdick*, R.J. Smeyne, C. Bocchiaro and J.I. Morgan. Dept. of Neurosci. Roche Institute of Molecular Biology, Nutley, NJ 07110.

We have previously shown that the gene for a Purkinje cell-specific marker, L7, can be used to drive expression of β-galactosidase in cerebellar Purkinje cells of transgenic mice. Although all Purkinje cells in the adult express both the transgene and endogenous gene, early developmental expression of both reveals a series of positive and negative bands. The number of bands increases in the medio-lateral direction during development until all Purkinje cells are positive. Truncation of the promoter region of the transgene disrupts this normal developmental pattern. Animals carrying these truncated constructs show precocious lateral expression in early dvelopment. There is also a delay in the normal "filling in" of the bands. This suggests a series of positive and negative elements in the promoter important for control of the banding pattern. In addition, these truncations have identified a minimal promoter sufficient for driving Purkinje cell-specific expression. This small promoter fragment contains within it a number of consensus binding sites for known families of developmental control genes and these sites can be footprinted by purified proteins from these families or by crude cerebellar nuclear extracts. By specifically mutating these elements we are assaying their affect on cell-specific transgene expression. We are using a PCR strategy to identify the cerebellar transcription factors themselves.

404.7

A MOLECULAR APPROACH TO CEREBELLAR COMPARTMENTATION: THE CLONING OF ZEBRIN II

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The Zebrins are Purkinje cell-specific antigens that represent striking examples of the parasagittal organization of the cerebellar cortex. Zebrin staining by immunohistochemistry appears as sharp anterior to posterior bands in the vermal region and islands (or less well registered bands) in the hemispheres. The bands have been likened to "compartments" and probably reflect a basic organizing principle of the cerebellum. To study the molecular genetic basis of Zebrin's periodic pattern of expression, we have cloned Zebrin II, a 32 kd intracellular antigen, from a cDNA expression library of postnatal day 20 C57BL/6J mouse cerebellum. Several independent recombinants expressed a single class of cDNA recognized by the Zebrin II monoclonal antibody. Partial sequence from these clones reveals a near perfect identity to that previously published for rat and human aldolase C, one of the three known aldolase isozymes. Aldolase is a glycolytic enzyme that hydrolyzes fructose-1,6-bisphosphate, and previous reports show that the expression of aldolase C is heterogeneously restricted to Purkinje cells of the cerebellum; no higher-order organization of its expression has been previously recognized. To determine whether the banded pattern of Zebrin/aldolase antigen in cerebellum was due to differential transcription, *in situ* hybridization of horizontal sections of mouse brain was performed. A strong and specific signal appears in the cerebellar Purkinje cell layer, with regional heterogeneity. The registration of this heterogeneous pattern with that of Zebrin II bands will be assessed.

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404.8

FURTHER CHARACTERIZATION OF MEANDER TAIL, A GENETIC MUTATION AFFECTING CEREBELLAR MORPHOGENESIS. C. Fletcher°, N. Heintz°, M.E. Hatten‡ and C. Mason‡*. ‡Dept. of Path., Columbia Univ. of Phys. and Surgeons, N.Y., N.Y. 10032 and °The Howard Hughes Med. Inst., The Rockefeller University, N.Y., N.Y. 10021.

We have previously described the effects of a genetic mutation on the cellular and foliar structure of the mouse cerebellum (Ross et al., PNAS 87:4189, '90). We now present further analysis of this phenotype, including a three dimensional reconstruction of the mutant cerebellum and the cellular details of early postnatal development. Frontal and coronal sections were examined to determine the lateral extent of the affected region. These sections show that the affected region includes the more medial anterior lobes (the lingula cerebelli, lobulus centralis, and the culmen) as well as lateral aspects of the declive. Specifically, the foreshortened declive fails to bifurcate laterally. The meander tail cerebellum is not only disorganized in the anterior lobes, but is significantly reduced in size. This was examined by three dimensional reconstruction of the mutant cerebellum. This analysis demonstrates that a rather large volume, comprising the "anterior" cerebellum, is completely missing. Thus, the mea cerebellum is a posterior half-cerebellum. We have also analyzed the appearance of the phenotypic features. There is a progressive failure of organization, with altered foliation and glial cell structure apparent at P0. The cells in the anterior external granule layer do not proliferate and the Purkinje cells never organize into a monolayer. By P7 the mutant phenotype is fully established. These results further implicate the regional specification of the developing cerebellum.

404.9

DEVELOPMENTAL REGULATION OF THE POU-HOMEODOMAIN GENE SCIP/TST-1 DURING CEREBRAL CORTICAL DEVELOPMENT. G.D. Frantz, L.M. Burstein, A.P. Bohner and S.K. McConnell*, Department of Biological Sciences, Stanford University, Stanford, CA 94305.

Homeodomain genes have been implicated in the generation of patterning in the mammalian CNS, but most reports have focused on segmental organization in the hindbrain. Many forebrain structures, in contrast, are patterned into layers of neurons. He et al. (Nature 340:35-42,1989) have reported that the POU-homeodomain gene Tst-1 [also known as SCIP (Monuki et al., Neuron 3:783-793, 1989) or Oct-6 (Suzuki et al., EMBO J. 9:3723-3732, 1990)] is specifically expressed in the deep layers of the rat cerebral cortex. We have examined in detail the expression of the mRNA encoding SCIP/Tst-1 using probes to both the SCIP 3' untranslated region and the POU domain of Tst-1; both probes show similar patterns of hybridization but the SCIP probe provides a somewhat cleaner signal. In the adult rat, SCIP/Tst-1 mRNA is strongly expressed in layer 5 throughout the cerebral cortex, with little labeling in other layers. The signal is clearly localized to pyramidal neurons in layer 5, which are easily identified by their large pale nuclei and low density. Expression is also apparent in other brain regions, particularly in the CA1 region of the hippocampus. The regulation of SCIP/Tst-1 gene expression during cortical development is quite complex. By E20 a strong signal is seen in the top half of the cortical plate; at this time layer 5 and 6 neurons occupy their final positions in the cortical plate but upper-layer neurons are still migrating (Berry and Rogers, J. Anat. 99:691-709, 1965). Thus the label is most likely in layer 5. During postnatal cortical development, however, SCIP/Tst-1 expression is not restricted to layer 5 neurons. In postnatal rats through at least P17, we observe a bilaminar pattern of hybridization that includes the upper layers 2/3 as well as layer 5. During later life, labeling of the upper cortical layers decreases and the adult pattern is achieved. Although SCIP/Tst-1 gene expression is not strictly confined to layer 5 pyramidal neurons during the course of cortical development, these results show that neurons within an individual layer of the adult cortex can exhibit a unique molecular phenotype. Whether SCIP/Tst-1 gene expression is involved in the generation of cell commitment to a particular laminar phenotype remains unclear.

We thank G. Lemke and M.G. Rosenfeld for providing the SCIP and Tst-1 probes. Supported by PEW Scholars, Searle Scholars, NSF PYI, and NIH EY06342.

404.10

NEURON SPECIFIC ENOLASE PATCHES IN THE DEVELOPING RAT STRIATUM. W.F. Silverman* and Y. Solberg. Unit of Morphology, Corob Center for Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel.

One of the initial events signaling the development of patch and matrix domains in the embryonic striatum is the appearance of *islands* or patches of dopamine (DA) terminals originating in a subset of mesostriatal neurons. Cells in the patches subsequently produce opiate receptors and substance-P (SP). A complementary pattern, the *matrix*, is produced postnatally following ingrowth of fibers from the remaining DA cells into the areas between the patches, followed by synthesis of neurotensin, calcium binding protein and other peptides by neurons there. This sequence of neuropeptide expression preceded by the ingrowth of DA terminals suggests an inductive role for the mesostriatal projection. We have examined the ontogeny of immunoreactivity for neuron-specific enolase (NSE), a metabolic enzyme-marker for synaptogenesis and neuronal activity with respect to that of tyrosine hydroxylase, SP and neurotensin in the striatum and ventral mesencephalon of the developing rat at the light and ultrastructural levels. The most striking findings were that NSE appeared exclusively in striatal patches until the 2nd postnatal day, where it co-localized with SP in neuronal perikarya; after P2 NSE also appeared in neurotensin-positive cells in the matrix. The data obtained support the hypothesis that DA fibers establish connections in the striatum, and induce metabolic activity and expression of neuropeptides, initially in the patches and then in the matrix. Supported by The Israel Ministry of Science and Technology.

404.11

THE DISTRIBUTION OF CHOLINERGIC NEURONS CHANGES FROM A PREFERENCE FOR THE PATCH COMPARTMENT TO A HOMOGENENOUS DISTRIBUTION IN THE DEVELOPING STRIATUM. E.H.S. van Vulpen* and D. van der Kooy. Neurobiology Research Group, Dept. Anatomy, University of Toronto, Toronto, Ontario, M5S 1A8.

Striatal cholinergic interneurons in the rat are generated early in development (E12-E17) and choline acetyl transferase activity (CHAT) can be detected as early as E13.5 in the striatum. Early born neurons in the striatum preferentially end up in the patches and late born neurons in the matrix compartment. This birthdate data leads one to expect that the cholinergic neurons will be located mainly in the patches. However, in the adult rat these large sized cholinergic cell bodies are distributed more or less homogenously in the two compartments of the striatum. To study the distributions of cholinergic cells during development, a retrograde tracer was injected in the substantia nigra at postnatal day (PND) 2 to label the patches. The pups were sacrificed after different survival times and vibratome sections were cut and stained with an antibody against CHAT. The results show that early in development (PND 3), cholinergic neurons are located preferentially in the patches. Within the patches they are located primarily near the border between patch and matrix. At PND 17, an increased proportion of cholinergic neurons are found in a small region of the matrix just around the patches. Thus, we hypothesize that during early postnatal development some cholinergic neurons may migrate out of the patches or perhaps cholinergic neurons in the patches undergo cell death. An additional possibility is that some cholinergic neurons in the matrix do not express CHAT until relatively late in development. Absolute counts of the number of CHAT staining cells in relation to other striatal neurons during development may help to discriminate among these possibilities.

404.12

DEVELOPMENT OF MORPHOLOGICAL CORTICAL COLUMNS IN HUMAN CORTEX. E. Armstrong* and D. Buxhoeveden, A.F.I.P., Washington, D.C. and Anthropology Department, University of Chicago, Chicago, IL.

A new method that quantifies the morphology of cortical cell columns was used to analyze the changes from single-cell ontogenetic columns to the adult configuration in Tpt of the human brain. Nissl stained coronal sections of normal brains from the Yakovlev Collection were digitized. Seven adult, three children and nine fetal brains were analyzed.

Results indicate that in layer III the cells continue to grow apart horizontally, possibly as a result of the growing space between cell columns until late in maturation. The adult configuration had not been reached by 11 years. On the other hand, the pattern of the cellular arrangement is established as early as 29 or 32 gestational weeks. Laminae II and IV differ from III in that the adult pattern of cell arrangement develops much later.

The data show the complexity of anatomical plasticity. Even a small structure like a morphological cell column has nonuniform rates of development. Furthermore in the language area, anatomical plasticity extends into late childhood. This raises interesting questions about the evolution of language and the importance of late childhood for human behavior.

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404.13

ANONYMOUS MARKERS OF CELLULAR SIGNALLING DURING CORTICAL DEVELOPMENT. W.E. Kaufmann* and P.F. Worley. Neuropathology Laboratory and Departments of Neuroscience and Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Cellular interactions are critical during brain development and play a role in neuronal migration and cytodifferentiation. Histochemical markers that are sensitive to these intercellular signals will be extremely useful for studying normal and aberrant neocortical histogenesis. Differential screening techniques have been used to identify two clones representing previously uncharacterized genes that are expressed in brain as early as E15 and that are rapidly induced by synaptic activity in the adolescent brain. Both are expressed primarily in cortex and hippocampus but exhibit distinct laminar patterns. At E21-P1, clone # 8 is expressed in highest levels in the most superficial layers of the neocortex and in pyramidal (CA1-CA4) hippocampal neurons. By contrast, at the same developmental stage, clone # 59 is expressed in all layers in the dorsomedial (somatosensory and cingulate) neocortex and the dentate gyrus. Our preliminary observations suggest that histochemical markers for these genes may be useful to study the role of activity in development of discrete populations of cortical neurons.

404.14

POSTNATAL DEVELOPMENT OF THE STRIATAL MOSAIC IN RHESUS MONKEY. L.J. Martin* D.M. Spicer and L.C. Cork. Johns Hopkins Univ. Sch. Med., Baltimore, MD. 21205.

The postnatal striatal ontogeny in *Macaca mulatta* was studied immunocytochemically in 6 male monkeys (1 day and 1,4,6,9,12 months), 2 female monkeys (1 and 4 months), and 2 adults. Sections were concurrently processed for substance P (SP), leucine-enkephalin (LENK), tyrosine hydroxylase (TH), calbindin (CAL), choline acetyltransferase or Nissl. In the first postnatal year, monkey striatum undergoes considerable changes in cyto- and chemoarchitecture. Striatal maturation progresses ventral to dorsal and medial to lateral. Early postnatally, neuronal density decreases significantly; ingrowth of dopaminergic nigrostriatal afferents (islands enriched in TH processes) overlap patches enriched in SP neurons but devoid of LENK and CAL neurons. Regions destined to become matrix initially contain sparse TH processes and few SP neurons but are densely populated with LENK neurons. After 4 months, TH afferents densely innervate matrix; SP neurons and processes increase in matrix; LENK neurons decrease in matrix and increase in patches; cholinergic neurons decrease. At 12 months, striatal chemoarchitecture is similar to, but does not completely match, adult patterns. Developmental patterns of male and female monkeys are similar, but striatal maturation is accelerated in females. Maturation of primate striatum is dynamic, gender-specific with synchronous changes in neuronal density, afferent ingrowth, and neuronal phenotype expression.

404.15

EXCITATORY AMINO ACID RECEPTORS COLOCALIZE WITH ACETYLCHOLINESTERASE-RICH STRIOSOMES IN FETAL CAT STRIATUM. Leon S. Dure IV*, Anne B. Young, John B. Penney, Massachusetts General Hospital, Boston, MA. 02114

Afferent connections to mammalian striatum are compartmentalized within caudate and putamen. These compartments, termed striosomes and matrix, differ with respect to efferent connections as well, allowing for modulation and integration of striatal neurotransmission. Striosomes and matrix may be identified by differences in acetylcholinesterase (AChE) activity The primary corticostriatal neurotransmitters are excitatory amino acids (EAA), which are divided pharmacologically into *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole propionic acid (AMPA), kainic acid (KA), and phosphoinositol-related metabotropic receptor (metabotropic 1) subtypes depending on agonist affinity. We have previously demonstrated in the adult human that AMPA binding sites are primarily localized in matrix, and KA binding sites are localized in striosomes. To investigate the distribution of EAA binding sites among striatal compartments during development, we have studied binding to receptor subtypes in fetal cat striatum using quantitative *in vitro* receptor autoradiography. Using AChE as a marker for striosomes, we have determined that NMDA, AMPA, KA, and metabotropic 1 binding sites are located primarily in the AChE-rich striosomal compartment during the latter half of gestation in the fetal cat. Since EAA are known excitotoxins, these findings may in part explain the inhomogeneous striatal pathology observed as a sequel of perinatal hypoxia/ischemia in the human. This work supported by NICHD grant 1K11HD00983.

404.16

STRIOSOMAL ORGANIZATION OF DOPAMINE TERMINALS AND ACETYLCHOLESTERASE DURING POSTNATAL DEVELOPMENT OF THE RAT. H.K. Happe*, L. Arqueros and L.C. Murrin, Dept. of Pharmacology, Univ. of Nebraska Med. Ctr., Omaha, NE 68198-6260.

In postnatal striatum several neurochemical markers are concentrated in striosomes. We examined the organization of dopamine (DA) uptake sites by autoradiography with [^{125}I]RTI-55 and acetylcholinesterase histochemistry (AChE). [^{125}I]RTI binding is found in striosomes on day 1. Binding in striosomes and matrix increases through day 7 with 40% more sites in striosomes. By day 10 matrix sites have increased so striosomes are less apparent. There is a lateral to medial and a rostral to caudal gradient in the appearance of striosomes. On day 1 the head of the striatum has only a lateral subcallosal streak, but by day 3 striosomes are found. In the body of the striatum both the callosal streak and lateral patches are seen on day 1. On day 3 patches appear in a gradient from dorso-lateral to the ventromedial region. More caudally there is no callosal streak but there are patches on day 1 and a large single patch in the ventro-medial region. In the tail of the striatum there is a single streak of [^{125}I]RTI binding extending ventro-dorsally near the center of the structure. This streak is present day 1 through day 10. AChE corresponds extremely well to [^{125}I]RTI binding sites at day 1. The close match continues through day 5 in the lateral striatum and through day 10 in the more caudal regions. By days 7 and 10 AChE increases from lateral to medial, however correspondence of patches can still be observed in the body of the striatum. In the central and medial regions there is a lack of prominent patches for AChE staining. It has been suggested that AChE may be produced by DA neurons. However 6-hydroxydopamine lesions on day 2 deplete >95% of striatal DA by day 4 but do not affect the distribution or apparent density of AChE. Therefore AChE staining is not likely to be on DA neurons and may be a useful marker for cholinergic terminals during early postnatal development. Supported by NS23975 and the Burroughs Wellcome Fdn.

404.17

DEVELOPMENT OF TOPOGRAPHY IN THE GENICULOCORTICAL PROJECTION OF THE RAT. Gavin Dixon* Department of Physiology, University of Sydney NSW 2006 Australia.

Relay neurons of the adult rat dorsal lateral geniculate nucleus (DLG) project onto area 17 of the cerebral cortex with a high degree of topographic order. To study the timecourse of development of this precision, small particles (~20μm diameter) of DiI and DiA were placed superficially into rostral and caudal sectors (respectively) of presumptive area 17 of albino rats at three ages: post-conceptional days (PCD) 20, 24 and 30. Coronal sections through the DLG at PCD20 revealed two large, diffuse and overlapping populations of single-labelled relay cells. By PCD24 two distinct, partially-overlapping groups of single-labelled cells were oriented along separate lines of projection within the DLG. Further, when taking into account the cortical position of the dye placements, these groups were located in the topographically appropriate parts of the DLG as determined electrophysiologically in the adult animal. These features were observed at PCD30, at which time the geniculo-cortical projection was first observed to arborize within cortical layer 4. However, compared to PCD24, each group of labelled cells had a smaller locus (as seen in coronal section) with respect to the areal size of the DLG. These findings indicate that considerable order exists within the geniculocortical projection several days before afferents reach cortical layer 4, the main termination zone within area 17.

404.18

MOUSE CEREBRAL NEOCORTEX IS ORGANISED INTO DEVELOPMENTAL MODULES. S-S Tan*, Embryology Laboratory, Department of Anatomy, The University of Melbourne, Parkville 3052, Victoria, Australia.

Cell lineage plays an important role during development of the mammalian neocortex, providing a link between cell division and cell migration. Transgenic mice carrying the *lacZ* marker gene provide a genetic method of marking progenitors cells in the ventricular zone. Following X-chromosome inactivation, the marker in half of these cells is switched off, generating functional genetic mosaicism in the neocortex. The resulting pattern did not show a "salt and pepper" mixture of marked and unmarked cells, instead the neocortex was delineated into broad bands of variable widths which we termed developmental modules. These bands were first seen in the ventricular zone layer but later extended into the cortical plate. In the adult brain, each module appeared either as a blue or clear band with clearly defined borders, suggesting that a module has arisen from radial cell migration. However, there was also a significant degree of horizontal migration across modules, these were seen as blue cells in clear areas, and vice versa. These horizontally-migrating cells were mostly neurons, and they comprised about one-third of the total cortical cells in a given area. Taken together, the results suggest that the cortex is basically assembled by modules whose final positions reflect the initial mosaicism of the germinal neuroepithelium. It would also appear that the neuroepithelium is not completely devoid of form-generating capability, and that the non-dispersing modules may serve to provide a stabilizing scaffold upon which the growing cortex is built. More importantly, this study would provide support for the radial column hypothesis, while also affirming the presence of other cortical cells that are capable of extensive horizontal migration.

GLIA AND OTHER NON-NEURONAL CELLS III

405.1

GLIAL CELL RESPONSE TO SUBSTRATE-BOUND ADHESION MOLECULES. H.R. Payne* and V. Lemmon. Department of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

During neurogenesis and regeneration, both soluble and nondiffusible factors provide environmental cues for glial cell development. We examined the influences of purified extracellular matrix molecules and cell adhesion molecules on the development and proliferation of glial cells from neonatal rat optic nerves. Dissociated optic nerve glia were plated on nitrocellulose-bound fibronectin, laminin, collagen type IV, L1, n-cadherin, and NCAM. Cultures were grown in chemically-defined medium to promote formation of oligodendrocytes. Other cultures were grown in 10% serum to support type-1 astrocytes and the differentiation of progenitor cells to type-2 astrocytes. Short term adhesion assays were used to measure cell affinity for the different substrates. In these experiments, glial cell types displayed characteristic patterns of substrate preference. The glial cells developed distinctive morphologies on different substrates after incubation for 4 days. Measurements of BUdR incorporation showed that the substrates did not significantly influence cell proliferation rates. Our results indicate that progenitor cells, oligodendrocytes, type-1 astrocytes, and type-2 astrocytes possess different complements of receptors for the adhesion molecules in their environment. Transduction of this substate-receptor binding signal may induce cytoskeletal alterations that produce the observed morphological differences in glial cells.

405.2

NEURITE OUTGROWTH OF OLFACTORY SENSORY AXONS ON IMMORTAL OLFACTORY BULB ASTROCYTE AND ENSHEATHING CELL LINES. M. N. Goodman*, J. Silver, J. W. Jacobberger. Depts. Genetics and Neurosciences, Case Western Reserve Univ., Cleveland, OH 44106.

Rat primary olfactory synapses are located in glomeruli and can be regenerated during adulthood. It has not been determined whether the unusual structure or the regenerative capacity of the glomerular synapses is due to unique growth properties of the olfactory sensory neurons (OSNs), to unique properties of the olfactory bulb (OB) glia that surround the OSN axons and glomeruli, or both. However, the organization and phenotypes of two types of OB glia, OB astrocytes and ensheathing cells, suggest that the formation of neural circuitry in the OB, as in other areas of the nervous system, is controlled by glial-neuronal interactions. OB astrocytes resemble type-1 astrocytes, and some of the OB astrocytes encircle the glomeruli and express the molecules J1 and chondroitin sulfate proteoglycan (CSPG) that have been associated with cordones or axon barriers. Ensheathing cells resemble Schwann cells and ensheath OSN axons from the glial limitans to the glomeruli. Further, OB astrocyte cell lines with the characteristics of the glomeruli-encircling glia promote very low neurite outgrowth by chick retinal ganglion neurons (CRGNs), as expected of glia which form barriers, while other OB astrocyte lines and ensheathing cell lines support high CRGN outgrowth, as expected of glia that form axon-growth permissive pathways. In this study, rat OSN neurite outgrowth has been tested over the same cell lines. The results are that neonatal OB astrocyte lines that express J1 and CSPG support very low OSN outgrowth, while other neonatal astrocyte lines and ensheathing cell lines support higher outgrowth, and adult ensheathing lines support higher OSN outgrowth than any other OB glial cell lines. Together, the chick and OSN outgrowth results are consistant with the following hypotheses: 1) during development, some OB astrocytes may form cordones that delineate the glomeruli, while ensheathing cells guide the axons to the glomeruli, and 2) during adulthood, ensheathing cells and perhaps also some of the OB astrocytes permit OSN axon growth, allowing regeneration of the glomerular synapses.

405.3

THE NG2 PROTEOGLYCAN INHIBITS NEURITE OUTGROWTH FROM CEREBELLAR GRANULE NEURONS. C.Dou* and J.M.Levine, Dept. of Neurobiology and Behavior,SUNY at Stony Brook,Stony Brook,NY 11794

The NG2 antigen is a high molecular weight, chondroitin-sulfate proteoglycan. It is a cell surface marker for O2A progenitor cells *in vitro* and identifies a population of developing glial cells *in vivo*, some of which differentiate into oligodendrocytes. Proteoglycans can play important roles in the regulation of axon elongation. Therefore, we tested the ability of immuno-affinity purified NG2 to either support or inhibit neurite elongation *in vitro*.

Tissue culture surfaces were coated with poly-L-lysine followed by either laminin alone ($0.3\mu g/cm^2$) or laminin mixed with NG2 ($6\mu g/cm^2$). Cerebellar granule neurons were isolated from postnatal day 5 and 6 rat pups on discontinuous Percoll gradients and seeded onto these surfaces in media supplemented with bFGF. After 24hr, the cultures were fixed and the extent of cell attachment and neurite outgrowth quantitated.The neurons attached equally well to both substrates, however, 66% of the cells extended neurites on laminin, while only 23% of the cells grew processes on the laminin plus NG2 coated surfaces. The extent of neurite outgrowth also differed between the 2 types of surfaces. On laminin, the mean neurite length was 92.5±11.4 microns whereas on laminin plus NG2, mean neurite length was 54±12.2 microns. Digestion of NG2 with chondroitinase ABC did not remove the growth-inhibitory activity. When the laminin concentration was increased to $8\mu g/cm^2$, NG2 no longer inhibited neurite outgrowth. The glycosaminoglycans, chondroitin sulfate and hyaluronic acid , each inhibited neurite outgrowth when used at $15\mu g/cm^2$ in this assay. These data suggest that NG2 is one of a number of proteoglycans that can inhibit neurite outgrowth. Cells that carry the NG2 proteoglycan on their surfaces within developing neural tissues may provide an unfavorable substrate for axon elongation.

405.4

INCREASED EXPRESSION OF THE NG2 PROTEOGLYCAN AFTER BRAIN INJURY. J.M.Levine* and A.K.Levine, Dept.of Neurobiology and Behavior,SUNY at Stony Brook,Stony Brook,NY 11794

The NG2 antigen is a chondroitin-sulfate proteoglycan that is a cell surface marker for neonatal O2A progenitor cells *in vitro*. It also marks a population of unusual glial cells in adult animals. To determine whether these adult cells participate in the glial reaction to injury, we made small puncture lesions in the cerebelli of anesthetized adult rats and analyzed the distribution of NG2-immunoreactivity at intervals after the lesion. The distribution of the NG2 proteoglycan was compared to that of markers for astrocytes, ramified microglia and monocytes.

At 24hr after lesion, NG2-positive cells adjacent to the damaged area had enlarged processes. By 48hr post-lesion, the number of NG2-positive cells had increased. These cells had large cell bodies with punctate deposits of NG2-immunoreactivity at their surfaces. At this same time, ED1-positive monocytes invaded the damaged tissue and the number of OX42-positive microglia increased. NG2-immunoreactivity in the damaged tissue continued to increase up to 5-7 days post-lesion and declined thereafter. At 4-5 days post-lesion, GFAP immunoreactivity increased as an astrocytic scar formed around the damaged area. An increased density of NG2-positive fibers persisted within the damaged tissue for up to 45 days post-lesion. These changes in NG2-immunoreactivity were confined to the damaged tissue; areas distant from the lesion site appeared normal. The increase in NG2-positive cells at the lesion site is likely due to cell division since NG2-positive cells bodies became labeled with silver grains after administration of a pulse of ^3H-thymidine at 24 and 48hr post-lesion. These studies demonstrate that the NG2-positive glia of adult animals participate in the reaction to brain injury. The NG2 proteoglycan inhibits neurite outgrowth *in vitro* (Dou and Levine,this volume); increased expression of NG2 after injury may contribute to the failure of CNS neurons to regenerate successfully.

405.5

Differential effects of median and lateral mesencephalic astrocytes on neuritic growth. L.A. Cavalcante*, V. Moura-Neto, J. Garcia and S.L. Carvalho. Instituto de Biofisica, UFRJ, 21941 Rio de Janeiro, RJ, Brazil.

Radial glia in median and lateral sectors of the mesencephalon are heterogeneous in several respects (Barradas et al., Glia 2:103, 1989). In this study, we have tested for regional differences of mesencephalic astrocytes on neuritic growth. Dissociated cells from either median or lateral sectors of mouse embryo mesencephali (MMN or LMN) were (1) cultivated onto confluent cultures of astrocytes from either sector (MMG or LMG) for 2 days and co-cultures reacted with an anti-MAP2 antibody, (2) cultivated onto poly-l-lysine in serum-free medium with addition of conditioned medium from MMG or LMG cultures. Both MMN and LMN on LMG grow long and varicose neurites whereas both MMN and LMN on MMG tend to aggregate and show diminutive neurites. These features are also mimicked by conditioned medium from LMG or MMG. These results suggest (1) permissive vs. non-permissive effects of LMG vs. MMG on neuritic growth, respectively, (2) mediation of effects, at least, partially by soluble factors.

(Support: CNPq, FINEP, CEPG/UFRJ)

405.6

CULTURED ENSHEATHING GLIA FROM ADULT RAT OLFACTORY BULB ENFOLD OLFACTORY NEURITES. A.Ramón-Cueto, J.Pérez, P. Bovolenta* and M. Nieto-Sampedro. Cajal Institute, 37 Doctor Arce, 28002 Madrid, Spain.

Ensheathing glia is a type of macroglia exclusively present in the olfactory bulb. To gain insight into the mechanisms underlying axonal regeneration in the olfactory bulb, we have studied ensheathing glia and its interaction with olfactory neurites in culture at the ultrastructural level. Three morphologically and ultrastructurally different cell types were identified in secondary cultures from adult rat olfactory bulb: process bearing cells, macrophage-like cells and endothelial-like cells. Examination of these cultures showed that only process bearing cells were immunoreactive for GFAP and NGF receptor (NGFR). These cells shared immunocytochemical and ultrastructural properties with those described for ensheathing glia *in vivo* and were the only type capable of enfolding olfactory neurites *in vitro*. Therefore, process bearing cells are ensheathing cells. When cells from adult rat olfactory bulb were co-cultured with olfactory epithelium explants, olfactory neurites preferentially grew over NGFR positive cells. Olfactory neurites were NGFR-positive when growing either alone or over NGFR positive cells, but lost NGFR-immunoreactivity when enfolded by ensheathing cells. NGFR immunoreactivity disappeared from both, neurite and glial contact surfaces, but was kept in the glial membrane not involved in ensheathing in a comparable way to that descibed for Schwann cells *in vivo*. In summary, ensheathing cells retained *in vitro* both, the *in vivo* ultrastructure and the ability to ensheath olfactory neurites.

405.7

COAGGREGATION OF NEURONS AND GLIAL CELLS IN RAPHE AND CORTICAL CELL CULTURES. F. C. Zhou* and S. Bledsoe. Indiana Univ. Sch. Med., Dept. Anatomy, Indianapolis, IN 46202.

5-HT and cortical neurons from fetal brain have distinct morphological and aggregational patterns in cell culture. Raphe and cortical cells from E14 fetal brain were plated on 24-well plates coated with poly-D-lysine and laminin. Immunostained 5-HT neurons dispersed along with raphe cells throughout the well, while cortical cells form large aggregations after 2 days in culture (DIC). Mainly, diffused fibers were formed in raphe culture, and fasciculated fibers in cortical culture at 14 DIC, with distinct cell aggregation pattern described above. These cultures, when stained for astrocytic marker glial fibrillary acidic protein (GFAP), showed distinct astrocytic patterns closely resembling corresponding neuronal groups. Astrocytes aggregated around neurons with a diameter 3-5 times that of neuronal aggregation. Neuron-glial interactions may play a role in formation of distinct morphology. To test if glia and/or neurons from one brain area can influence another, raphe cells were co-cultured with cortical cells. A mixture of aggregated and diffused neurons as well as astrocytes were found, with 5-HT neurons diffused and non-5-HT neurons aggregated. These results suggest (a) glial cells from raphe and cortex which shared same GFAP marker are morphologically and functionally different, (b) interactions between neurons and corresponding glial cells are regional specific, and (c) specific aggregations between glial cells and neurons are probably contact-based and are not altered by diffusible factors.

405.8

BIOPHYSICAL PROPERTIES OF GAP JUNCTION CHANNELS BETWEEN SCHWANN CELLS. M. Chanson, K. Chandross, R. Dermietzel, J. Kessler and D.C. Spray.* Dept. of Neuroscience, A. Einstein Coll. Med., Bronx, N.Y. 10461.

Observations *in vivo* and *in vitro* suggest that direct intercellular communication participates in the control of proliferation and differentiation of Schwann cells. To characterize the gap junction channels expressed by these cells, we applied the dual whole cell voltage clamp technique on primary and cultured cell pairs. Because macroscopic junctional conductance (g_j) of Schwann cells is very low, averaging 500 ±120 pS (mean±SEM, n=32 cell pairs), and is steeply dependent on transjunctional voltage (V_j), recording of unitary junctional conductance (γ_j) without the use of uncoupling agents was possible. Frequency histograms of γ_j values revealed a distribution well described by a single Gaussian curve with a mean of about 40 pS (n=2,193 events, 25 experiments). This observation indicates that the junctional current (I_j) is carried through only one type of channel. Therefore, relaxation of I_j elicited by V_j can be modeled by a Boltzmann relation; best fit values for Boltzmann parameters were V_0 (V_j at which g_j is reduced by half) = 15 mV, g_{min}/g_{max} (ratio of minimal to maximal g_j) <0.1 and n (the number of equivalent charges moving through the field) =3. Compared to V_0 values for other gap junctions, Schwann cell junctions are among the most voltage-sensitive. Time courses of junctional current relaxations were always well fit (r^2>0.9) by a single exponential for V_j ±10 to 60 mV. Calculation of rate constants indicates that the fraction of channels that are in the open state is predominately determined by the closing rate constant. The connexin (Cx) protein encoding these channels is unknown but differs from Cx43, 32 and 26 as determined by immunostaining with specific antibodies and Northern blot analysis with specific cDNA probes. Molecular cloning of the cDNA encoding this novel gap junction channel is underway.

405.9

GROWTH FACTOR INDUCED PHENOTYPIC CHANGES IN CULTURED SCHWANN CELLS INCLUDE ALTERED GAP JUNCTIONAL CONDUCTANCE. K.J. Chandross*, M. Chanson, D.C. Spray, J.A. Kessler. Albert Einstein College of Medicine, Bronx, New York 10461.

Schwann cell proliferation, morphology, and gene expression are altered after nerve injury. These changes are reproduced in vitro by agents which elevate intracellular levels of cAMP and by growth factors which are released after nerve damage. We have examined changes in gap junctional conductance in cultured Schwann cell pairs which occurred in association with other phenotypic changes. After treatment with forskolin (F:2uM) in combination with bovine pituitary extract (BPE:10ug/ml), the cells became more spindle shaped, and cell proliferation and low affinity nerve growth factor receptor (NGF-R) expression were increased. Junctional conductance was significantly elevated from 0.56 nS$\pm$0.11(SEM, n=27) before treatment to 1.23 nS$\pm$0.24(SEM, n=19)($p < 0.01$). By contrast, after exposure to transforming growth factor beta, (TGFβ_1:10ng/ml) cells displayed a flatter, multipolar morphology, cell proliferation was not increased and NGF-R expression was decreased. Further, compared to control values, TGFβ_1 significantly reduced cell coupling in a time dependent manner; after 48 hours of treatment junctional conductance was 0.05 nS$\pm$0.03(SEM, n=7)($p < 0.03$). Neither F+BPE nor TGFβ_1 altered the characteristics of the gap junction channels (i.e. voltage dependence or unitary conductance), suggesting that changes in coupling resulting from these treatments reflected the number but not the type of channels that were expressed. Electrical or chemical coupling may help to coordinate Schwann cell responses to injury and other stimuli. These observations indicate that the strength of intercellular communication between Schwann cells changes in response to alterations in the cellular milieux providing a possible mechanism for modulating functional interactions between Schwann cells and their environment.

405.10

THE EFFECT OF CENTRAL AXOTOMY ON REMOTE MICROGLIA IN THE RAT SPINAL CORD. S. Hong,*P.J. Reier, W.J. Streit, Depts. Neurological Surg. and Neuroscience, Univ. Florida Coll. Med., Gainesville, FL, USA.

Microglial responses in the rat spinal cord following a hemisection at T9 were examined at levels rostral and caudal to the lesion. Microglia were visualized histochemically using the *Griffonia simplicifolia* B_4-isolectin (GSA). Expression of Ia-antigens on microglia was shown immunohistochemically with OX-6 mAB. In addition, astrocytes were visualized by GFAP staining. Lectin staining showed two distinct patterns of the microglial response. First, activated microglia appeared 1 day post-injury (DPI) and were widespread in both the white and gray matter rostral and caudal (C4-C5, T4-T5, T12 and L1) to the level of the lesion. Activated microglia remained prominent for up to 2 weeks in the white matter and 4 weeks in the spinal gray areas. The second pattern of microglial reactivity was confined to the area of fiber tracts undergoing degeneration. The onset of this response was as early as 3 DPI and lasted as long as 2 months. In the dorsal funiculus rostral to the level of the lesion the microglial reaction was confined to the fasciculus gracilis (FG) of the lesioned side without involving the adjacent corticospinal tract (CST). Caudally, the response was confined to the degenerating CST without involvement of the FG. By 5 days post-injury, a subpopulation of activated microglial cells showed expression of Ia antigen. The localized activation of microglia in regions of degenerating white matter (i.e. FG rostral to the lesion), and not in adjacent, intact regions (i.e. CST rostral to the lesion) suggests a non-diffusible triggering mechanism for the initiation of the degeneration-induced microglial response. The astrocytic reaction, on the other hand, showed a delayed onset (5 DPI), mostly in the degenerating fiber tracts, and the reaction was less intense than the microglial response during the early survival times. At longer survival times (>4 weeks), however, microglia and astrocytes displayed a largely parallel response in temporal and spatial distribution patterns. (Supported by NIH P01-NS 27511)

405.11

MICROGLIAL RESPONSES TO NEUROTOXIC ABLATION OF SEROTONERGIC AXON TERMINALS. M.A. Wilson* and M.E. Molliver. Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

Microglial cells are a heterogeneous class of non-neuronal cells in the CNS which respond to neuronal damage. Microglia exhibit a number of characteristic features which are altered in response to CNS injury: "activated" microglia undergo morphologic changes, up-regulation or novel expression of macrophage/monocyte antigens, and may proliferate and become phagocytic[1]. The response of microglia varies, depending on the nature of the particular injury; e.g., neuronal death vs. axotomy[2].

We have examined the response of microglial cells in the forebrain to the selective chemical axotomy of 5-HT axon terminals induced by p-chloroamphetamine (PCA). Rats were treated with PCA (10 mg/kg, s.c., x2) and sacrificed 3 days, 6 days, 3, 6, or 9 weeks later. This drug destroys fine 5-HT axon terminals in the forebrain, but spares preterminal 5-HT axons and cell bodies. Antisera directed against 6 microglial antigens were utilized as markers for microglial activation in PCA treated rats, untreated controls, and in rats with unilateral facial nerve transection. Morphologic changes in microglial processes and increases in staining for two of the antigens were detectable 6 days after PCA treatment. Three weeks after PCA, increased expression of four of the microglial antigens was detected, as well as increased branching of microglial processes and changes in the intracellular distribution of one of the antigens. By 6 weeks after treatment, these alterations were less marked; at nine weeks the microglia in treated animals resembled those in controls.

[1]Perry & Gordon '91; [2]Streit et al. '89. (Support: NIDA DA04431, 271-90-7408)

405.12

A QUANTITATIVE ANALYSIS OF MICROGLIAL AND ASTROGLIAL CELL REACTIONS IN PRIMARY SENSORY PROJECTION AREAS FOLLOWING PERIPHERAL NERVE INJURY IN THE ADULT RAT. P. Eriksson, J. Persson, M. Svensson, J. Arvidsson, C. Molander and H. Aldskogius*. Dept. Anat., Karolinska Institutet, Box 60400, S-104 01, Stockholm, Sweden.

Recently, it has become clear that proliferation of microglial cells and hypertrophy of astrocytes may be a general component in association with the central processes of peripherally axotomized sensory ganglion cells. For that purpose, by the use of a computerized scanning system, we have analyzed the time course for the microglial (antibody OX-42) and astroglial (antibodies to GFA and in situ hybridisation for mRNA-GFA) cell reaction in the L4 dorsal horn, the column of Clarke and the gracile nucleus after sciatic nerve transection as well as in the trigeminal nucleus after infraorbital nerve transection. In all areas examined the microglial cell reaction started 24 to 48 hours and peaked one to two weeks postoperatively. Thereafter there was a gradual decline in the reaction over a period of several months. The astroglial cell reaction seemed to parallell the microglial cell reaction. These observations demonstrate that peripheral nerve injury induces a rapid and prominent response among microglial and astroglial cells in all the appropriate primary sensory projection areas.

405.13

CALCIUM PERMEABLE CHANNELS IN MICROGLIA. M. Jia, M. X. Li, C. A. Colton, and D. L. Gilbert*. Lab. of Biophysics, NINDS, NIH, Bethesda, MD 20892 and Dept. of Physiology and Biophysics, Georgetown Univ. Med. School, Washington, DC 20007.

Cytosolic calcium is important for the activation of microglia. An influx of calcium through calcium-permeable channels may be a means of increasing cytosolic calcium. To study these channels, whole cell recording and single cell recording experiments were performed on cultured rat microglia cells. The bath solution was (in mM): 145 KCl, 1 $CaCl_2$, 1.8 $MgCl_2$, and 10 HEPES; the patch solution was (in mM): 110 $BaCl_2$ and 10 HEPES. Non-inactivating inward currents were observed at hyperpolarized membrane potentials. The single channel conductance was about 8 pS. The open probability was voltage-independent. We also report on a voltage-gated L-type calcium channel utilising a whole-cell recording mode. The bath solution was (in mM): 120 TEA-Cl, 15 $CaCl_2$, 2 $MgCl_2$, 10 TRIS-HEPES; the pipette solution was (in mM): 120 CsCl, 20 TEA-Cl, 10 Cs-HEPES, 2 $MgCl_2$, and 10 EGTA. Small long-lasting inward currents were activated with depolarizing pulses at a holding potential of -40 mV. The currents were enhanced with 1 µM Bay K 8644. Due to a lack of an outward K^+ current, a slight activation could result in a sustained membrane depolarization (H. Kettenmann et al., 1990. J. Neurosci. Res. 26:278-287). Increased K^+ from injured surrounding cells could possibly depolarize the microglia and activate this L-type channel.

405.14

POTASSIUM MODULATION OF MICROGLIAL SUPEROXIDE RADICAL ION PRODUCTION. C. A. Colton*, J. Keri, and D. L. Gilbert. Lab. of Biophysics, NINDS, NIH, Bethesda, MD 20892 and Dept. of Physiology and Biophysics, Georgetown Univ. Med. School, Washington, DC 20007.

Microglia are the resident CNS macrophages and are found at sites of trauma in the brain. Since injured cells release potassium into the extracellular fluid, we have examined the effect of high extracellular potassium on microglial function. Using a cytochrome c reduction assay in the presence and absence of superoxide dismutase (SOD), we have measured the production of oxyradicals by resting and stimulated cultured neonatal rat microglia. In order to maintain a normal ionic strength and osmolarity, potassium was substituted for sodium in all experiments. When potassium concentration was changed from a normal of 5 mM to a high of 55 mM, there was no change in resting superoxide radical ion production. A significant increase was seen, however, when phorbol myristate acetate (PMA) was used to activate the microglia to produce the superoxide radical ion. In the presence of 5 µg/ml PMA, potassium concentrations of 25 and 55 mM initiated a 115 percent and a 127 percent increase, respectively, over the PMA stimulated release in normal potassium. This enhancement was partially blocked by 10 µM nifedipine. These data suggest that changes in extracellular potassium at sites of injury may serve to modulate microglial release of oxyradicals.

405.15

MODULATION OF MOUSE MICROGLIA FORM AND IMMUNE REACTIVITY BY GOLDFISH OPTIC NERVE FACTOR(S).

Robert W. Keane*[1] and G.W. Perry[2]. [1]Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, FL 33101 and [2]Program in Complex Systems and Brain Sciences, Florida Atlantic Univesity, Boca Raton, FL 33431.

Damage to central nervous system tissue is associated with infiltration of immune cells and the activation of microglia at the site of injury. The factors regulating activation of microglia have not been characterized. We have utilized the goldfish optic nerve (GFON), a well-characterized neural system which demonstrates a vigorous regenerative capacity following injury, to establish whether this neural tissue produces substance(s) that affect microglia morphology and immune reactivity. Microglia purified from mouse brain were grown in medium conditioned (CM) by GFON, optic tectum, vagal lobe, cerebrum and cerebellum, and medium conditioned by rat optic and sciatic nerves. Microglia maintained in GFON-CM assumed an ameboid morphology, whereas microglia grown in media conditioned by the other nerves produced long crenellated processes that resembled the ramified microglial form. Microglia maintained in all types of CM functioned as antigen-presenting cells in a MHC-restricted manner when tested on conalbumin-specific T_{helper} cells, except for microglia maintained in GFON-CM. The failure of these microglia to present antigen was not changed by treatment of the GFON-CM with neutralizing antibodies against TGF-β or TNF-α. These studies demonstrate that microglia form and immune reactivity can be modulated by factor(s) released by GFON.

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MOTOR SYSTEMS I

406.1

CHANGES IN LOCOMOTION AND NEUROMUSCULAR DEVELOPEMENT IN THE NEONATAL RAT ACCOMPANY SIMULATED WEIGHTLESSNESS. K.D. Walton[1], J. Jacoby*[2], K. Ko[2], S.A. Williams[1] and R. Llinas[1], Depts. Physiology & Biophysics and Ophthalmology, NYU Medical Center, 550 First Ave., NY, NY, USA, 10016

Tail suspension of rat pups postnatal day 8 (P8) to P13 leads to abnormal swimming and stepping (Walton et al, Neurosci. Soc. Abst. 16, 937, 1991). This study asked if: (1) irreversible deficits in motor skills result from an extended period of susp. and (2) susp. alters hindlimb (HL) muscle devel. Pups were susp. P8-P13, P8-P21, P13-P21, or P13-P31. Each susp. (S) animal had a non-suspended companion (C). Free walking was videotaped with high speed camera (nac, 200 fps) and analyzed with Peak 2D motion analysis system. Swimming was unaffected in pups S on P13. Stepping in all S was slow with hyperextension of foot and ankle joints. Animals taken down on P13 or P21 recovered. Deficits persisted in those S until P31, e.g. maximum ankle extension on P60 was $128 \pm 2.8°$ in S ($n=6$) and $97.6 \pm 3.2°$ in C ($n=6$) ($p<0.001$). Effects of susp on HL muscle devel. were complex. The % slow twitch fibers in soleus (SOL) was decreased in S pups at the end of suspension (P13, P21, P31), as assayed by a McAb for type 1 (slow/beta) myosin HC. Slow fibers in SOL were signif. smaller and neonatal myosin expression in SOL and peroneus muscles was different in S pups. Pups S from P13-P31 showed persistence of neonatal myosin on P31. Studies of righting reflexes (Skorina et al, Neurosci. Soc. Abst. 1992) and swimming, suggest a set of critical periods for motor development. Patterns of changes in devel. muscle should be interpreted in terms of these critical periods. (Supp: NASA, and NIH EY06232 to J.Jacoby)

406.2

HINDLIMB SUSPENSION IN NEONATAL RATS LEADS TO PERMANENT DEFICITS IN AIR RIGHTING REFLEXES. Jane Skorina[1], Kerry D. Walton*[2], Dean Hillman[2] and Rodolfo Llinás[2], Depts. of [2]Physiology & Biophysics and [1]Otolaryngology, NYU Medical Center, 550 First Ave, NY, NY U.S.A. 10016

Unloading the hindlimbs (HL) of rats by tail suspension from P8 to P21 leads to reversible slowing of the air righting reflex (ARR) (Walton et al, IBRO Absts. 1991). In this study rats were suspended from P13 to P31 to find if the critical period for development of the ARR was the same as that for other motor behaviors and if extending the suspension period would lead to persistent deficits. The pups were suspended by their tails at 30°, and each suspended (S) animal had a non-suspended companion (C). The ARR, elicited by dropping the animals from 50cm onto a padded surface, was videotaped at 200fps. Righting of the head (H), forelimbs (FL), and HL were measured. Righting of the HL was slower in S than C pups on P19 (241.8 ± 5.1 msec compared to 212.3 ± 11.9 msec, $p \leq 0.004$). On P31 the mean S and C HL righting times were significantly different at the $p \leq 0.0001$ level (250 ± 3.1 and 221 ± 6.4msec) Righting times for the H and FL were not significantly different in the two groups. The differences in HL righting persisted at P60 indicating the existence of a critical period in the development of the central vestibular system (Dieter's nucleus and the vestibulo-spinal tract) beginning near P14 and ending by P31, a period different than that for swimming, but similar to that for walking These data suggest the existence of a set of "critical periods" organized in a hierarchical manner corresponding to the acquisition of particular motor skills. Preliminary morphological results indicate that the density of neurotransmitters, specifically glycine, may be decreased in Dieter's nucleus in S animals. Supported by NASA.

406.3

A KINEMATIC AND ELECTROMYOGRAPHIC STUDY OF LOCOMOTION IN THE KITTEN. L. Girard*, T. Cabana, and T. Drew, Depts. Physiology and Biology, Université de Montréal, Québec, Canada, H3C 3J7.

To better understand the developmental processes of locomotion, a detailed kinematic and electromyographic (EMG) analysis has been undertaken in the kitten. Eight (8) kittens between the ages of 2 and 3 weeks were chronically implanted for the recording of EMG activity from flexor and extensor muscles of the fore- and/or hindlimbs. Video recordings were always made simultaneously with the EMG recordings. Most kittens were capable of steady treadmill locomotion at a slow speed ($0.1\ m.s^{-1}$) at 3 weeks of age, but they had difficulty in supporting their weight, and the hindlimbs were placed in abduction. Analyses of the changes in joint angle, at this age, showed that there was a pronounced yield at the beginning of stance at the ankle and, to a lesser degree, at the elbow. By 6 weeks of age, joint angles resembled those measured in adult cats. Even at 3 weeks of age the EMG recordings resembled closely those seen in the adult cat, with flexor and extensor muscles showing strict alternation, with little evidence of co-contraction. Although at 3 weeks there were some subtle differences in the relative timing of several of the muscles, in particular with respect to the flexor muscles of the hindlimb, these disappeared by 6 weeks. These results suggest that kittens are capable of generating a normal pattern of locomotor activity at an early age, and that many of the differences in the locomotor capacities of young kittens, compared to adults, are due to their inability to fully support their weight. Supported by the FCAR and the FRSQ.

406.4

EARLY CHANGES OF PARVALBUMIN-IMMUNOREACTIVE PRIMARY AFFERENT FIBERS IN THE RAT SPINAL CORD FOLLOWING NEONATAL NERVE INJURY. L.Greensmith, J. Dekkers & R. Navarrete* Dep. Anatomy, Charing Cross & Wesminster Med. Sch. and Univ. College London, U.K.

Neonatal nerve injury leads to rapid changes in motoneuron synaptic excitation from afferents in the injured nerve (Navarrete, J.Physiol. 438:220P,'91). Here we have studied the distribution of primary afferent fibres around injured motoneurons using a monoclonal antibody to parvalbumin (PV) which preferentially labels large diameter primary afferent fibres (Zhang et al, J.Comp.Neurol. 302:715,'90). Motoneurons were pre-labelled by injection of fluorescent tracers into ankle flexor muscles at birth (P0). At P2, the common peroneal (CP) or the sciatic nerve was crushed unilaterally. At P7 and P14 spinal cords were processed for PV immunocytochemistry. In some cases, motoneurons were injected intracellularly prior to processing. At 7 and 14 days after sciatic crush, the density of PV staining in the ventral horn was markedly decreased on the lesioned side. After CP crush, decreased PV staining was largely confined to the area occupied by the CP motoneuron pool. On both sides of the spinal cord some PV immunoreactive fibres were found in close aposition to the soma and dendrites of labelled flexor motoneurons. We are mapping the distribution of PV-immunoreactive fibers on the somatodendritic surface of injured and uninjured motoneurons.

These results show that neonatal nerve injury causes a rapid decrease in PV-immunoreactive primary afferent fibres around lesioned flexor motoneurons. This may, in part, be attributed to death of dorsal root ganglion neurons.

406.5

DOES BUOYANCY MASK THE POTENTIAL FOR COORDINATED MOTILITY *IN OVO*? S.H. Chambers* and N.S. Bradley. School of Physical and Occupational Therapy, McGill Univ., Montreal, QC, Canada H3G 1Y5.

Spontaneous embryonic motility is characterized by orderly recruitment of agonist and antagonist muscles but corresponding coordinated movement may be masked by mechanical variables such as buoyancy (Bradley & Bekoff, *Devel. Psychobiol.* '91). As part of ongoing kinematic studies, we are now examining motility in chick embryos under conditions of reduced buoyancy *in ovo*.

Amniotic fluid (2 ml) was extracted and continuous video recordings (1 hr) were made on embryonic day 9 for each of 5 embryos. Video records of entire movement sequences (40 s) for 2 embryos were computer analyzed at 60 Hz, the data filtered and corrected for movement out of plane, to measure concurrent wing and leg activity and to compare findings to data for 4 control embryos.

Preliminary findings indicate that reduced buoyancy does not alter the primary features of motility. Movement sequences consist of 5-11 cycle periods (peak extension) for both wing and leg in experimental and control embryos. Time between movement episodes (152 s) and movement duration per episode (33 s) is also similar between groups. Further, linear trend analyses for experimental embryos suggest that relative timing of shoulder/elbow (r^2=0.51) excursions and hip/knee/ankle (r^2=0.43) are similar to controls (r^2=0.47;0.44). While reduced buoyancy does not appear to alter primary features of motility, experimental embryos are not posturally displaced during movements or amniotic contractions and their movements appear more orderly as compared to controls. This work was supported by FCAR and NSERC.

406.6

CHRONIC SPINAL GAP TRANSECTION IN CHICK EMBRYOS: A KINEMATIC ANALYSIS. N.S. Bradley* and S.H. Chambers. School of Physical and Occupational Therapy, McGill Univ., Montreal, QC, Canada H3G 1Y5.

EMG (Bradley and Bekoff, *J. Neurobiol.* '92) and observation studies (Oppenheim, *J. Comp. Neurol.* '75) suggest that development of motility is altered in the absence of descending neural inputs in chick embryos. Thus, a kinematic study was undertaken to examine these changes as part of a series of studies underway to explore the interaction of neural and extraneural factors in motor development.

Midthoracic spinal transections were performed on embryonic day 2 (E2) and continuous video recordings (1 hr) made on E9 *in ovo* for each of 9 embryos with histologically verified transections (silver stain, 12μ sections). Video records of entire movement sequences (90 s) for 2 embryos were computer analyzed at 60 Hz, the data filtered and corrected for movement out of plane, to measure concurrent wing and leg activity and to compare findings to data for 4 control embryos.

Linear trend analyses for spinal embryos suggest that relative timing of shoulder/elbow (r^2=0.35) excursions and hip/knee/ankle (r^2=0.45) are similar to controls (r^2=0.47;0.44). However, leg cycle periods (peak extension) are shorter for spinals (1-3s) vs controls (2-7s). Also, movement sequences for spinals contain more cycles (leg 16-25; wing 16-20) vs controls (leg 3-9; wing 4-12). While leg and wing intra- and inter-episode durations are similar between groups, spinal embryos are more active per episode (49 vs 27%) due to disassociation of wing and leg activation. These findings suggest that loss of descending neural control significantly alters temporal features of both intra- and interlimb coordination. This work was supported by FCAR and NSERC.

406.7

THE EFFECT OF VARYING THE SEGMENTAL LEVEL OF SPINAL CORD HEMISECTION ON REACTIVE SYNAPTOGENESIS IN THE PHRENIC NUCLEUS. H.G. Goshgarian* and X.-J. Yu. Dept. of Anatomy, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

Previous results have shown that a spinal cord hemisection at C2 causes an increase in the number of multiple synapses (MS) contacting phrenic motoneuron profiles at the C3-C6 levels of the spinal cord. The reactive synaptogenesis may be caused by lesioning the descending bulbospinal respiratory pathways to the phrenic nucleus or by the generalized effects of spinal cord injury (i.e., edema, ischemia, and the interruption of other inputs) in the immediate area of the phrenic nucleus. To evaluate these possibilities, we carried out a hemisection at either C2, C7, or T4 in 3 groups of rats and allowed a survival period of 7 days. The number of terminals forming multiple synapses and the number of synaptic active zones (SAZ) contacting HRP labeled phrenic motoneurons profiles were counted at EM levels in the phrenic nucleus ipsilateral to hemisection. Seven days post hemisection the percent decrease in synaptic active zones was 86% (C2), 90.7% (C7), and 91.6% (T4) as compared to controls (100%). In spite of the decrease in SAZ, the number of multiple synapses increased after cervical hemisections (35.5 ± 5.2 (C2), 34.0 ± 7.4 (C7) as compared to controls (28.3 ± 3.3), but the number of multiple synapses decreased following the T4 lesion (22.8 ± 4.4). These results suggest that the degree of reactive synaptogenesis in the phrenic nucleus is dependent upon the distance of the spinal cord hemisection site from the target neurons.

406.8

THE EFFECT OF A CONDITIONING LESION AT DIFFERENT LEVELS IN THE SPINAL CORD ON THE QUANTITATIVE ASSESSMENT OF CROSSED PHRENIC NERVE ACTIVITY IN RATS. X.-J. Yu* and H.G. Goshgarian. Dept. of Anatomy, Wayne State University Sch. of Med., Detroit, MI 48201.

Previous studies have demonstrated a rather weak expression of the crossed phrenic reflex (CPR) following C2 spinal cord hemisection and an immediate contralateral phrenicotomy in young adult rats (Exp. Neurol. 111:244-250, 1991). There is a significant increase in reflex activity, however, if there is a delay following C2 hemisection before contralateral phrenicotomy. The present study was carried out to determine if conditioning lesions at either the C7 or T4 levels of the spinal cord may also influence the CPR when a postlesion delay of seven days is allowed before the reflex is induced. There were 4 groups of rats: a control group (left C2 hemisection and right phrenicotomy with no interoperative delay), and 3 experimental groups each receiving a C2, C7, or T4 hemisection with a 7 day delay before induction of the reflex . Quantitative assessment of the CPR was accomplished by determining the mean integrated area of phrenic nerve compound action potentials under standardized recording conditions. The results showed that the CPR is significantly increased in the C2 hemisection group (97 ± 30mm^2, P=0.0003), C7 hemisection group (57 ± 14mm^2, P=0.01), and T4 hemisection group (31 ± 8 mm^2, P=0.04) as compared to reflex activity in the control group (12 ± 2 mm^2). These results suggest that a conditioning lesion in the spinal cord causes an increase in CPR activity. Furthermore, the amount of the increase is dependent upon the spinal cord level of the conditioning lesion.

406.9

ULTRASTRUCTURAL CHANGES IN THE RAT PHRENIC NUCLEUS 2 HOURS AFTER SPINAL HEMISECTION. M.A. Sperry*and H.G. Goshgarian. Dept. of Anatomy, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

This study extends our earlier analysis of injury-induced morphological changes in the rat phrenic nucleus (JCN, 284:519,1989) by examining the nucleus at 2 hrs. post-hemisection (p.h.). Phrenic motoneurons were identified at EM levels by horseradish peroxidase labeling. Micrographs were analyzed qualitatively and quantitatively in both normal and spinal hemisected rats. Our results showed a significant increase in the percentage of dendrodendritic appositions from a normal level of 4.73±0.18% to 8.58±0.54% at 2 hrs. p.h. Although there was not a significant increase in the mean percentage of single and multiple synapses at 2 hrs. p.h. (as first seen at 4 hrs. p.h. in our earlier study), a new finding showed a significant increase in the mean lengths of asymmetrical and symmetrical synaptic active zones from normal lengths of 0.372±.009μm and 0.404±.007μm to 0.410±.011μm and 0.513±.032μm respectively in hemisected rats. Lengthening of active zones in the phrenic nucleus could enhance synaptic efficacy by increasing contact area, and thus may be part of the mechanism underlying functional recovery in our spinal cord injury model.

406.10

DECREASED NUMBER OF DOPAMINERGIC SUBSTANTIA NIGRA NEURONS AFTER NEONATAL QUINOLINATE STRIATAL LESION. A. Macaya*, E.M. Janec, D.C. De Vivo, R.E. Burke. Departments of Pediatric Neurology and Neurology, Columbia Univ., New York, NY 10032.

We have reported that a neonatal axon-sparing striatal injury induced by hypoxia-ischemia results in a diminished adult number of substantia nigra (SN) tyrosine hydroxylase (TH)-positive neurons (Soc Neurosci, 1991). This decrease occurs in the absence of direct nigral injury. To examine the specific role of striatal injury, we made selective axon-sparing striatal lesions in the newborn rat with quinolinate (40, 80, 120 and 480 nmols). We then counted the number of SN TH-positive neurons and measured by image analysis the area of SN pars compacta when animals reached adulthood (12-16 weeks). Striatal lesions resulted in a decrease in the number of TH-positive neurons in the ipsilateral SN, and the decrease was correlated with the reduction in striatal area (r=0.76, p<.01). There was no direct injury to the SN. At 50-60% striatal area loss, there was a 25-30% reduction in SN TH-positive neurons. The area of SN pars compacta (SNpc) in animals with 120 (N=5) and 480 (N=10) nmol lesions was reduced 12% and 15%, respectively (p<.01) and this reduction also correlated with the loss of striatal area (r=0.63, p<.01). In animals with the 480 nmol lesion, there was no alteration in individual TH-positive neuron size determined by image analysis (C:173±3; E:167±3 um^2) or neuron packing density (C:713±38; E:717±44 neurons/mm^2) in the SN on the experimental side. We conclude that the adult number of SN dopaminergic neurons depends on developmental support by the striatum. We hypothesize that the smaller striatum provides less trophic support to SNpc, resulting in an accentuated developmental cell death, and a diminished adult number of neurons.
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406.11

REHABILITATION OF MOTOR DISFUNCTIONS IN PATIENTS WITH INFANTILE CEREBRAL PALSY. M.S.Sinyaya[*2], O.V. Bogdanov [1], D.U.Pinchuc [1], A.M.Shelyakin [1]. Institute of Exper .Med.[2]Pavlov Institute of Physiology of the Acad.Sci.RAN, St.Petersburg,Russia, 199034.

Previous studies demonstrated that one of the main factors of normal formation and development of structure of central nervous system is afferentation from motor apparatus. In recent studies we used the local stimulation of the periferal neuro-muscular system and polarization of the motor cortex. Using this data we developed a method of functional biocontrol (FBC) which aids to increase the rehabilitation of motor disfunctions. Application of this method had high clinical efficiency. We observed the reconstruction of movement, normalization of EEG, EMG, reflex responses of spinal motoneurons and normalization of the habituation process on long-term stimulation of sensory systems. We have shown that this method restors the functional asymmetry in the cortex and spinal cord. Using the method of FBC allows to provide rehabilitation and to decrease the period of treatments 1,5- 2,5 time.

406.12

CALBINDIN D-28k DOES NOT SEEM TO BE CO-LOCALIZED WITH TYROSINE HYDROXYLASE IN CULTURES OF DISSOCIATED RAT SUBSTANTIA NIGRA NEURONS. P.-Y. Côté*, E. Roy, F. Tardif, P.J. Bédard and A. Parent, Neurobiology Res. Center, Fac. of Med., Laval Univ., Québec, Canada.

Despite its wide distribution in the central nervous system, the role of calbindin D-28k (CB), a calcium-binding protein, is still poorly understood. Recently, Lavoie et al. (1991) have suggested a possible neuroprotective role for this protein, in the primate model of Parkinson's disease since tyrosine hydroxylase (TH)-positive neurons containing CB seem to be less severely affected by the toxin 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridin (MPTP) than those who do not contain CB. The aim of this study was to investigate the ontological appearance of CB in neurons of rat substantia nigra and the degree of co-localization of CB with TH-positive cells *in vitro*. In sterile conditions, ventral midbrain tissue containing groups A9 and A10 was dissected from rat embryos at 14 days gestation, enzymatically and mechanically dissociated, then plated on 13 mm-culture disks coated with poly-L-ornithine as substrate. These cultures were grown from 1 hour to 10 days before being fixed by cold paraformaldehyde. The culture disks were then immunohistochemically reacted following standard procedures to reveal TH- and CB-immunoreactive profiles.

TH-positive neurons are present in all cell cultures. In cultures grown for 1 hour, round to oval cell bodies without neurites display TH-immunoreactivity. Immunoreactive neurites appear in cultures grown 6 hours. The immunoreactive neurons form clusters and have the characteristic shape of dopaminergic neurons, being mostly polygonal and elongated with two main processes arising at each pole of the cell body. In contrast, the immunoreactivity for CB appears in cultures grown for 6 days. CB-immunoreactive cells are oval, smaller than the TH-positive neurons and also tend to aggregate in the form of clusters. In double-labelling experiments *in vitro*, CB-positive cells were clearly distinct from the TH-positive cells. [Supported by FRSQ and MRC and Canadian Network of Centres of Excellence].

406.13

THE EFFECTS OF NEONATAL CORTICAL OR CEREBELLAR LESIONS ON THE NUMBERS OF CORTICOSPINAL (CS) NEURONS IN ADULT RATS. D.L. O'Donoghue*, +D.R. Humphrey and C.R.D. Poff. Dept. Anatomical Sciences, the University of Oklahoma Health Science Center, Oklahoma City, OK 73190; +Lab. of Neurophysiology, Emory University, Atlanta, GA 30322.

Previously, we showed that 40,000 cortical cells have transient spinal axons that are eliminated by the second postnatal week. The present study determined if increased numbers of CS cells could be retained into adulthood when the terminal fields for growing corticofugal axons are modified. Targets available to growing axons were expanded in the spinal cord by the ablation of the opposite cortex. Brain stem targets, such as the basilar pontine nuclei, were removed after cerebellectomy. Aspiration lesions were performed on postnatal day (PND) 2 prior to cortical axons reaching the mid-cervical (C5) spinal cord or the development of corticopontine projections. The left frontal and parietal cortices or the cerebellum were removed from pups anesthetized by hypothermia. Pups matured to PND 30 when they were re-anesthetized with methoxyflurane. Fast Blue (FB) soaked pledgets were inserted into the CS tract at C5 of all animals. Serial section analysis of FB labeled cells indicate that neonatal cortical lesions do not alter the distribution or number labeled CS cells as compared to litter-mate controls. Cerebellectomy, although incomplete in cases, caused qualitative and quantitative increases in the distribution and number of CS cells that could be labeled. These data indicate that enlarged spinal target areas do not result in more CS cells. By contrast, the loss of either the brain stem targets (i.e. basilar pons), or the ascending inputs from the cerebellum, resulted in an increased number of labeled CS neurons. Neonatal hemispherectomy or cerebellectomy result in similar alterations in microstimulation maps of evoked forelimb movements from the cerebral motor cortex. However, the present study indicates the substrate for these alterations in stimulation mapping are different. (Grant support by OU BRSG funds (DOD) & NIH-NS20146 (DRH).

REGENERATION III

407.1

REGENERATING FROG OPTIC NERVE AXONS: DO GLIA GUIDE THE GROWING TIPS? P.M. Orkand* and R.E. Blanco. Inst. of Neurobiology and Dept. of Anatomy, Univ. of Puerto Rico Sch. of Med., San Juan, PR 00901

Unlike those of mammals, injured optic nerves of frogs can regenerate and eventually form functional connections. Since frog retinal ganglion cell (RGC) axons are slow to degenerate after separation from their cell bodies, the regenerating tips may follow the course of the degenerating axons after a crush injury. In preliminary experiments aimed at studying the relationship of glial cells with regenerating axons in the absence of the degenerating ones, we looked at the structure of the proximal tip of the optic nerve 1-12 weeks following transection and deflection of the distal stump.

At early time periods, there is macrophage activity at the cut end, and glial cells are phagocytosing debris of retrograde degeneration in the nerve stump, where regenerating axons begin to appear. After 4 weeks, bundles of putative regenerating fibers lie along phagocytic cells and astrocytes that appear to have migrated into the cut end. Their identification will be confirmed by HRP fillings of RGCs.

Supported by NIH NS07464 and NSF EPSCoR II.

407.2

LEPTOMENINGEAL CELLS CAN INDUCE CHANGES IN ASTROCYTES IN VITRO. R. Ness and S. David*. Centre for Research in Neuroscience, Montreal General Hospital Research Institute and McGill University, Montreal, Canada.

Leptomeningeal (Lm) cells rapidly migrate into penetrating wounds of the CNS. Astrocytes in the parenchyma of the CNS that interface with these Lm cells transform to form the glia limitans. We have used an in vitro approach to study whether direct interactions with Lm cells induce morphological and functional changes in astrocytes.

DiI labeled astrocytes purified from the neonatal rat cerebral cortex were plated onto monolayers of Lm cells, astrocytes or polylysine coated glass coverslips. 18-20 hours later the cultures were fixed and the size of the DiI labeled astrocytes determined using an IBAS image analysis system. Astrocytes were smaller in size when plated on Lm as compared to astrocytes or polylysine. When neurite outgrowth on these astrocytes was assessed by plating purified cerebellar granule cells, there was a 40% reduction in the ability of the astrocytes plated on Lm to support neurite growth, compared to astrocytes plated on astrocyte monolayers. These results suggest that leptomeningeal cells can induce both morphological and functional changes in astrocytes, and may account for some of the astrocytic changes at the site of CNS lesions. (Supported by MRC, RHMMLF, FRSQ and FCAR)

407.3

ASTROGLIAL RESPONSE TO REGENERATING CNS AXONS PROMOTED BY PNS TISSUE TRANSPLANTATION IN INJURED RAT SPINAL CORD. M. Hasegawa*, H. Taguchi, N. Uchiyama, T. Yamashima, J. Yamashita. Dept. of Neurosurg., Kanazawa Univ. Sch. of Med., Kanazawa, 920, Japan

We have recently developed a useful model to promote axonal regeneration in the unilaterally injured rat spinal cord (Advance in Neurotrauma Research 3: 41-44, 1991). Central neurons in the injured rat (female Wistar rat, 90-190 g body weight) spinal cord showed regrowth and remyelination in the autografted PNS bridge of peroneal nerve. HRP study demonstrated that the regenerated and remyelinated axons originated from the neurites of the spinal cord. In this paper, we examined relationship between astroglia and regenerated nerve fibers in this model. The examination of the graft-spinal cord interface revealed that GFAP-positive zone did not inhibit the regrowth of CNS axon into the graft. The A2B5 antibody-positive cells were less conspicuous at the graft-inserted site of the spinal cord, whereas glial processes at the anterolateral column located far from the graft-inserted site were clearly stained with the A2B5 antibody. Electron microscopically, in the graft close to the graft-inserted site, many processes of Schwann cells as well as some of astrocytic processes were attached to the surface of basal lamina scaffolds. Regenerating axons were always engulfed by the processes of Schwann cells. There was neither invasion of astrocytic process into the periaxonal space between myelin and axolemma, nor phagocytic activity of PNS myelin by astrocytes during the period of observation. In the spinal cord, regenerated nerve fibers with PNS type myelin could be seen especially along the perivascular space. Astrocytic processes were closely apposed to the regenerated axons with or without glial basal lamina. These results indicate that astrocytic reaction does not impede the regeneration, rather astrocytes, especially type-1, might support the regeneration of CNS nerve fibers in the milieu of this model.

407.4

THE FUNCTIONAL PROPERTIES OF PURIFIED POPULATIONS OF CULTURED HUMAN SCHWANN CELLS STUDIED IN VIVO. A.D.O. Levi[1], V. Guenard[1], P. Aebischer[2], R.P. Bunge[1]*. [1]The Miami Project and Dept. of Neurological Surgery, Univ. of Miami School of Medicine, Miami, FL 33136 and [2]Brown Univ., Artificial Organ Laboratory, Providence, RI 02912.

Methods are now available to purify populations of Schwann cells (SCs) derived from adult human nerve (Morrissey et al, J. Neurosci. 11:2433-2442, 1991). To determine the functional capacity of human SCs isolated in culture, these cells were introduced into an immunodeficient rat using a peripheral nerve injury model. Permselective guidance channels were seeded with 30:70 solution of Matrigel[R]: DMEM (M/D) with or without human SCs at a density of 80 million cells/ml. Channels were implanted within an 8 mm gap of the transected sciatic nerve of nude female rats for a period of 4 weeks. The number of myelinated axons and cable surface area was uniformly greater in channels seeded with human SCs when compared to channels containing M/D only. Survival of the transplanted human Schwann cells was established by dissociating explants taken from the regenerated cable after 5 days in culture and immune staining for primate and rat specific nerve growth factor receptor and S100. These results also indicated that the regenerated cable contained a mixture of human and rat (host) SCs. Myelination of regenerating rat axons appeared to be occurring by both donor and host SCs. We are using immune staining for HNK-1, an antigen found in human but not rat myelin, to establish the presence of human myelin segments within the regenerating cable. These studies indicate that purified populations of cultured human SCs survive and enhance axonal regeneration when transplanted into the injured peripheral nervous system of the immune deficient rat. (Supported by NS19923 [NIH/NINDS], Gliatech, Inc., and The Miami Project. Dr. A. Levi is a Fellow of the Medical Research Council of Canada.)

407.5

INTERACTION OF CENTRAL AXONS WITH SCHWANN CELLS OF PERIPHERAL NERVOUS TISSUE. W.K.D. CHAU, P. DOCKERY, K.-F. SO* and K.C. LAU. Dept. of Anatomy, University of Hong Kong, 5 Sassoon Rd., Pokfulam, Hong Kong.

Optic nerve of SD rat aged 6 weeks was cut intra-orbitally and replaced by a sciatic nerve graft. After 9 months, the graft containing the regenerating optic axons was processed for electron microscopic examination. Various morphometric measurements were made on the myelinated fibers of the regenerating (n=6), aged-matched normal optic (n=6) and sciatic (n=6) nerves.

The relationship between myelin and axon was examined by plotting the myelin area against the axon area (based on perimeter). It was found that the mean slope of linear regression line for the regenerating group (0.54± 0.043) showed a closer resemblance to that of the optic (0.64±0.024) rather than to the sciatic group (1.29± 0.081). The mean g-ratio (axon diameter/fiber diameter), which indicates the relative sheath thickness, was different among the three groups. However, there is a tendency for the regenerating group to overlap with the optic group (eg. 16%, 64% and 98% of sciatic, regenerating and optic fibers respectively have a g-ratio above 0.68).

Thus, central axons regenerating in peripheral nerve environment seem to be able to alter the myelin production of the Schwann cells in such a way that it supports the idea of axon-dependence in the process of myelination.

407.6

MAPPING THE ROLE OF TRANSPLANTED ADULT RAT SCHWANN CELLS IN PROMOTING VENTRAL ROOT REGENERATION M.L. McCormack[1]*, T.K. Morrissey[2], D.C. Xiong[1], R.P. Bunge[2], and P. Aebischer[1] Brown University, Providence, RI[1], and The Miami Project, Univ. of Miami School of Medicine, Miami, FL[2]

Semipermeable guidance channels do not support the regeneration of ventral root axons across a large gap in adult rats. The guidance channel's luminal environment was modified using inbred adult rat-derived primary Schwann cells (SC) seeded in a laminin-containing gel (LCG) to provide an axonal outgrowth-promoting substrate. Adult Fisher rat-derived SCs were cultured and used at passages 0 to 5 post-dissociation. Culture purity ranged from 94 - 46% SCs. Semipermeable channels were seeded with SCs (5-6 x 10^7 cells/ml) in LCG, or the LCG alone, and tested in a L5V root 8 mm gap injury model in adult male Fisher rats. Twenty-one rats received SC grafts and 8 rats received LCG grafts. Regeneration was assessed at 4 weeks post-implantation by counting the total number of axons on cross sections at the channel's midpoint. Regenerated cables were found in 19 out of 21 of the SC graft recipients (mean total axons = 2630 ± 270), while only 2 of the LCG grafts resulted in cables containing a small number of axons (mean = 302 ± 225). Passage number and SC purity did not significantly affect the total number of axons in the regenerated cables. In order to delineate the role that the grafted SCs play in the regeneration process, a recombinant retrovirus encoding human placental alkaline phosphatase (AP) was used to generate a population of AP-expressing primary adult inbred rat SC cultures which were then tested in the L5 ventral root model. Histochemical analysis of the regenerated cables at 4 weeks revealed the presence of AP-positive cells and demonstrated the ability of transplanted SCs to myelinate and ensheath host axons.

407.7

SUPPRESSION OF MYELINATION EXTENDS THE PERMISSIVE PERIOD FOR FUNCTIONAL REPAIR FOLLOWING INJURY OF THE EMBRYONIC CHICK SPINAL CORD. H.S. Keirstead, S.J. Hasan, G.D. Muir and J.D. Steeves*. Depts. of Zoology & Anatomy, UBC, Vancouver, B.C., V6T 1Z4.

Previous studies have demonstrated that a transected embryonic chick spinal cord loses it's capacity for anatomical repair and functional recovery on embryonic day (E) 13. The onset of spinal cord myelination is coincident with this transition from permissive to restrictive repair periods. To assess a possible inhibitory role of myelin in regeneration, we have delayed the onset of myelination (dysmyelination). In such an unmyelinated embryonic cord, the subsequent assessment of spinal cord repair after a restrictive period transection (eg. E15) would serve as a test of whether myelin inhibits anatomical and functional repair. On E9-E12, direct injection into the thoracic spinal cord of a galactocerebroside monoclonal antibody with a source of serum complement resulted in dysmyelination until E17, as determined by myelin basic protein immunocytochemistry. A subsequent thoracic cord transection as late as E15 (i.e. during the normal restrictive period for repair) resulted in neuroanatomical repair and functional recovery (normally myelinated embryos showed no anatomical repair or functional recovery after an E15 transection). Retrograde labelling of brainstem regions, after lumbar injection of a fluorescent tracer, indicated that anatomical repair of brainstem-spinal projections in dysmyelinated, transected chicks was indistinguishable from unoperated control animals. Behavioral and physiological observations from dysmyelinated chicks were also indistinguishable from unoperated control hatchlings. This indicates that that the myelination process is inhibitory to the repair of transected spinal cord in embryonic chick. (supported by Canadian NCE for Neural Regeneration and Functional Recovery)

407.8

PROTEINS ASSOCIATED WITH SPINAL CORD INJURY, MYELINATION, AND THE TRANSITION FROM PERMISSIVE TO RESTRICTIVE PERIODS FOR SPINAL CORD REPAIR. Douglas W. Ethell* and John D. Steeves. Depts. of Zoology & Anatomy, UBC, Vancouver, B.C., V6T 1Z4.

Previous studies in our lab have established that embryonic chick loses its capacity for substantial spinal cord regeneration around embryonic day (E) 13. Correlating with this transition, we have examined changes in protein expression using high resolution two dimensional gel electrophoresis (2D). We have identified 2 sets of proteins; early neural proteins (ENPs) which are continuously present during spinal cord development between E7-E12 and then decrease to relatively low levels, and late neural proteins (LNPs) which are only expressed at high levels after E13. Some of these ENPs and LNPs may play direct or indirect roles in establishing permissive or non-permissive environments for the outgrowth of axotomized fibers. Spinal cord myelin also appears at E13 and has been suggested to inhibit axonal regeneration. We have also examined changes in the levels of these ENPs and LNPs after: 1) spinal cord injury (transection) and 2) delaying the onset of myelination (dysmyelination).

Embryos were subjected to complete spinal cord transections during the permissive (E10) and restrictive (E15) periods for axonal repair. Expression profiles of the ENPs and LNPs were examined for recovery periods, in increments of 2 days, and compared with the unperturbed developmental expression profiles. Two of the ENPs appear to be up-regulated in response to spinal cord injury. In dysmyelinated animals, the higher expression of some LNPs was delayed until well after E13, suggesting that they are myelin associated. These preliminary studies have used high resolution silver staining to visualize the proteins. Currently, we are attempting to confirm and expand on these results with isotopic labelling techniques. (Supported by the MRC of Canada)

407.9

RESPONSE OF MACROPHAGES IN DORSAL ROOT GANGLION TO PERIPHERAL NERVE INJURY. X. Lu and P.M. Richardson*. Div. of Neurosurgery, Montreal Gen. Hosp. & McGill University, Canada H3G 1A4 The signals following peripheral nerve injury that induce a regenerative programme in the nerve cell bodies may involve cells in the vicinity of nerve cell bodies. Previously, the recruitment of inflammatory cells to a dorsal root ganglion was shown to stimulate axonal regeneration in the corresponding dorsal root. More recently, immunohistochemistry with monoclonal antibodies to a cytoplasmic macrophage marker (ED-1), the Ia antigen (OX-6), and the complement 3 receptor (OX-42) were performed on cryostat sections of fifth lumbar dorsal root ganglia removed 0-16 days after sciatic nerve transection. With all three antibodies, immunopositive cells were present in normal ganglia, the total number being several thousand per ganglion. Immunopositive cells had increased in number by 4 days after injury and were 3-8 times more numerous than normal after 16 days. The higher counts could result from recruitment of macrophages and/or increased immunoreactivity of resident macrophages. The modest inflammatory response surrounding sensory nerve cell bodies is a potential stimulus to regeneration of their axons.

407.10

RESPONSES OF RETINAL GANGLION CELLS TO AXOTOMY: DIFFERENCES WITH AND SIMILARITIES TO PERIPHERAL NEURONS. M. R. Wells* and U. Vaidya. Nerve Regeneration Research Laboratory, Dept. of Veterans Affairs Med. Center, Northport, N.Y. 11768 and Dept. of Neurology, SUNY, Stony Brook, N.Y. 11794.

The reaction of retinal ganglion cells to intraorbital crush lesions of the optic nerve was investigated over the first two weeks after injury using the binding of [^{3}H]Actinomycin D (Act. D) to nuclei of neurons in tissue sections. Changes in nuclear binding of Act. D reflect structural changes in chromatin associated with transcriptional activity. Adult, male,Wistar rats received intraorbital crushes of the optic nerve and were utilized at 0,1,2,3,4,5,7,8,9, 11, and 14 days after injury. Binding of nuclei for Act. D decreased sharply at one day after injury followed by a transient increase to above normal levels at days 2-3. The remaining pattern of response was characterized by below normal binding 4-7, 9 and 14 days, separated by increases to normal levels at 8 and 11 days after injury. Alterations of Act.D binding were also observed in the contralateral eye. Below normal binding was observed at 3-4 and 9 days and a primary increase in binding at 7 days after the operation. Except for magnitude, the basic pattern of Act. D binding to surviving axotomized retinal ganglion cells is similar to dorsal root ganglion neurons after axotomy. Binding ratios of retinal ganglion cells were characterized by statistically normal or below normal binding as contrasted to the large increases in binding of dorsal root ganglion neurons after axotomy. The sharp decrease in binding at 1 day after injury was also unique to retinal ganglion cells. The results suggest that the basic pattern of response of surviving central and peripheral neurons to axon injury is similar, but the magnitude of the response is much less in central neurons.

407.11

SEAL FORMATION IN TWO TRANSECTED GIANT AXONS SUGGESTS TWO MODELS OF SEALING. Todd L. Krause, Harvey M. Fishman,*Martis L. Ballinger, and George D. Bittner. University of Texas at Austin, 78712 and University of Texas Medical Branch at Galveston, 77555.

Severed axons could seal according to the following two models: **MODEL 1.** Constriction at a cut end reduces the opening and increases the resistance of the conducting path between axoplasm and external solution; sealing is completed if axolemmal fusion occurs. **MODEL 2.** Injury-induced vesicles migrate to the cut end, occlude it, and subsequently fuse with each other and the axolemma to form a complete seal. Previous studies have not distinguished between these two models; in fact, we now show they have not determined whether complete sealing indeed occurs. To distinguish between these two models and to determine whether complete sealing has occurred, we have used several electrophysiological measures (membrane potential, complex impedance, and injury current) together with video-enhanced, light, and electron microscopic observations to assess sealing in giant (80-500 μm diameter) axons of squid (Lolgio pealei and Sepioteuthis lessoniana) and earthworm (Lumbricus terrestris). We found recovery of resting potential and input resistance (R_{in}) in both axons. However, injury current (I_i) persisted at a substantial level relative to precut level (background) in squid giant axon (GA) for 2.5 hr following transection whereas I_i in earthworm medial giant axon (MA) decayed to background within 30 min. Further, the decay of I_i to background in MGAs correlated with the time course of R_{in} recovery. Morphological observations of the cut end, together with the electrical data, indicate that MGAs seal completely by vesicle plugging of a slightly constricted end whereas squid GAs constrict at the cut end to produce an increase in the resistance but the end remains open. Support: ONR (N00014-90-J-113), TX ATP, and NSF (ECS-891-5178).

407.12

SIGNALS THAT INDUCE SPROUTING IN THE CENTRAL NERVOUS SYSTEM: SPROUTING IS DELAYED IN A MUTANT MOUSE EXHIBITING DELAYED WALLERIAN DEGENERATION. O. Steward*. Departments of Neuroscience and Neurosurgery, Univ. of Virginia, Charlottesville, VA 22908.

This study evaluates whether sprouting in the CNS is initiated by signals related to the degeneration of presynaptic axons. We evaluate the time course of sprouting of cholinergic septohippocampal fibers after unilateral entorhinal cortex lesions in a substrain of mice carrying a mutation which leads to a substantial delay in the onset of Wallerian degeneration. This is the "Ola" mutation, which has been characterized in detail by Perry and colleagues (Perry, V.H., Lunn, E.R., Brown, M.C., Cahusac, S., and Gordon, S. Eur. J. Neurosci., 2: 408-413, 1990). It is thought that axonal degeneration is delayed because the mutation affects a signaling mechanism for macrophage activation.

We assessed the time course of Wallerian degeneration after entorhinal cortex lesions using the Fink-Heimer technique. Cholinergic sprouting was evaluated using a histochemical technique for acetyl-cholinesterase (AChE).

In normal control mice, both the time course of Wallerian degeneration, and the time course of cholinergic sprouting after EC lesions occur with a time course that is comparable to that described in rats. Argyrophilic degeneration debris was prominent by 4 days postlesion, and increases in AChE staining were well-developed by 8-10 days. In mice carrying the Ola mutation, however, argyrophilic degeneration debris was not detectable at 4 or 6 days postlesion, began to appear in the dentate gyrus by 8 days postlesion, and did not become prominent until 12 days. Increases in AChE staining in the molecular layer of the dentate gyrus were not detectable even at 12 days postlesion, but developed gradually after 14 days. These results indicate that the signals which initiate at least one form of CNS sprouting are related to the degeneration of presynaptic axons. Supported by NIH grant NS12333.

407.13

PROTEINS FROM SCIATIC NERVE CONTAIN A SIGNAL PEPTIDE THAT MEDIATES TRANSPORT THROUGH THE AXON TO THE CELL BODY AND INTO THE NUCLEUS. C. C. Huang, R. Schmied, R. T. Ambron, and C. Noback*. Otolaryngology, Anatomy and Cell Biology, Columbia University, New York, NY 10032.

Axons undergo structural changes in response to environmental clues or injury. While these changes require transcription, it is not clear how the needs of the periphery are communicated, often over long distances, to the nucleus. Using exogenous protein constructs, we discovered a pathway in Aplysia neurons that conveys proteins retrogradely from the axon periphery to the nucleus (J Neurosci, in press.) Access to the transport system depends upon a signal peptide (sp), H_2N-Pro-Lys-Lys-Lys-Arg-Lys-COOH. To obtain a probe that might be used to identify endogenous axonal molecules that use this pathway, we coupled sp to keyhole limpet hemocyanin and injected the conjugate into rabbits. A polyclonal antibody fraction was obtained which was then affinity purified using immobilized sp. When proteins extracted from sciatic nerves were separated by SDS-PAGE; a soluble 35 Kd peptide (sp35) was the major constituent detected by the antibody. Interestingly, when these soluble proteins were subjected to gel filtration on Sephacryl S-200, the major immunoreactive fractions, detected by dot-blot assay, were found in a high molecular weight (105 Kd) fraction. However, Western blots showed that this fraction contains sp35. To look for transport, the 105 Kd proteins were coupled to rhodamine (TRITC) and microinjected into axons of Aplysia neurons in vitro. 24 h later, TRITC-protein was found in the nucleus. In contrast, sp35 excised from a gel after SDS-PAGE was not transported after injection. These results indicate that endogenous sp-containing proteins exist in mammalian nerve, and suggest that these proteins must be in their native form, or complexed to a carrier molecule, in order to be transported.

407.14

THE APPEARANCE OF PRE-MITOTIC MICROGLIA ASSOCIATED WITH AXOTOMIZED MOTOR NEURON PERIKARYA IS INDEPENDENT OF AXON STUMP LENGTH

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Following axonal injury tritiated thymidine labeled cells, identified as microglia, appear in the region of the perikaryon. These cells are implicated in the separation of synapses from the motor neuron that is seen following axotomy. We report that the timing and relative peak abundance of these labeled cells does not vary with location of the axonal lesion.

At age 3 weeks both tibialis anterior muscles of Sprague-Dawley rats were injected with 1% fast blue. At age 8 weeks the left innervating nerve was severed, either by ventral root section of L4 and L5 (13 mm from the spinal cord) or the peroneal nerve at the level of the fibular head. One, 2 and 4 days later, 4 hours following intraperitoneal injection of 5 mCi/gm tritiated thymidine, the animal was perfused. The number of labeled cells touching, or within one perikaryal diameter to, fluoresently labeled motor neurons were counted on each side. At day 2 neuron-associated labeled cells were abundant (0.99 / neuron for proximal axotomy, 1.17 / neuron for distal axotomy). At 1 and 4 days labeled cells were less profuse, although variable for the proximal axotomy group. Labeled cells on the control side were rare at all time points. These findings are consistent with the hypothesis that neuron-associated microglia are regulated by factors emanating from the terminal axon or target muscle.

408.1

A SOLUTION TO THE CRIMPING PROBLEM IN THE CYLINDRICAL MODELLING OF BIOLOGICAL STRUCTURES:G. Sun, Z. Xu and P. Ramm*. Imaging Research Inc., Brock University, St. Catharines, Ontario, Canada L2S 3A1

Many biological structures, such as blood vessels and neuronal processes may be modelled in two or three dimensions as cylinders. From the computer model, morphometric and volumetric measurements may be derived. A typical approach to cylindrical modelling consists of two steps: 1) define the skeleton of the cylinder, which has a main axis (a 3D polyline) and a planar cross-section at each joint of the polyline; 2) define the surface of the cylinder by connecting each pair of adjacent cross-sections. Using this approach, the model is limited to the reconstruction of structures which yield a smooth main axis. The major difficulty arises from the so-called "crimping problem". That is, adjacent cross sections intersect each other at high curvature segments of the main axis. Our algorithm solves the crimping problem, and faithfully reconstructs the original cylindrical structure. The algorithm includes four major steps: 1) find the main axis and cross sections; 2) find all the crimping sections; 3) replace the crimping sections by interpolation; 4) define the cylindrical surface by connecting adjacent surfaces. The resulting cylindrical model provides continuous and true-to-life sweeping of cross sections along the main axis. It can be easily extended to 2D cylindrical modelling, in which it is very useful for the analysis of density distributions across structures containing abrupt changes in axis orientation. For example, accurate measurements of reaction product density in a tortuous neuronal process may be obtained. These density measurements may be taken from regions as small as one pixel in width, and integrated across any height.

408.2

A COMPARISON OF STEREOLOGICAL ESTIMATES OF MOTONEURON SOMAL VOLUME VERSUS DIRECT 3-D MEASUREMENTS USING CONFOCAL MICROSCOPY. Y.S. Prakash* and G.C. Sieck. Mayo Clinic and Foundation, Rochester, MN 55905.

Previous studies on motoneuronal morphometry have assumed geometry and orientation of neuronal soma in estimating somal volumes, based on two-dimensional (2D) measurements. The "optical sectioning" property of the confocal microscope allows direct 3D measurements. We retrogradely labelled phrenic and lumbar (medial gastrocnemius) motoneuron pools in adult and 21-day old rats, using fluorescent dyes. Motoneurons in the two pools are known to differ in shape and size. Series of digital images ("optical slices") of labelled motoneurons were obtained using a Bio-Rad Laser Confocal microscope. Minimal overlap between adjacent optical slices was ensured. From these images, direct measurements of somal axis dimensions in three orthogonal directions were taken using ANALYZE®. Somal volumes were directly measured using a volume rendering program, with minimal image manipulation. These volumes were compared to estimates based on a variety of geometrical shapes used in previous studies. The 2D paramaters were assumed to be those measured in the plane of section, in keeping with previous techniques. In the adult phrenic pool we found significant errors (upto 300% overestimation) in estimated volume assuming prolate spheroidal shape in cases where the minor axis was not equal to the third dimension. These errors were less apparent in the adult lumbar pool. Significant errors also arose in cases where the orientation of soma differed from the general population within the pool. In the 21-day phrenic pool, there were upto 100% underestimation of somal volume, while in the 21-day lumbar pool errors were dependent on individual somal features. We conclude that, using confocal microscopy, it is possible to obtain true volumes of neuronal soma without the assumption of geometrical shape and orientation. Supported by NIH grants HL34817 and HL37680.

408.3

COMBINATION OF INTRACELLULAR STAINING OF RETROGRADELY LABELED NEURONS AND ANTEROGRADE FLUORESCENT TRACING: USE OF THE CONFOCAL LASER SCANNING MICROSCOPE. C-J Shi and M.D. Cassell*. Dept. of Anatomy, Univ. of Iowa, Med. College, Iowa, IA 52242.

This report describes a combined retrograde tracing, intracellular injection and anterograde fluorescence labeling method using the application of confocal laser scanning microscopy to simultaneously view the morphology of identified projection neurons and the distribution of anterogradely labeled fibers and terminals to characterize the anatomical relationship between these two elements. With this approach, the retrograde tracer Fast Blue was injected into the bed nucleus of stria terminalis (BNST) and the anterograde tracer tetramethylrhodamine-conjugated dextran was injected into the insular cortex in adult rats. After one week survival time, the brains were fixed and sectioned on a vibratome. Individual BNST projecting neurons identified in the amygdaloid complex on 120 um thick sections were intracellularly injected with Lucifer Yellow under visual control and analysed with confocal laser scanning microscopy. The results demonstrate that images from very thin optical sections can clearly show potential synaptic contacts between anterograde labeling and intracellularly labeled projecting neurons. Stacked images from optical sections show, in very great detail, the morphology of projection neurons in three-dimensions. In comparison with other combinations, the present method provides a more simple and efficient means to trace three successive components of a putative neuron chain.
Supported by NS25139.

408.4

THREE-DIMENSIONAL TERRITORIAL COVERAGE BY GENICULATE NEURONS USING COMPUTERIZED LIGHT AND CONFOCAL MICROSCOPY. J. Gemmill*, L-A. Coleman and M.J. Friedlander. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294 USA.

The relationship of growth and territorial coverage of three-dimensional (3D) space by dendrites during development can be quantified using computerized microscopy. We estimated the 3D coverage of Area 18 projecting dorsal lateral geniculate nucleus (LGNd) neurons and compared coverage during postnatal development. LGNd volume was calculated using camera lucida drawings of wet mounted serial sections of the LGNd and then integrating over section thickness using Simpson's rule. Green and red fluorescently labeled latex microspheres were retrogradely transported to the LGNd neuronal somata from cortical injection sites. Cell densities were estimated by the disector method applied to optical sections from image volumes captured on a confocal microscope and compared with total densities obtained from tissue counterstained with Acridine Orange and analyzed in the same manner. Complete dendritic arbors of neurons at each age were analyzed directly from slides using a 3D computer reconstruction system; complete branches also were measured for diameter and spine location. The volume within the LGNd occupied by an individual neuron's dendritic arborization was estimated by finding the spherical volume occupied by the dendrites, computing 3D branch angles for all branches, and using a tortuousity measure for individual dendritic segments. Total LGNd volume, density of cells, and individual cell volume metrics can be used together to describe coverage and overlap within an anatomically defined region such as the LGNd.
Supported by NIH EY05116 (MJF) and the Lucille P. Markey Foundation.

408.5

THREE DIMENSIONAL IMAGING OF FACIAL NUCLEUS MOTOR NEURONS USING LASER SCANNING CONFOCAL MICROSCOPY. B. F. Lofthus*[1], N. H. Donegan[1] and T. H. Brown[1,2]. Depts. of Psychol.[1] and Cell. & Molec. Physiol.[2], Yale Univ., New Haven, CT 06520.

The rat eye blink reflex is useful for neurophysiological studies of adaptive gain control and Pavlovian conditioning. We have been interested in methods that might help elucidate the neurobiology of use-dependent plasticity in this reflex. For circuit tracing, one promising approach involves laser scanning confocal microscopy (LSCM) applied to thick brain slices.

The lid closure during a reflex blink is generated by a passive downward force plus active contraction of the orbicularis occuli (oo), whose motor neurons are located in the facial motor nucleus (FMN). The oo receives innervation from 2 branches of the facial nerve, the upper zygomatic and the temporal. *In vivo* injections of tetramethyl rhodamine or fluorescein into these 2 branches resulted in retrograde labeling of motor neurons in the FMN. After fixation, the FMN was transversely sliced (400 μm thick sections), mounted and coverslipped. Optical sections were taken through the FMN (at 0.5 - 1.0 μm intervals) using a Bio-Rad MRC 600 LSCM system and a long-working distance (500 μm) 63X objective (NA = 1.25).

The amount of detail discerned with LSCM was significantly better than with conventional microscopy. Optical sectioning enabled convenient 3D imaging, which is important for cell classification, morphometric analysis, and dual labeling of pre- and postsynaptic structures. We are currently exploring other dyes that may be useful with LSCM in elucidating the reflex circuitry and its sites of modulation. In particular, we are examining projections onto this circuitry from the amygdala, which has been implicated in conditioned potentiation of the eye blink reflex. *Supported by an NIH grant (THB) and an NSF fellowship (BFL).*

408.6

THREE-DIMENSIONAL RECONSTRUCTION OF NEURONS USING CONFOCAL MICROSCOPY AND VOLUME RENDERING. D. Birt*, C. Cepeda, S.H. Chandler(1), N.A. Buchwald and M.S. Levine. Mental Retardation Research Center and Department of Physiological Science(1), UCLA, Los Angeles, CA 90024.

Our laboratory has been using confocal microscopy to visualize neurons in three-dimensions. Intracellular recordings and dye injection (Lucifer Yellow (LY) or biocytin) were performed in neurons obtained from brain slices of neostriatum or neocortex of rats or trigeminal motor nucleus of guinea pigs. LY-filled cells were examined in whole mounts while biocytin-filled cells were examined in 60-80 μm thick sections processed by standard immunofluorescent techniques. Neurons were first optically sectioned with a laser scanning confocal microscope (Biorad MRC600). Sections were obtained at intervals of 1-5 μm (z-axis) with the confocal aperture set at its minimal diameter. Depending upon the numerical aperture and the magnification of the objective, optical section thickness varied from 1-4 μm. Three-dimensional visualization was obtained as a series of optical sections, stereo pairs or by volume rendering. Volume rendering to permit three-dimensional imaging was performed with commercially available software (VoxBlast, VayTek, Inc.) which uses alpha blending to create a three-dimensional projection. The software permits rotation around any axis, changes in the source of illumination and changes in the transparency of the image. Rotation around different axes provides a clearer picture of the three-dimensional characteristics of the neuron. Changes in source of illumination and transparency allow shadowing and surface landmarks to be visualized. Three-dimensional reconstructions permit perspectives to be reconstructed that were difficult or impossible to view in the serial optical sections or stereo pairs.
Supported by USPHS HD 05958.

408.7

***IN-VIVO* CONFOCAL MICROSCOPY OF THE RABBIT OPTIC NERVE HEAD**

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The tandem scanning confocal microscope (TSM) allows repeated, noninvasive examination of living tissue, in real-time, with the advantage of serial optical sections, superior contrast, lateral and vertical resolution. We used a reconfigured TSM to examine the optic disc in the rabbit in two modes: as a scanning ophthalmoscope and as an ex-vivo imaging technique to investigate the human lamina cribrosa. Using a Noran TSM with a 35mm camera objective lens, and a Goldmann 3-mirror fundus contact lens we examined the retina, and optic disc of the rabbit. At this low magnification of 30x, we were able to ascertain the depth of the optic cup and to perform densitometry of the nerve fiber layer at the neuroretinal rim. Following transection of the optic nerve, we monitored the development of optic atrophy by repeated reflectometry. The fibrous structure and astrocyte content of the lamina cribrosa has been described in histological studies. However, the relationship of its fibers to the axons exiting the optic nerve have not been established. Additionally, these studies did not measure the lamina cribrosa pores in absolute units, nor examine serial levels of the lamina. Furthermore, fixation, sectioning, and freeze fracture induce shrinkage artefacts in this delicate web-like tissue. We used the TSM with a 25x water immersion lens to map the 3-dimensional architecture of the fibrous trabeculae in normal, unfixed and unstained eye bank eyes. We performed morphometry of the anterior 400μm of the lamina cribrosa, which showed a wide variation in the size (30-150μm in diameter, 6000-10,000μm^2 in area), configuration and complex interconnections of canals in the lamina. 3-dimensional volume rendering of the serial optical sections was also performed.

408.8

***IN-VIVO* MONITORING OF CORNEAL NERVE INJURY BY TANDEM SCANNING CONFOCAL MICROSCOPY**

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With its superior contrast, lateral and vertical resolution, the tandem scanning confocal microscope (TSM) allows repeated, noninvasive examination of the cornea in vivo. We used a reconfigured TSM to examine the nerves in the rabbit, following two modes of injury (mechanical and laser damage) and in human contact lens wearers. In NZW rabbits, the positions of large myelinated limbal nerves were identified and marked. They were then transected by penetrating circular (4mm) perilimbal corneal incisions. Using a reconfigured TSM and a 25x water immersion objective lens, we then examined the eyes at regular intervals. Within 24 hours after wounding, ruptured axon pieces could be observed. Reinnervation of the denervated cornea was observed by regenerating nerves that penetrated the limbal scar tissue. Long, large caliber, dense neurites that coursed perpendicularly to the wound margins. The recovery of corneal sensation in the affected quadrant was also monitored by aesthesiometry. The regenerating neurites. Secondary degeneration was observed after one week. Computerized morphometric evaluation of the corneal neural density was also performed after image capture using a CCD camera. Excimer laser photorefractive keratectomy was performed, with the ablation of the anterior 75μm of corneal stroma. The regeneration of epithelial nerve endings were observed with the TSM. Histology, using the gold chloride impregnation technique was used to confirm these observations.The epithelial nerves of human contact lens wearers were compared with those of non-contact lens wearers.

408.9

CONFOCAL FLUORESCENCE IMAGING OF $[Ca^{2+}]_i$-TRANSIENTS IN ACUTE RAT-BRAIN SLICES USING FLUO-3-AM AND A SMALL VOLUME SUBMERGED CHAMBER SYSTEM.

A.Them, A.Villringer, U.R.Büttner*, U.Dirnagl. Dept. of Neurology, University of Munich, 8000 Munich 70, F.R.G.

In order to establish a simple system for the imaging of intracellular-ion concentrations in brain slices using fluorescent dyes we developed a small volume slice chamber. The chamber volume is < 0.5 ml, affording rapid (< 5 s) exchange of the bathing medium and small amounts of dyes. Imaging was performed with a confocal laser scanning microscope (Bio-Rad MRC 600; KrAr-Laser), equipped with a Zeiss x40, NA 0.75 water immersion objective corrected for a cover slip. Wistar rats (age 2-4 days and 2-4 weeks) were anesthetized with halothane and decapitated. The brain was removed in cold artificial cerebrospinal fluid (ACSF) and sliced to 500 μm using a vibratome. Slices were incubated with FLUO-3-AM (1, 10, or 50 μM in ACSF; DMSO/Pluronic (3:1) < 0.1%; Molecular Probes) for 15, 30, 60 or 180 min. $[Ca^{2+}]_i$-changes were stimulated by switching the bathing medium to L-GLU (500μM), high K^+ (50mM), or low pO_2 (< 170mmHg) containing ACSF. Cells were resolved up to a depth of 50 μm. Signal intensity increased with loading time and dye concentration. Density of loaded cell-bodies was highest in the neonatal slices, where spontaneous $[Ca^{2+}]_i$-transients were imaged. In the cortex and hippocampus, the density of loaded cells appeared similar. Only neonatal slices responded to stimulation and best results were obtained with minimal dye concentrations and loading time (1μM, 15min) resulting in a low basal fluorescence level and strong increase after stimulus onset. We conclude that Ca^{2+}-imaging in acute brain slices is feasible. This system may allow the investigation of intracellular-ion concentration dynamics under various experimental conditions.

408.10

THREE-DIMENSIONAL ANALYSES OF GRANULE NEURONS IN THE RAT DENTATE GYRUS LABELED WITH FLUORESCENT DYES.

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Efforts to analyze the 3-D structure of fluorescently-labeled neurons have been hindered by the lifespan of the fluorochrome. Dense labels, which are more difficult to use, have been required. Here we report a technique that allows neurons labeled with DiI or Lucifer Yellow (LY) to be quantified using a computer-microscope system equipped with epi-fluorescence optics.

Granule neurons in 400-um-thick slices of the rat dentate gyrus were labeled either by retrograde transport of DiI in fixed tissue (Claiborne et al., Neurosci. Abstr. 17: 35) or by intracellular injection of LY in vitro or in fixed tissue (Felthauser & Claiborne, Neurosci. Lett. 118: 249). To minimize bleaching of the dye, the intensity of the mercury excitation (Nikon G2A or Omega LY filter) was decreased using neutral density filters, and the final field area of excitation was reduced. To compensate for the concurrent decrease in fluorochrome emission, we used a Hamamatsu C2400-08 camera and a Rolyn KG2 heat filter. With these modifications, all labeled dendrites could be digitized.

Total dendritic length per neuron ranged from 366 to 1524 um (n=9) for DiI-labeled granule neurons from 3-day-old rat pups, and from 412 to 2235 um (n=5) for neurons from 8-day-old pups, reflecting the wide variability in granule cell structure at these ages; from 1470 to 2950 um (n=6) for LY-injected granule neurons from 14-day-old pups; and from 4241 to 5356 um (n=4) for LY-injected cells from 40- to 60-day-old rats. The last two results are similar to our previous data on HRP-labeled granule neurons (Rihn & Claiborne, Dev. Br. Res. 54: 115), illustrating the reliability of the system for 3-D analysis of fluorescently-labeled neurons. (Supported by MBRS and ONR.)

408.11

QUANTITATIVE OPTICAL IMAGING OF THE DIFFUSION OF DEXTRANS OF DIFFERENT MOLECULAR WEIGHTS IN RAT CEREBRAL CORTEX.

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Many neuroactive substances have molecular weights of 1 - 100 kDa and it is important to know how they diffuse in brain tissue. We have developed a quantitative optical imaging system and analyzed the diffusion of 3, 10, 40, and 70 kDa fluorescent dextran molecules in the cortex.

Dextrans tagged with the fluorescent dye Texas Red (Molecular Probes, OR) were pressure-injected into rat cortical slices maintained in a perfused chamber at 34°C and imaged as they spread in the tissue, using a compound epi-fluorescent microscope with a 10x water-immersion objective. About 20 images were taken with at 2 - 10 second intervals and recorded by a cooled CCD camera (Photometrics, AZ) with 576 x 384 pixels and 14 bits and transferred to a 486 PC. The apparent diffusion coefficient, D^*, was determined by fitting an integral expression relating the measured 2-dimensional image intensity to the 3-dimensional dextran concentration. Measurements in dilute agarose gel provided a reference value of D.

Values of the ratio, $(D/D^*)^{1/2}$, for the 3 and 10 kDa dextrans were consistent with tortuosities derived from tetramethylammonium measurements in cortex (Cserr et al. J. Physiol., 442: 277, 1991) but the 40 and 70 kDa dextrans showed markedly larger ratios. This suggests that extracellular space may not be uniform but has constrictions that hinder diffusion of molecules above a critical size that lies in the range 10 - 40 kDa. This implies that the concept of tortuosity, as a simple parameter describing the diffusion properties of the extracellular space, is valid only for molecules below this critical size. Supported by NIH Grant NS 28642.

408.12

CCD-IMAGING OF VOLTAGE SENSITIVE DYE SIGNALS FROM WHOLE BRAIN OF LOWER VERTEBRATES

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We used the fluorescent potentiometric membrane probe Di-4-ANEPPS to image distributed neural activity *in vivo* and in isolated whole brain preparations of frog, lamprey and turtle. Shot noise limited images were obtained with a CCD at a rate of 13 Hz and processed to produce movies of the spatio-temporal patterns of voltage changes. Small arrays of diodes were used to measure voltage changes with higher temporal resolution from selected regions. Simultaneous recording of optical and electrical signals was possible for periods >8 hours without light-induced toxicity.

Optical recordings reliably showed the initiation and propagation of neural responses that were evoked by stimulation of cranial nerves or brainstem structures, by disinhibition with picrotoxin (PTX) and by light or odor stimuli. Optical signals generally differed from field potential recordings by the presence of long lasting slow components, that often persisted many seconds after a stimulus. In contrast, a close correspondence between the time course of optical signals and intracellularly measured voltage was seen suggesting the slow components are not glial in origin. Spontaneous activity induced by PTX originated in various brain regions and propagated along several defined paths (e.g. ant. dorsal telencephalon to thalamus/ hypothalamus). Repeated initiation of these "epileptiform" events from the same sites was observed over the course of several hours. Telencephalon initiated events occurred more frequently and recovered faster than those in diencephalon, tectum, cerebellum or brainstem. Odors or olfactory nerve shock produced optically recorded oscillatory activity in olfactory bulb and activity extending into ipsilateral and contralateral caudal telencephalon.

408.13

SIMULTANEOUS KINETIC IMAGING OF INTRACELLULAR CALCIUM AND pH IN SINGLE MELANOTROPES. Stephen J. Morris*, Diane M. Beatty†, and Bibie M. Chronwall†. Div. of Molecular Biology and Biochemistry and †Div. of Cell Biology and Biophysics, Univ. of Missouri-Kansas City, Kansas City, MO 64110

There is a growing interest in the use of multiple fluorescence probes to analyze the relationships between $[Ca^{2+}]_i$ and pH_i. We have designed an epifluorescence video microscope for dual excitation and four wavelength emission which will simultaneously capture all four emission images at 405, 475, 575 and 640 nm from the two ratio dyes indo 1 (for $[Ca^{2+}]_i$) and SNARF 1 (for pH_i) at video rates (*SPIE Proc* **1428**:148,(1991), *Optical Microscopy: New Technologies and Applications*. B Herman and JJ Lemasters, eds, Academic Press (1992-in press)).

Popular dyes for measuring intracellular calcium, such as indo 1 and fura 2, have pH-dependent Kd's; thus changes in pH_i can be misinterpreted as changes in $[Ca^{2+}]_i$. SNARF sensitivity to pH between 6.5 and 8.0 was unchanged by $[Ca^{2+}]$. The indo/Ca^{2+} Kd, examined at 20° and 37°C, shifted more than ten fold between pH 6.5 and 8.0. Rat pituitary melanotropes, grown in explant cultures, were double-labelled and Ca^{2+}/pH interactions were examined during exposure to various stimuli. SNARF ratio maps were used to correct the pH-dependent changes in local cell calcium. Under most circumstances, pH correction modified the apparent $[Ca^{2+}]_i$; small changes in $[Ca^{2+}]_i$ disappeared or were greatly attenuated and kinetics changed. Cells were later positively identified as melanotropes by immunohistochemistry.

K^+-induced depolarization of melanotropes produced increases in $[Ca^{2+}]_i$ which were closely coupled to reductions in pH_i. Chronically applied dopamine agonists suppressed this activity, which returned when the drugs were removed. Cells responded to both NH_4Cl alkalinization and subsequent acidification upon NH_4Cl withdrawal with calcium transients from intracellular stores. We conclude that the new video microscope will be an invaluable tool for the study of intracellular dynamics.

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408.14

DO GLIA CELLS REGULATE PERINEURAL CALCIUM? D.Manor, N. Moran and M. Segal*, Neurobiology Dept., The Weizmann Inst., Rehovot 76100, ISRAEL.

We studied the effects of rapid changes of $[Ca]_o$ and $[K]_o$ on $[Ca]_i$, imaged with Fluo-3, in cultured neural cells, using a confocal laser scanning microscope. In constant, 2.8 mM $[K]_o$, changing $[Ca]_o$ (range 0 - 10 mM) caused a proportional shift in $[Ca]_i$ (range 50 - 150 nM). The effect was more prominent in C6 glioma and primary glia than in cultured hippocampal neurons. $[Ca]_i$ increased 2.5 fold in C6 cells with half maximum at 2.5 mM $[Ca]_o$ and with T1/2 of 15 s. In neurons too, $[Ca]_o$ modulated $[Ca]_i$, but with a lower $[Ca]_i$ range (30-100 nM) and had a slower time course ($T_{1/2}$ > 50 sec.). The two cell types differed more strikingly in their response to $[K]_o$ variations. As expected, high $[K]_o$ triggered a marked elevation of $[Ca]_i$ in neurons, which was prevented by verapamil. In contrast, increasing $[K]_o$ in glia resulted in a marked decrease of $[Ca]_i$. Significant variations in $[Ca]_i$ could be observed also in the physiological range of $[K]_o$. $[Ca]_o$ control of $[Ca]_i$ was unaffected by verapamil, cadmium, sodium vanadate, ouabain or low sodium. However, a 10 s perfusion with 10 mM Lanthanum (La) induced a 5 min suppression of basal $[Ca]_i$. In neurons, La efects lasted up to a minute. Involvement of intracellular calcium stores in this regulation in glia is suggested by a marked reduction of $[Ca]_i$ responses to 5-HT_2 and α1-NA ligands - known Ca mobilizers - during $[Ca]_i$ reduction by high $[K]_o$ or La. These results suggest that glial cells possess unique, fast $[Ca]_i$-based $[Ca]_o$ regulating mechanisms.

408.15

THREE-DIMENSIONAL RECONSTRUCTION OF LARGE SUBCELLULAR STRUCTURES: A METHOD FOR COMBINING AXIAL TILT TOMOGRAPHY WITH SERIAL THICK SECTIONS G. E. Soto, M. E. Martone, S. Lamont, S. J. Young*, T. J. Deerinck, M. H. Ellisman. San Diego Microscopy & Imaging Resource, Dept.Neurosci., UCSD, La Jolla, CA 92093-0608

Cells and cellular organelles can extend for tens of microns and therefore cannot be encompassed in a single thin section normally examined with conventional electron microscopy. Even with the use of high voltage electron microscopy, section thickness is limited to no more than a few microns. In an effort to overcome these limitations we have been exploring a method of linking serial thick sections by first extracting their three-dimensional (3D) information using axial tilt tomography and then aligning and linking the resulting serial volumes to form a single volume. We have used this method to investigate the 3D structure of the Golgi apparatus in dorsal root ganglion neurons . Briefly, four 2µm serial sections were cut from an osmium impregnated bullfrog dorsal root ganglion (Lindsey et al., J. Neurosci. 12:3111, 1985). A tomographic volume was derived for each section from a single axis tilt series taken through ±60 degrees in 2 degree increments with a JEOL 4000 intermediate voltage electron microscope operated at 400KeV. Alignment of the serial volumes was accomplished by slicing each volume into individual planes orthogonal to the depth dimension and registering the last slice of one volume to the first slice of the next with the aid of fiducial marks . The aligned slices of the 4 volumes were then merged into a single volume. The resulting volume was rendered and examined using the program ANALYZE (Robb et al., IEEE Trans. Med Image. MI-8:217, 1989). The continuity of components of the Golgi apparatus could be observed through a depth of 8µm with spatial resolution near to 100nm. This method appears promising for characterizing the 3D organization of subcellular structures over many microns while maintaining resolution sufficient to discern fine structural detail. While the alignment of the serial volumes appeared accurate, we are currently validating this technique by using a specimen of known 3D geometry. We are also exploring the use of this technique to investigate the continuity of neuronal endomembrane system components in bullfrog dorsal root ganglion and in cerebellar Purkinje cell dendrites.

408.16

TOWARDS A DIGITAL THREE-DIMENSIONAL NEUROANATOMIC ATLAS OF THE HUMAN BRAIN.

B. Quinn*, K. Ambach, & A.W. Toga, Laboratory of Neuro Imaging, Dept. of Neurology, UCLA Medical School, Los Angeles CA 90024.

We have generated high resolution, full color three-dimensional (3D) data sets from human brains cryosectioned with the cranium intact. Whole human heads were perfused via the common carotid and vertebral arteries with formol-saline fixative delivered by a constant-pressure perfusion system, followed by post-fixation perfusion using glycerol and polyvinylpyrrolidone, which act as cryoprotectant and plasticizing agents. After decalcification of the cranium, specimens were pre-chilled to near freezing and then rapidly frozen in isopentane chilled with liquid nitrogen. Frozen specimens were sectioned on an automated PMV Cryo-Microtome equipped with a Digistat 1024^2 high resolution camera. Color images were captured from the specimen blockface at serial intervals of 400 or 800 microns and reassembled into a 3D volume. An important feature of this system is direct volume reconstruction from in-register images without need for complex registration algorithms. The reconstructed volume data set could be oriented in a Cartesian coordinate system and extensively manipulated for visual display including planar transection and surface modeling. The ultimate objective of digital reconstruction is the development of a digital 3D neuroanatomic atlas of a prototypic human brain intact in the cranium through application of warping algorithms to an array of multiple specimens.

408.17

A HISTOLOGICAL APPROACH TOWARDS A HUMAN BRAIN REFERENCE ATLAS FOR COMPUTER ASSISTED IMAGING TECHNIQUES. J.K. Mai, T.A. Voß, J. Assheuer, L. Lanta, T. Sievert and L. Teckhaus (SPON: European Neuroscience Association). Abt. für Neuroanatomie, Heinrich-Heine Universität, D-4000 Düsseldorf 1; MPI für Systemphysiologie, D-4600 Dortmund 1, FRG.

In spite of high resolution obtained by modern imaging techniques it is necessary to supplement these image data with histological and histochemical data from the microscopical examination of brain sections. In order to integrate the two different sets of data we have created a 3-D human-brain-reference-atlas. This atlas is based on one paraffin-embedded brain (death to fixation interval: 2h), which was serially sectioned vertical to the intercommissural line. Cytomorphologic and morphometric studies of this brain and correlations with immunohistochemical findings have been published by numerous authors. From 630 sections contourlines representing pial and ventricular surfaces, as well as subcortical nuclei and their subdivisions, were extracted manually. The contourlines were adjusted into a 3-D metric grid. Points of intersection between the contourlines and the grid were used for cubic spline-approximation. The resulting curves with damped extrema were transformed after estimating deformation and shrinkage due to tissue preparation. The smoothed contourlines were used for 3-D reconstruction. Contourlines of main thalamic subnuclei have been quantitatively compared, regarding to the interindividual variability, with those derived from paraffin-embedded brains (applying the same procedure of in-vitro - in-vivo transformation; 25 hemispheres) and MR Imaging (18 hemispheres).

408.18

THREE-DIMENSIONAL STRUCTURE OF LOCAL PARENCHYMAL MICROVASCULAR SYSTEMS IN RAT BRAIN. J.A. DeMaro, W. Finnegan, V. Acuff, G. Richardson, D. Bereczki, L. Wei, and J. Fenstermacher*. Dept. Neurological Surgery, SUNY, Stony Brook, NY 11794-8122

Past methodologies for analyzing cerebral microvascular system structure have employed single histological sections and stereological theory. Such approaches do not yield information on microvascular branching, length, and tortuosity. Since there is reason to suspect that some of these features vary among brain areas, three-dimensional reconstructions of local microvascular systems were made using serial histological sections and an image analysis system.(MCID M1, Imaging Res. Inc) The results indicate that cerebral microvascular systems vary considerably in branching and tortuosity. For example, the capillaries in the hippocampus are fairly straight, long, and arise by simple division of the terminal arteriole, whereas those in the paraventricular nucleus form tortuous networks of many relatively short capillaries. Microvascular system organization, thus, seems to vary among brain areas, possibly in accordance with the structure and function of the surrounding tissue.

408.19

An Improved Approach to Quantitative *In Situ* Hybridization. J.T. McCabe, E.F. Wheeler* and R.P. Bolender Dept. of Anatomy & Cell Biology, USUHS, Bethesda, MD and Depts. of Physiology & Biophysics, Biological Structure, Univ. of Washington, Seattle, WA

In neuroscience research, *in situ* hybridization is often the method of choice for measurement of mRNA levels. Compared with other procedures used to assess gene activity, this method allows one to study changes at the morphological level, and estimate both the amount of mRNA/cell and the number of cells containing the mRNA of interest. Quantification of *in situ* hybridization results, however, is not a simple procedure. If cell volume changes, estimates of the number of labeled cells/brain region and amount of mRNA/cell can be grossly inaccurate. We have developed a quantitative morphological method that estimates the total number of labeled cells/structure, and converts the conventionally used parameter, *grain density* (grains/cell profile area), to both grains/cell and grains per total cell population: $N = V \times N_A \times 1/t$, where N = total number of grains/cell, V = cell volume, N_A = grains/cell profile, and t = the effective depth of the radioactive source (*Anatomical Record*, **231**: 407-15, 1991). Our presentation demonstrates how conventional approaches can lead to inaccuracy and misinterpretation, and how the proposed method gives reliable estimates of the number of labeled cells, mRNA levels/cell, and mRNA levels/brain region. Supported by Grants USPHS NS25913 and USUHS RO70AL to JTM.

408.20

DISTRIBUTION OF AROMATIC L-AMINO ACID DECARBOXYLASE mRNA IN MOUSE BRAIN.

M.J.Eaton,[1]*, K.P.Gudehithlu[1], C.P.Silvia[1], M.Hadjiconstantinou[1,2,3], and N.H.Neff[1,3], Depts of Pharmacology[1], Psychiatry[2] and Neuroscience Program[3], Ohio State University College of Medicine, Columbus, OH 43210.

A 286 bp DNA sequence for mouse-brain aromatic L-amino acid decarboxylase (AAAD) was obtained from a mouse brain cDNA library using oligonucleotide probes prepared from a complete bovine adrenal AAAD sequence. Antisense riboprobes for the bovine adrenal and mouse-brain AAAD were labelled with digoxigenin and 20 μm sections of mouse brain used for non-radioactive in situ hybridization and anti-digoxigenin UTP immunohistochemistry. Brain from mice treated with MPTP, 30 mg/kg ip daily for 7 days, were also probed for AAAD mRNA. Both the full length bovine adrenal and mouse brain AAAD probes stained the cell nuclei known to synthesize dopamine, serotonin and norepinephrine. The substantia nigra, dorsal raphe nucleus, locus coeruleus and olfactory bulb contained the highest concentrations of AAAD mRNA. When MPTP-treated animals were examined, substantia nigra pars compacta had barely detectable staining for AAAD, while both locus coeruleus and dorsal raphe had levels of mRNA unchanged from those found in untreated mice. Ventral tegmental dopaminergic cells retained an intermediate level of AAAD staining. Histaminergic neurons in the hypothalamus and the Purkinje neurons in the cerebellum containing histidine decarboxylase and glutamic acid decarboxylase, respectively were unlabelled by these in situ methods. However, neurons of the deep layers of the frontal cortex, nuclei of the thalamus, and the pyramidal neurons of the hippocampus were moderately or strongly labelled for mouse AAAD mRNA. This specific probe for mouse brain AAAD message may be useful to monitor changes in expression of AAAD in models of Parkinson's disease.

CALCIUM CHANNEL TOXINS II

409.1

EFFECTS OF THE CALCIUM CHANNEL BLOCKER OMEGA-CONOTOXIN ON *IN VIVO* DOPAMINE EFFLUX IN RAT STRIATUM. C.A. Duva, C.D. Blaha* and A.G. Phillips. Dept. Psych., Univ. British Columbia, Vancouver, BC, Canada, V6T 1Z4.

The effects of omega-conotoxin (CTX, fraction GVIA), an N- and L-type voltage-sensitive calcium channel (VSCC) blocker, on basal dopamine (DA) efflux was investigated using *in vivo* electrochemistry (chronoamperometry, 1 s potential pulse/ 30 s). A 30 gauge cannula was positioned 0.5 mm adjacent to a stearate-graphite paste recording electrode and was stereotaxically implanted into the dorsomedial striatum of urethane anesthetized rats. Following at least 1 hr of baseline recordings, injection of CTX (0.1, 1 or 10 pmole/ 1 ul/ 4 min) produced a dose-dependent decrease in basal DA efflux. A maximal decrease in DA efflux was achieved within 10 min of injection of 10 pmole CTX. This decrease was followed by a rebound in DA efflux which increased significantly above preinjection values within 1 hr of CTX injection. Additional CTX (10 pmole) injections during this rebound phase produced decreases in DA efflux that were similar in magnitude and time-course as the first injection. These results provide some of the first *in vivo* evidence indicating that both N- and L-type calcium channels are involved in basal DA transmission and suggests that transient inhibition of these channels may lead to increased DA efflux. These findings are in contrast with *in vitro* studies which demonstrate an irreversible blockade of VSCCs by CTX. The mechanisms responsible for the transient and rebound effects of CTX are unknown at present, but may include a biphasic modulation of VSCCs or increased storage of DA at the presynaptic terminal.

409.2

NOREPINEPHRINE (NE) RELEASE FROM CARDIAC ADRENERGIC NERVE TERMINALS: AGE-RELATED DIFFERENCE IN OMEGA-CONOTOXIN INHIBITION. D.L. Snyder, V.J. Aloyo*, M.D. Johnson, and J. Roberts. Dept. of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Cardiac synaptosome (SYN) preparations from hearts of 6 and 24 mo old male F344 rats were used to investigate the effect of age on omega-conotoxin (CTX) inhibition of NE release. CTX is a specific N-type Ca^{2+} channel blocking agent. SYN were incubated in ^{3}H-NE, placed in a superfusion system, and then perfused for 30 min with various concentrations of CTX. The SYN were then depolarized with a 2 min pulse of buffer at 75 mM K^+ (concentration which produces maximum release) or 60 mM K^+. The mean fractional release of NE (% of total NE actually released) was significantly reduced in 24 mo old rats when compared to 6 mo old rats (N=3, see table). 6 mo old rats were more sensitive to CTX inhibition and showed a greater percent reduction in NE release at each level of CTX. These results suggesting that the adrenergic nerve terminals of 6 mo old rat hearts contain more CTX sensitive Ca^{2+} channels than at 24 mo agree with our previous finding that SYN from 6 mo old hearts contain more CTX binding sites than SYN from 24 mo old hearts. The loss of Ca^{2+} channels with age may be responsible for the age-related reduction in NE release.

		Mean fractional NE release (% inhibition by CTX)					
[CTX]		0	10 pM	100 pM	1 nM	10 nM	100 nM
6 mo	75 mM K+	4.9	4.5 (8)	3.7 (24)	3.3 (33)	3.3 (33)	2.5 (49)
24 mo	75 mM K+	4.0	4.2 (0)	3.8 (5)	3.6 (10)	3.1 (22)	2.9 (27)
6 mo	60 mM K+	4.8	4.0 (17)	3.4 (29)	2.4 (50)	1.6 (67)	2.0 (58)
24 mo	60 mM K+	4.0	3.2 (20)	3.3 (17)	3.0 (25)	2.3 (42)	2.3 (42)

409.3

BLOCK OF MAMMALIAN CALCIUM CHANNELS EXPRESSED IN *XENOPUS* OOCYTES BY ω-AGATOXINS. A. Kondo, J. D. Mills*, and M. E. Adams. Departments of Entomology and Neuroscience, University of California, Riverside, CA 92521.

ω-Agatoxins from *Agelenopsis aperta* spider venom are potent and selective blockers of voltage-activated calcium channels. We are using these toxins as probes in comparative studies of Ca^{2+} channels expressed in *Xenopus* oocytes. Oocytes injected with mRNA prepared from rat heart, whole brain, and cerebellum displayed voltage activated currents through Ca^{2+} channels using Ba^{2+} as a charge carrier. TEA, 4-AP, and niflumic acid were used to block potassium and chloride currents. Oocytes expressing mRNA from heart muscle showed Ba^{2+} currents with kinetics typical of L-type Ca^{2+} channels. Exposure to 100-200 nM ω-Aga-IIIA resulted in 50-90% block of high threshold current, with little or no effect observed on low threshold currents. Expression of mRNA from whole brain resulted in Ba^{2+} currents that were blocked as much as 70% by 200 nM ω-Aga-IIIA, but only 15-25% by 400 nM ω-Aga-IVA. In contrast, oocytes injected with cerebellar mRNA displayed Ba^{2+} currents that were substantially blocked by both ω-Aga-IIIA and ω-Aga-IVA. Based on the selectivity of ω-Aga-IVA, (see Mintz et al., this meeting), these results suggest that P-type channels are relatively more abundant in the cerebellum than in whole brain.

Supported by NIH grant NS24472.

409.4

A NOVEL PEPTIDE NEUROTOXIN SELECTIVELY BLOCKS MYOCARDIAL L-TYPE CALCIUM CURRENT. J.J. McArdle, Y.-F. Xiao, S.P. Aiken*, L.C. Sellin, J.J. Schmidt, and S.A. Weinstein. Dept. Pharmacology & Toxicology, New Jersey Medical School (UMDNJ), Newark, NJ 07103-2714, and Dept. Toxinology, USAMRIID, Frederick, MD 21701-5011.

Peptide I (WTX-I) from the venom of *Trimeresurus wagleri* appears to act presynaptically to interfere with synaptic transmission (Pharm. & Tox. 1992, in press). In order to explore the interaction of WTX-I with ion channels, we examined its effect on L-type Ca^{2+} (I_{Ca}) and transient K^+ (I_{to}) currents, as well Na^+ (I_{Na}) currents recorded from myocytes isolated from the left ventricle of 10-11 week old rats. Ionic currents were recorded with the whole cell configuration of the patch voltage-clamp technique. Bath application of 6 to 20 μM WTX-I had no detectable effect on I_{to} or I_{Na}. In contrast, 2, 4, and 6 μM WTX-I reduced I_{Ca} to 68.4 ± 15.4 % (n=3), 47.7 ± 6.0 % (11), and 10.1 ± 10.1 % (3) of the respective control values. This inhibitory effect on I_{Ca} could not be reversed by application of 5 μM isoproterenol. Recovery occurred after washout of the WTX-I containing solution. These findings suggest that WTX-I selectively blocks L-type Ca^{2+} channels and that stimulation of β-adrenergic receptor-modulated phosphorylation of this channel cannot overcome the blocking effect of WTX-I. Supported by grants from the NIAAA (AA08025) and the American Heart Association (90-G-27).

409.5

The cytochemical distribution of ω-Conotoxin binding sites in the Electrosensory Lateral Line Lobe (ELL) of *Apteronotus leptorhynchus*. R.W. Turner* and R. Hawkes
Neuroscience Research Group, Univ. of Calgary, Alberta T2N 4N1

The ω-conotoxin GVIA (ω-CgTX; *Conus geographus*) binds to a subset of high threshold voltage-operated calcium channels (VOCCS). ω-CgTX-binding sites in the ELL were determined using a monoclonal antibody against ω-CgTX (mab anti-ω-CgTX). 500 μm ELL slices maintained alive in cold oxygenated ACSF were exposed to 1nM - 1μM ω-CgTX (20 min), washed (3 X 10 min), and fixed in 4% PARA (2 hrs). 100 μm slices were cut and incubated overnight in mab anti-ω-CgTX (1: 0.66) and processed using the ABC technique and HRP label. The intensity of immunoperoxidase label was variable across a section but consistent in cellular distribution. Omission of ω-CgTX from the first incubation prevented all labelling. Intense staining was observed in spherical cells, but also in somata of polymorphic, granule and pyramidal cells. Pyramidal cell basal dendrites were positive but all apical dendrites beyond ~150 μm were negative. Punctate membrane-associated label was detected on spherical, polymorphic, and granule cell somata, and in association with synaptic contacts on pyramidal cells. Preliminary *in vitro* slice recordings reveal that pyramidal cells are insensitive to ω-CgTX unless exhibiting oscillatory discharge that incorporates synaptic circuitry. These data reveal a localized distribution of physiologically relevant ω-CgTX-binding sites (putative VOCCS) among cell classes of the ELL.

409.6

BIOLOGICAL CHARACTERIZATION OF THE NOVEL CALCIUM CHANNEL BLOCKER ω-AGA-IVA IN MAMMALIAN CNS. L.D. Hirning[1*], L.D. Artman[1], N. Alasti[1], D. Phillips[2], R.A. Volkmann[2], N.A. Saccomano[2] and A.L. Mueller[1]. [1]NPS Pharmaceuticals, 420 Chipeta Way, Salt Lake City, UT 84108 and [2]Pfizer Central Research, Pfizer, Inc., Groton, CT 06340.

The venom of the funnel-web spider *Agelenopsis aperta* contains a number of peptides which block voltage-sensitive Ca^{2+} channels (VSCC). Sequential chemical purification of crude peptide fraction AG1 gives rise to a single pure peptide called ω-Aga-IVA which was recently isolated and characterized independently by M. Adams and his colleagues (Nature 355: 827, 1992). ω-Aga-IVA possesses novel inhibitory activity on dihydropyridine (DHP)- and ω-CgTx-insensitive VSCC in a variety of mammalian CNS preparations. First, ω-Aga-IVA blocks transmission at the Schaffer collateral-CA1 pyramidal cell synapse in rat hippocampal slices potently and with high efficacy. Synaptic transmission in this pathway is resistant to blockade by DHPs and is blocked only partially by ω-CgTx. Second, the release of ^{3}H-GABA from hippocampal minces is resistant to blockade by DHPs and ω-CgTx. ω-Aga-IVA blocks GABA release in this system to the same extent as does 100 μM Cd^{2+}. Third, ω-Aga-IVA blocks DHP- and ω-CgTx-resistant current in acutely dissociated rat cerebellar Purkinje cells ("P-channels"). Finally, stimulation of N1E-115 cells in culture with 60 mM KCl produces transient and sustained increases in intracellular Ca^{2+} as measured with fura-2. The transient response is largely DHP-resistant but nearly completely blocked by 100 nM ω-CgTx. ω-Aga-IVA at 100 nM is ineffective, again demonstrating that ω-CgTx and ω-Aga-IVA target different populations of VSCC. ω-Aga-IVA is a unique probe with which to study the function of this novel type of VSCC in the mammalian CNS.

409.7

ω-GRAMMOTOXIN SIA: A SPIDER VENOM PEPTIDE INHIBITOR OF ω-CONOTOXIN GVIA-SENSITIVE AND -INSENSITIVE VOLTAGE-SENSITIVE CALCIUM CHANNEL RESPONSES. R. A. Keith*, P. A. DeFeo, M. B. Horn, T. J. Mangano and R. A. Lampe. Dept. of Pharmacology, ICI Americas, Inc., Wilmington, DE 19897.

A purified fraction from *Grammostola spatulata* venom was recently shown to inhibit neuronal voltage-sensitive calcium channel (VSCC) responses that were insensitive to ω-conotoxin GVIA (ω-CgTx), a selective inhibitor of N-type VSCC (Keith et al., Pharmacol. Commun., in press). The present report characterizes further the VSCC profile of this purified fraction, termed ω-grammotoxin SIA (ω-GsTx). ω-GsTx caused a concentration-dependent and near complete inhibition of K^{+}-evoked $^{45}Ca^{++}$ influx in both rat and chick synaptosomes with IC_{50} values ≅ 200 nM. In contrast, ω-CgTx potently inhibited chick synaptosomal $^{45}Ca^{++}$ influx (IC_{50} ≅ 20 nM) but was virtually inactive against rat synaptosomal $^{45}Ca^{++}$ influx (IC_{50} > 10,000 nM). ω-GsTx also caused a concentration-dependent and near complete inhibition of K^{+}-evoked release of ^{3}H-norepinephrine (NE) from rat hippocampal and chick cortical brain slices and K^{+}-evoked release of ^{3}H-D-aspartate from rat hippocampal brain slices (IC_{50} ≅ 150 nM). ω-CgTx caused a potent and complete inhibition of chick brain slice ^{3}H-NE release (IC_{50} ≅ 50 nM), a potent but incomplete inhibition of rat brain slice ^{3}H-NE release (IC_{50} ≅ 5 nM, 60% maximum inhibition), and was virtually inactive against rat brain ^{3}H-D-aspartate release (no effect at 3000 nM). ω-GsTx (750 nM) did not significantly displace ^{125}I-ω-CgTx, ^{3}H-PN 200-110 or ^{3}H-D-888 binding to rat synaptosomal membrane fragments. The results suggest that ω-GsTx is a potent inhibitor of ω-CgTx-sensitive and -insensitive neuronal VSCC by a mechanism that is distinct from previously identified VSCC antagonists.

409.8

ISOLATION AND STRUCTURAL CHARACTERIZATION OF ω-GRAMMOTOXIN SIA, A NOVEL PEPTIDE INHIBITOR OF NEURONAL VOLTAGE-SENSITIVE CALCIUM CHANNELS. R. A. Lampe*, M. M. Lo, M. Davison, M. B. Horn, A. Verticelli, P. A. DeFeo, J. L. Herman and R. A. Keith. Depts. of Pharmacology, Structural Chemistry and Biotechnology, ICI Pharmaceuticals, Wilmington, DE 19897.

ω-Grammotoxin SIA (ω-GsTx), a peptidergic blocker of voltage-sensitive calcium channels (VSCC), was purified from *Grammostola spatula* (tarantula spider) venom by reverse phase HPLC (RP-HPLC). Three RP-HPLC purification steps, using gradient and isocratic elutions from a C-8 Zorbax Rx semi-preparative column, were required to obtain this single, apparently homogeneous, peptidergic species. Biological activity was monitored by determining the inhibition of K^{+}-stimulated influx of $^{45}Ca^{++}$ into rat brain synaptosomes *vs.* lack of inhibition of ^{125}I-ω-conotoxin GVIA binding to rat hippocampal membranes. Fast atom bombardment mass spectrometry (FAB-MS) indicated an average molecular mass of 4110 daltons for the protonated molecular ion. Exposure of the peptidergic entity to reducing agents on the FAB probe led to an increase of 6 amu indicating the presence of 3 disulfide bridges or 6 Cys residues. N-Terminal Edman sequence analysis of the reduced, pyridylethylated peptide was carried out on the intact species and on the COO^{-} terminal fragment following tryptic digestion. Primary sequence data confirms the existence of 6 Cys residues, and 36 residues in total, with a calculated molecular mass of 4109 for the amidated COO- terminal species. Synthetic preparation of ω-GsTx is currently underway to provide undeniable demonstration of the biological profile obtained for the purified peptide. This biological profile includes the blockade of N-, as well as non-L/non-N-type, VSCC responses, with no inhibition of binding of known Ca^{++} channel ligands. Biological data are presented in a companion abstract.

409.9

NOVEL CONOPEPTIDE INHIBITORS OF MAMMALIAN PRESYNAPTIC Ca CHANNELS DERIVED FROM cDNA CLONING AND PEPTIDE SYNTHESIS. D. R. Hillyard, V.D. Monje, S. Gaur, L. Nadasdi, G. Miljanich, J. Ramachandran, and B. M. Olivera*
Depts. of Pathology and Biology, U. of Utah, Salt Lake City, UT 84132; Neurex Corp., 3760 Haven Ave., Menlo Park, CA 94025.

The venoms of fish-hunting cone snails contain potent peptide inhibitors of presynaptic neuronal calcium channels. However, previously characterized peptides such as ω-conotoxin GVIA, target only a subset of mammalian CNS Ca channels. We used a molecular biological approach to derive new ω-conopeptides which inhibit an expanded subset of mammalian CNS Ca channels. Oligonucleotide probes directed to codons of conserved amino acids of the Ca channel-targeting conopeptides MVIIA and MVIIB were used to identify cDNAs encoding two new Ca channel ligands. cDNA sequencing was used to guide synthesis of peptides MVIIC and MVIID which inhibit synaptic transmission which is ω-GVIA-resistant. Several ω-GVIA-resistant Ca currents, i.e., depolarization-induced ^{45}Ca uptake in rat synaptosomes, "P" currents in cerebellar Purkinje cells and a subset of ω-GVIA-resistant channels in CA1 hippocampal cells are sensitive to ω-MVIIC. In addition, in several systems, ω-MVIIC inhibits a component of neurotransmitter release that is resistant to ω-GVIA or MVIIA (see abstracts by Gaur *et al.*, and Newcomb and Palma). Accordingly, these new peptides offer important new tools for the study of Ca channel subtypes.

409.10

NEOMYCIN DISPLACEMENT OF [^{125}I]-ω-CONOTOXIN GVI-A BINDING IS NOT UNIFORM ACROSS NEUROANATOMICAL REGIONS: EVIDENCE FROM AUTORADIOGRAPHIC STUDIES. F. Filloux[1*], B.M. Olivera[2] and J.M. McIntosh[3], Depts Neurol[1], Pediatr[1], Biol[2] and Psychiat[3], Univ. Utah, Salt Lake City, UT 84132.

Previous studies have demonstrated that neomycin (NEO) inhibits [^{125}I]-ω-Conotoxin GVI-A (*ω-CgTx) binding to brain membranes suggesting that NEO may block or modulate N-type Ca^{++} channels. Our early observations, however, have indicated that the ability of NEO to displace *ω-CgTx from slide-mounted tissue sections is not uniform across brain regions. This issue has been investigated further. 10μM sagittal slide-mounted tissue sections from rat brain were labeled with *ω-CgTx at 4 concentrations (40, 80, 200 and 500 pM). Adjacent tissue sections were labeled in the presence of 0.1 mM NEO or 250 nM unlabeled ω-CgTx (the latter to define non-specific binding). At each concentration of *ω-CgTx, NEO produced the same asymmetric pattern of regional displacement of radioligand binding; e.g., at 200 pM *ω-CgTx, NEO displacement varied from greatest to least as follows: granule cell layer, cerebellum (94% displacement)> granule cell layer, dentate gyrus (84%)> thalamus (77%)> molecular layer, cerebellum (58%)> neocortex (47%)> substantia nigra reticulata (38%). These results confirm that the displacement of *ω-CgTx by NEO varies considerably across neuroanatomical areas. Such findings may indicate that the combined use of NEO and *ω-CgTx may delineate subtypes of Ca^{++} channels which occur in differing proportions in distinct brain regions.

409.11

CALCIUM CHANNEL ANTAGONISTS [^{125}I]ω-Aga-IIIA AND [^{125}I]ω-CgTx: AN AUTORADIOGRAPHIC COMPARISON OF BINDING SITES IN RAT BRAIN. J.M. McIntosh[1]*, M.E. Adams[2], B.M. Olivera[3] and F. Filloux[4], Departments of Psychiatry[1], Biology[3], Pediatrics[4] and Neurology[4], University of Utah, Salt Lake City, Utah, 84132; and Departments of Entomology and Neuroscience[2], University of California, Riverside, California, 92521.

Specific binding of the calcium channel ligands [^{125}I]ω-agatoxin IIIA (*ω-Aga-IIIA) and [^{125}I]ω-conotoxin GVIA (*ω-CgTx) was compared autoradiographically using sagittal sections from rat brain. Binding patterns for these two ligands were generally similar, but notable differences were detectable particularly in the cerebellum and hippocampus. Specific *ω-Aga-IIIA binding was greatest in the granule cell layers of the cerebellum and of the dentate gyrus. In contrast, binding of *ω-CgTx was most intense in the molecular layers of these structures. 250 nM ω-CgTx inhibits less than 33% of *ω-Aga-IIIA binding while 40 nM ω-Aga-IIIA inhibits greater than 92% of *ω-CgTx binding. The P-type calcium channel antagonist ω-Aga-IVA (40 nM) blocked only 11% of *ω-Aga-IIIA binding and 2% of *ω-CgTx binding. These data suggest that ω-CgTx binding sites are a subset of ω-Aga-IIIA sites and that the combined use of agatoxins and conotoxins may be useful for discriminating between calcium channel subtypes.

409.12

A POLYPEPTIDE FRACTION ISOLATED FROM *CONUS MAGUS* VENOM EXHIBITS NOVEL CALCIUM CHANNEL ACTIVITY IN NEURONAL PREPARATIONS. S.J. Stoehr†, A.B. Giordani‡, S.R. Naisbitt°, J.M. McIntosh°, D.J. Dooley†, B.M. Olivera°, and D.A. Downs*†. Departments of Neuroscience† and Chemistry‡, Parke-Davis, Pharmaceutical Research Div., Warner Lambert, Ann Arbor, MI 48106; Departments of Psychiatry° and Biology°, University of Utah, Salt Lake City, UT 84112.

Natural peptides purified from the venoms of numerous poisonous organisms have become important tools for understanding neuronal ion channels. We have isolated two polypeptides from venom of the marine snail *Conus magus* which interact with L-type Ca^{2+} channels. An aqueous extract from crude venom was tested in a [^{3}H]-isradipine binding assay to assess L-type Ca^{2+} channel affinity. The *C. magus* extract was at least ten times more effective at inhibiting [^{3}H]-isradipine binding to rat neocortical membranes than extracts from eight other *Conus* species (relative IC_{50} = 1/400,000 dilution). We used two reverse phase chromatography steps to isolate the active component. In each step, the inhibitory activity eluted as a single peak (absorbance at 214 nm), resulting in a ~300-fold purification of the material. This material inhibited [^{3}H]-isradipine binding to both neocortical and crude skeletal muscle membranes. Intracerebroventricular injection of this fraction into mice resulted in arched backs and extended extremities, an effect similar to that seen with the L-type Ca^{2+} channel activator Bay K 8644. This fraction also blocked K^+-induced $^{45}Ca^{2+}$ flux into rat brain synaptosomes, indicating a functional interaction with presynaptic Ca^{2+} channels. When the fraction was further purified using reverse phase chromatography with isocratic elution, the fraction separated into two peaks, and both inhibited [^{3}H]-isradipine binding. The polypeptide molecular weights were found to be ~13.7 kDa by electrospray mass spectrophotometry. Sequencing efforts are underway to determine the identity of these polypeptides.

409.13

[^{125}I]-ω-CONOTOXIN MVIIA: A NEW, COMMERCIAL RADIOLIGAND FOR N-TYPE CALCIUM CHANNEL BINDING IN NEURONAL MEMBRANES. J.J. Geer*, S.J. Stoehr, and D.J. Dooley. Department of Neuroscience, Parke Davis, Pharmaceutical Research Div., Warner Lambert, Ann Arbor, MI 48106.

The ω-conotoxins GVIA and MVIIA, isolated from the marine snails *Conus geographus* and *Conus magus*, are important tools for identifying and blocking N-type calcium channels in neuronal preparations. Although [^{125}I]-GVIA is commercially available, its irreversible binding characteristics make true competitive binding studies difficult. We recently tested an experimental preparation of [^{125}I]-MVIIA (Amersham), and compared this ligand to [^{125}I]-GVIA. We report here the methods for and characteristics of [^{125}I]-MVIIA binding. Synthesized MVIIA (Peninsula) was iodinated by Amersham using an enzymatic method. Specific activity of the ligand was ~2000 Ci/mmol (74 TBq/mmol). Binding of [^{125}I]-MVIIA to crude rat neocortical membranes was carried out at 37° C in 0.5 ml buffer (50 mM HEPES-NaOH, 0.1% BSA; pH 7.4) for 30 minutes. Unbound ligand was removed by filtration through GFC filters presoaked in 0.1% polyethylenimine (PEI), followed by two washes with buffer supplemented with 0.2 M NaCl. Saturation analysis gave a K_D of 6 x 10^{-13} M and a B_{max} of 1200 fmoles/mg protein. Binding was linear from 10-800 ng protein using [^{125}I]-MVIIA at 3 pM. These results were comparable to those obtained with [^{125}I]-GVIA (K_D = 0.6 pM; B_{max} = 995 fmol/mg protein, 23°C). The association kinetics of both [^{125}I]-MVIIA and [^{125}I]-GVIA were rapid (MVIIA, $t_{½}$ = 2 min, 37°C, at 3 pM ; GVIA, $t_{½}$ = 4 min, 23°C, at 4 pM). However, the dissociation rates were markedly different. [^{125}I]-MVIIA was readily displaced by excess cold ligand ($t_{½}$ = 1 min). [^{125}I]-GVIA was not significantly displaced by cold GVIA in 4 hours. Commercial availability of this high affinity, reversible radioligand will allow for direct competitive analyses of novel N-type calcium channel modulators.

409.14

DISTRIBUTION OF OMEGA CONOTOXIN-SENSITIVE CALCIUM CHANNELS IN THE ADULT RAT BRAIN. R.E. Westenbroek*[1], J.W. Hell[1], S. Dubel[2], T.P. Snutch[2], and W.A. Catterall[1]. [1]Department of Pharmacology, University of Washington, Seattle, WA 98195, and [2]Biotechnology Laboratory and Departments of Zoology and Neuroscience, University of British Columbia, Vancouver, B.C., Canada, V6T1W5.

N-type channels are high-voltage-activated Ca channels which are blocked by ω-conotoxin GVIA (ω-CgTX) and have been implicated in generation of calcium-dependent action potentials and neurotransmitter release. A polyclonal anti-peptide antibody (CNB1) that recognizes the α_1 subunit of an ω-CgTx-sensitive Ca channel immunoprecipitates the high affinity receptor for ω-CgTX but not the dihydropyridine receptor of L-type Ca channels after solubilization from rat forebrain (Dubel et al., Proc. Natl. Acad. Sci. USA, in press). In the present study, CNB1 immunoprecipitated 43% of the total N-type calcium channels labeled with [^{125}I]ω-CgTX. Immunoblotting with CNB1 revealed proteins of 210 and 240 kDa. CNB1 was used in combination with the immunofluorescence and indirect peroxidase-anti-peroxidase techniques to investigate the distribution of N-type Ca channels in rat brain. Ca channels recognized by CNB1 are localized along dendritic processes and on occasional somata. For example, CNB1 immunoreactivity is localized along the full length of apical dendrites of hippocampal pyramidal neurons with little labeling of the somata, while somata of pyramidal cells in the dorsal cortex are frequently labeled and the staining of apical dendrites decreases as distance from the cell body increases. In addition, there is intense punctate staining of structures (possibly synapses) along the dendrites and on occasional cell bodies in the dorsal cortex, subiculum, and cerebellar Purkinje cells. These observations contrast with studies of L-type calcium channels which have been shown to be localized in a narrow zone around the periphery of neuronal somata and in proximal dendrites of projection neurons such as hippocampal pyramidal cells (Ahlijanian et al., Neuron 4:819-832, 1990; Westenbroek et al., Nature 347:281-284, 1990). These results indicate that the ω-CgTX-sensitive Ca channels recognized by CNB1 have a distinct distribution from L-type Ca channels in central neurons which may correlate with distinct functional roles for these channel subtypes.

409.15

Components of the electrically evoked increase in intracellular calcium in chick dorsal root ganglion and sympathetic ganglion neurons. Yi Zhou,* and W. D. Branton. Department of Physiology, University of Minnesota, Minneapolis, MN. 55455.

We measured changes in Fluo-3 fluorescence as an indicator of changes in intracellular calcium in primary cultures of chick DRG and sympathetic ganglion neurons. Ca^{++} rises were induced via trains of electrical stimuli delivered thru extracellular electrodes placed in the recording chamber. A 1-2 sec train at 10 hz produced a rapid and reversible rise in internal Ca^{++} in both DRG and sympathetic ganglion cells. The response was prevented by removal of external Ca^{++} and by 200 nM TTX, indicating that it was dependent on calcium influx and on the generation of sodium dependent action potentials. In DRG cells 89% (+/- 2%; n=15) of the Ca^{++} rise was prevented by brief incubation with omega Conotoxin. The block appeared saturated at 1 uM toxin. 10 uM Nitrendipine blocked 13% (+/- 1%; n=7) 1 mM Amiloride blocked 4% (+/- 2%; n=7). This data suggests that the calcium influx in chick DRG cells under these conditions is largely through N type channels. T type channels appear to play a very minor role, since even relatively high concentrations of Amiloride block little of the Ca^{++} increase. These data appear to conflict with recent voltage clamp studies suggesting a significant role for T currents during the action potential. In sympathetic cells, Conotoxin blocked considerably less of the Ca^{++} rise (63% +/- 4%; n= 7). Very preliminary data suggest that Nitrendipine and Amiloride may each block more of the Ca^{++} rise in sympathetic cells relative to their effects in DRG cells. (Supported by NIH R01 GM42829. We particularly thank Stan Thayer for valuable advice and discussion.)

409.16

Toxins in *Plectreurys* spider venom are potent blockers of vertebrate calcium channels. E. A.Newman, Yi Zhou, M. S. Rudnick, and W. D. Branton*. Dept. of Physiology, University of Minnesota, Minneapolis, MN 55455.

We prepared a partially purified fraction of *Plectreurys tristes* spider venom for assay on vertebrate calcium channel preparations. Size exclusion on Sephadex G50 and step elution from C18 with 30% and 60% acetonitrile (0.1% TFA) yields a relatively hydrophobic fraction containing peptides with masses of a few thousand daltons. This fraction has been found in various previous assays to be primarily inhibitory. This inhibitory fraction represents approximately a one hundred fold purification from crude venom on the basis of mass, but it still contains many different peaks when analyzed on reverse phase by gradient elution. We assayed this fraction for activity in blocking electrically stimulated rises in internal Ca^{++} in primary cultures of chick dorsal root ganglion (DRG) and sympathetic ganglion neurons. The change in intracellular Ca^{++} induced by a train of brief extracellular voltage pulses was measured as an increase in the fluorescence of the Ca^{++} indicator dye Fluo-3. The inhibitory fraction of the venom blocked 87% (+/- 2%; n=-10) of the fluorescence increase in DRG cells, and 60% (+/- 2%; n=15) of the increase in sympathetic ganglion cells. The block saturated at a 1:1000 or greater dilution of toxin relative to crude venom and was not reversible during the time course of the experiments. This toxin activity is similar to omega Conotoxin and likely represents a potent block at least of N type channels. Preliminary voltage clamp data on DRG cells also show that this fraction blocks Ca^{++} currents. Purification and analysis of specific toxin components are in progress. (Supported by NIH R01 GM42829.)

409.17

A NOVEL CALCIUM CHANNEL BLOCKING CONOPEPTIDE, SNX-230 (M-VIIC), REVEALS MULTIPLE COMPONENTS OF NOREPINEPHRINE RELEASE. S. Gaur, L. Nadasdi, J. Bell, J. Ramachandran, and G. Miljanich*. NEUREX Corporation, Menlo Park, CA 94025.

We have synthesized the conopeptide designated SNX-230 corresponding to a novel ω-like conopeptide, M-VIIC, predicted from a c-DNA sequence obtained from a *Conus magus* venom gland expression library (see abstract by Hillyard, et al.). SNX-230 potently (20pM IC50) inhibits about 50% of release of radiolabeled norepinephrine (*NE) from K^+-depolarized rat hippocampal slices. It inhibits the remaining 50% with much lower potency (65nM IC50). In contrast, ω-conopeptide SNX-111 (synthetic M-VIIA from *C. magus* venom) inhibits a maximum of 65% of *NE release and with moderate potency (0.5nM IC50). To understand the relationship of the conopeptide-sensitive components of *NE release, slices were subjected to both peptides simultaneously: SNX-111 at a maximally (65%) effective concentration (100nM) and SNX-230 at a concentration (1nM) at which it exhibits only its high-potency (50%) inhibitory activity. Surprisingly, *inhibition of *NE release was not complete* - being only about 85% complete. One explanation is that there are four conopeptide-sensitive components of *NE release:

	total effect	comp.1 (35%)	comp.2 (20%)	comp.3 (15%)	comp. 4 (30%)
SNX-230, 1μM	100%	+	+	+	+
SNX-230, 1nM	50%		+		+
SNX-111, 100nM	65%	+			+
SNX-230 + SNX-111	85%	+	+		+

Thus, SNX-111 inhibits components 1 and 4 with about the same moderate potency (0.5nM IC50) and SNX-230 inhibits components 2 and 4 with high potency (20pM IC50) and components 1 and 3 with much lower potency (65nM IC50). Since these inhibitory effects are very likely due to blockade of calcium channels, we believe that the conopeptides SNX-111 and SNX-230 will be uniquely useful reagents in elucidating the roles of their calcium channel targets in neuronal function.

CALCIUM CHANNELS: PHYSIOLOGY II

410.1

LOCALIZATION OF CAFFEINE-SENSITIVE AND IP3-SENSITIVE CALCIUM RELEASE CHANNELS TO DISCRETE AREAS OF THE RAT BRAIN. A.H. Sharp*, P.S. McPherson±, C. Aoki#, T.M. Dawson, K.P. Campbell± and S.H. Snyder. Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205, +H.M.I., Univ. of Iowa, Iowa City, IA 52242 and #C.N.S., New York Univ., N. Y., NY 10003

Release of caffeine-sensitive and IP3-sensitive intracellular Ca2+ stores are mediated by distinct proteins identified as ryanodine receptor (RyR) and IP3 receptor (IP3R), respectively. Rises in intracellular Ca2+ can induce release of Ca2+ from the caffeine-sensitive pool via the RyR, a process known as Ca2+-induced Ca2+ release. We have localized caffeine-sensitive and IP3-sensitive Ca2+ release channels in the brain using specific antibodies raised against purified brain RyR and IP3R, respectively. RyR and IP3R were identified in overlapping populations of neurons in widespread areas of the brain but labeling was reciprocal in a number of areas. For example, while IP3R was enriched in cerebellar Purkinje cells and hippocampal CA1 pyramidal cells, RyR was present at relatively low levels in these cells. RyR was most enriched in the dentate gyrus and CA3/4 areas of the hippocampus, where IP3R was present at relatively low levels. In the cortex, RyR was localized to pyramidal neurons, some of which were not labeled by anti-IP3R antibodies. Electron microscopy in the hippocampus revealed the presence of RyR in axons and in dendritic spines receiving asymmetric synapses while IP3R was primarily identified in dendritic membranes forming symmetric synapses. These results suggest that the IP3- and caffeine-sensitive Ca2+ pools have roles in controlling intracellular Ca2+ levels that are in some cases distinct and that in other cases may interact to varying degrees depending on the neuron and on the subcellular compartment within a given neuron. Supported by MH09953 to AHS, DA00074 to SHS, 2-S07-RR07062-26 to CA and Pfizer Fellowship to TMD. KPC is HHMI investigator.

410.2

ACTION POTENTIAL-INDUCED ALTERATIONS IN CYTOSOLIC CALCIUM IN FURA-2-LOADED DISSOCIATED BULLFROG SYMPATHETIC NEURONS. T.J. Heppner* and J.F. Fiekers. Dept. Anatomy & Neurobiology, College of Medicine, University of Vermont, Burlington, VT, 05405, U.S.A.

The kinetics of $[Ca^{2+}]_i$ in response to direct electrical stimulation were examined in dissociated neurons from the 9th and 10th sympathetic ganglia of the bullfrog *Rana catesbeiana*. $[Ca^{2+}]_i$ was determined in individual neurons using a ratiometric analysis with fura-2. Simultaneous recording of action potentials and passive electrical properties were recorded with electrophysiological techniques. Basal levels of $[Ca^{2+}]_i$ ranged from 82 nM to 225 nM (n=19). Trains of action potentials (0.1 to 10.0 Hz) were evoked by depolarizing current injection. The rate of rise of $[Ca^{2+}]_i$ was increased in a frequency-dependent manner. At each stimulus frequency, a maximal level of $[Ca^{2+}]_i$ was obtained and maintained during the train. Basal levels were restored following cessation of stimulation. The addition of Co^{2+} (2 mM) to the extracellular solution abolished the stimulation-induced increase in $[Ca^{2+}]_i$. The $[Ca^{2+}]_i$ response to puffer applications of ACh was decreased during train-induced elevations of $[Ca^{2+}]_i$. These results indicate that the origin of $[Ca^{2+}]_i$ during action potential generation is extracellular and that these neurons are capable of buffering large changes in $[Ca^{2+}]_i$ during and following train stimulation. (Supported by NS 27319).

410.3

SPONTANEOUS CALCIUM OSCILLATIONS IN CULTURED RAT CHROMAFFIN CELLS. A.R. Wakade, D.A. Przywara* & T.D. Wakade. Dept. of Pharmacology, Wayne State University, Detroit, MI 48201.

Rat chromaffin cells (RCC) of 8-day-old pups were cultured and used to measure intracellular Ca^{2+} by Indo-1 dye method, to monitor membrane potential using patchclamp technique and to determine catecholamine release by labeling with $[^3H]$NE. Almost all cultured RCC exhibited spontaneous oscillations (OSC) in intracellular free Ca^{2+} ($[Ca^{2+}]_i$). The OSC were random and varied in frequency, duration and magnitude. Neither cadmium, nifedipine, lanthanum, ryanodine, thapsigargin nor tetrodotoxin or tetraethylammonium affected OSC. $CaCl_2$-free Krebs solution plus 0.5 mM EGTA completely blocked OSC and lowered $[Ca^{2+}]_i$ in RCC. Spontaneous release of $[^3H]$NE was reduced by more than 50% when Ca^{2+} was omitted from the external medium. Low temperature (≈5°C) caused maximum and sustained rise in $[Ca^{2+}]_i$. Whole cell current clamp recordings showed that RCC had unstable resting membrane potentials (Em) and spontaneous action potentials (AP) which varied in frequency. Spontaneous AP were depressed by TTX and blocked by TTX plus Ca^{2+}-channel blockers. However, Em continued to fluctuate in the presence of these blockers. We have ruled out the possibility that Ca^{2+} OSC are due to voltage- or receptor-operated Ca^{2+} channels, or release of Ca^{2+} by intracellular mechanisms. Passive influx of Ca^{2+} could generate OSC and initiate exocytosis in RCC.

410.4

$[Ca^{2+}]_i$ RELAXATIONS AND OSCILLATIONS FOLLOWING CHANGES IN VOLTAGE-DEPENDENT Ca^{2+} ENTRY AND Ca^{2+}-INDUCED Ca^{2+} RELEASE IN BULLFROG SYMPATHETIC NEURONS. D.D Friel* and R.W. Tsien, Dept. of Molec. and Cell. Physiol. Stanford Univ., Stanford, CA 94305.

We studied how $[Ca^{2+}]_i$ relaxes after sudden changes in voltage-dependent Ca^{2+} entry, and uptake and release from a caffeine- and ryanodine-sensitive store. High K^+ (30-50 mM) depolarized V_m and caused a steady $[Ca^{2+}]_i$ elevation, while caffeine (1-10 mM) produced a transient $[Ca^{2+}]_i$ rise. Restoring $[K^+]_o$ to 2 mM led to repolarization and a monotonic decline in $[Ca^{2+}]_i$; caffeine removal produced a transient $[Ca^{2+}]_i$ undershoot. Both relaxations are described by the sum of two decaying exponentials with the same τ_{fast} (~3-5 s) and τ_{slow} (~5-10 min) but with different amplitudes. This is consistent with a three-compartment scheme consisting of the external bath, the cytoplasm and an internal store, with pump and leak fluxes between compartments depending linearly on Ca^{2+} concentration, valid for small departures from the steady state. In this scheme, the τ's reflect the dynamical properties of the system (transport rates, relative cytosolic and store volumes) while the amplitudes additionally depend on the initial conditions. To account for $[Ca^{2+}]_i$ dynamics in the presence of caffeine, an internal Ca^{2+}-dependent leak was introduced. The resulting flux equations exhibit periodic solutions resembling $[Ca^{2+}]_i$ oscillations in the presence of caffeine and high K^+ (Friel and Tsien (1992), Neuron 8, 1-20): (1) ↑$[Ca^{2+}]_o$ → ↑ frequency (f); (2) ↑$[caff]_o$ → ↑f and ↓amplitude, and (3) Ca^{2+} removal has phase-dependent effects on $[Ca^{2+}]_i$. Therefore, a three compartment system with linear Ca^{2+} pumps and leaks and a single $[Ca^{2+}]_i$-dependent leak in the internal store predicts $[Ca^{2+}]_i$ oscillations like the observed ones; nonlinear pumps, etc. are not required. This provides a simple and testable model for caffeine-induced $[Ca^{2+}]_i$ oscillations in sympathetic neurons, and may be useful in the study of caffeine- and ryanodine-sensitive $[Ca^{2+}]_i$ oscillations induced by agonists in non-neuronal cells.

410.5

DISTRIBUTION OF CALSEQUESTRIN, RYANODINE AND IP3 RECEPTORS IN THE CHICK CEREBELLUM: A THREE-DIMENSIONAL IMMUNOLOCALIZATION STUDY. M. E. Martone*, V. M. Simpliciano, T. J. Deerinck, J. A. Airey‡, J. L. Sutko‡, and M. H. Ellisman. San Diego Microscopy and Imaging Resource, Dept. of Neurosciences, U.C.S.D., La Jolla, CA, 92093; ‡Pharmacology, Univ. of Nevada, Reno, NV 89557.

Many proteins involved in intracellular calcium regulation in skeletal or smooth muscle, e.g. the IP3 and ryanodine receptors, calsequestrin, Ca++ ATPase, have been found in brain where they are particularly abundant in the endomembrane system of cerebellar Purkinje neurons (Walton et al., *J. Cell. Biol. 113:* 1145, 1991; Villa et al., *J. Cell Biol. 113*: 779, 1991). While the muscle sarcoplasmic reticulum is planar and ordered relative to the sarcomere, the neuronal endomembrane system anastomoses in three dimensions (3D) presenting formidable obstacles to understanding the organization of calcium regulatory components. In the present study, the distribution of immunolabeling for calsequestrin (CS), the IP3 (IP3R) and ryanodine receptor (RR) was examined in chick cerebellum. Labeling for these proteins was determined in E18 to 4 week old chicks using laser-scanning confocal microscopy. Although labeling for all three proteins was extensively distributed within Purkinje cells, a unique pattern was found for each. Labeling for the IP3R was fairly evenly distributed throughout the cell body, dendrites and dendritic spines while labeling for RR and particularly CS was more discontinuous and not found in spines. The discontinuities in CS labeling appeared most prominent in older chicks. Preliminary analyses of double-labeled preparations suggest that the distribution of RR and CS labeling are not identical. In order to determine the structural basis for this difference, we have adapted a cryosection immunolabeling method for 3D studies using thick sections and high voltage electron microscopy. Labeling for IP3R with 5nm gold was achieved through 0.25 um sections, with good fine structure, by using extended incubation times and elevated temperatures. Through the use of single and double-labeling, we expect to resolve how the spatial distribution of these proteins relates to endomembrane system components thus adding important information to our understanding of the mechanisms by which these cells modulate intracellular calcium.

410.6

DETERMINATION OF THE 3-D CHANGES IN INTRACELLULAR Ca^{++} ACTIVITY IN DISSOCIATED RAT HIPPOCAMPAL NEURONES USING RATIOMETRIC CONFOCAL LASER SCANNING MICROSCOPY.

S.D.Jane, H.R.Parri, H.V.Wheal & J.E.Chad*. Dept. of Physiology and Pharmacology, Univ. of Souhampton, SO9 3TU, UK.

The increase in intracellular calcium ion activity (Cai) within neurones is a powerful and potentially dangerous signal, and is subject to a host of control systems. However, conventional investigative techniques have lacked the resolution to determine the true spatial and temporal extent of these changes.

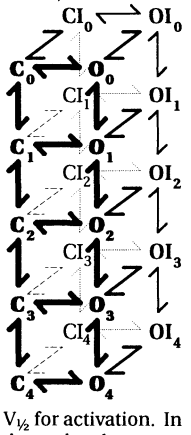
Confocal Laser Scanning Microscopy (CLSM) can resolve cellular morphology to a sub-micron level in three dimensions (3-D), revealing individual synaptic spines. This capability can be harnessed to the determination of changes in intracellular Ca^{++} ion activity within discrete volume elements (voxels), with the use of Ca^{++} sensitive fluorescent dyes. We have used the acetoxymethyl esters (AM) of the calcium-sensitive fluorescent dyes fluo-3 (10μM) and indo-1 (20μM), to load (60min,18°C) viable, dissociated rat hippocampal neurones. Indo-1, requires UV (360nm) excitation optics (argon ion laser), but can be used for ratiometric measurements.

The Cai responses of neurones to elevated K^+ (20mM), caffeine (20mM) and glutamate (50μM), were tested (objective lens Nikon x60 PlanApo, NA 1.4). The experimental design was to record z-series image data under control conditions, in the presence of the stimulus, after wash, in the presence of ionomycin, and finally with ionomycin and extracellular EGTA. The last two conditions give a calibration for maximal (saturated) and minimal Ca^{++} loading of the dye. Caffeine gave the most consistent data, with apparently widespread elevations in Cai (n=20/20 viable cells). Both glutamate and high K^+ also produced increases in Cai but less consistently. We are presently refining our techniques to unequivocally determine the subcellular localisation and extent of the changes in Cai due to different stimuli.

Supported by SERC, MRC and University of Southampton.

410.7

AN ALLOSTERIC MODEL FOR INACTIVATION OF VOLTAGE-DEPENDENT CHANNELS. Stephen W. Jones*. Dept. Physiol. & Biophys., Case Western Reserve Univ., Cleveland, OH 44106.



Activation kinetics of L-type calcium channels can be described based on the Monod-Wyman-Changeux model, with movement of a voltage sensor corresponding to ligand binding, and channel opening corresponding to activation of the protein; the channel opens more readily with more voltage sensors moved (Marks & Jones, *J. Gen. Physiol.* **99**, 367-390, 1992). A model where inactivation is also allosterically coupled to voltage sensor movement can explain (1) voltage-dependent macroscopic inactivation, (2) voltage-*in*dependent microscopic inactivation, (3) strong inactivation at depolarized voltages, but rapid recovery from inactivation at negative voltages, and (4) $V_{½}$ for inactivation more negative than $V_{½}$ for activation. In its simplest form, where inactivation rates depend on how many voltage sensors have moved but *not* on whether the channel is open or closed (or on voltage), the model has 9 free parameters.

410.8

Depolarizing afterpotentials and the T-type calcium current in hippocampal dentate granule neurons: experimental and computer simulation.

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Among intrinsic ionic events, post-spike potentials are known to play an important role in regulating neuronal excitability. In mature dentate granule (DG) neurons, a single action potential is followed by a depolarizing afterpotential (DAP). Ca^{2+}-dependant conductances have been suggested to contribute to the DAP in mature DG neurons and, play a role in post-spike depolarizing afterpotentials in other mammalian CNS neurons. We used whole cell and, perforated patch recording techniques to determine the ionic mechanisms underlying the DAP in hippocampal slices of postnatal day 10-17 rats. DAPs were generated either by antidromic stimulation of the mossy fiber pathway or with brief depolarizing current steps. DAPs display an all or none type of behaviour and, DAP like potentials can be generated in the presence of tetrodotoxin. Of the three types of calcium currents (transient low threshold or T-type; transient high threshold or N-type and, sustained high threshold or L-type) only the T-type calcium current displays strong voltage dependent inactivation, persists when evoked at 2 Hz and, is blocked by Ni^{2+}. That these properties are shared by the DAP, indicates that the T-type calcium current may play a role in the genesis of the DAP in immature DG neurons. From voltage clamp data we characterized the T-type current according to an m^3h Hodgkin-Huxley formulation and incorporated this current into a compartmental model of a DG neuron. Computer simulations of antidromically evoked action potentials indicate that the kinetics of the T-type calcium current are sufficient to generate a DAP like potential if the T-type calcium conductance has both a somatic and dendritic distribution.

Supported by the MRC.

410.9

THEORETICAL AND EXPERIMENTAL STUDIES OF PANCREATIC ISLET CELL BURST CURRENTS USING A BURST-WAVEFORM VOLTAGE-CLAMP COMMAND. L.S. Satin* and P.D. Smolen. Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA. 23298 and Biomathematics Research Branch, NIDDKD, Bethesda, MD. 20892

We recently suggested that a slowly-inactivating Ca current may act as a burst pacemaker mechanism in pancreatic islet B-cells (Cook, Satin and Hopkins (1991),TINS **14**:411-414). To test this hypothesis, a burst-waveform voltage command (BWC) that mimicked a B-cell burst was used to drive different theoretical models of bursting as well as voltage-clamped insulin-secreting HIT cells. The BWC started from a silent potential of -50 mV and slowly depolarized to a plateau of -30 mV from which spikes to -10 mV commenced. The spiking phase lasted for 7 seconds and was followed by repolarization to the next interburst. To simplify the analysis of Ca current, 20 msec test pulses to +20 mV were superimposed on the BWC at 1 Hz. *Results:* Models that incorporated slow Ca current inactivation predicted slow Ca current decay during the burst-waveform plateau and recovery during the interburst. Application of the same BWC to HIT cells whose Ca current was pharmacologically isolated (Satin and Cook, 1989; Pflugers Archiv **414**:1-10) showed that Ca current initially activated and then slowly inactivated during the BWC, supporting the recent models. In addition, analysis of spike currents suggests that fast inactivation may contribute to spike repolarization. Application of BWC appears to be a powerful new approach for understanding the bursting mechanisms of excitable cells.

410.10

ISOLATION OF NOVEL NEURONAL CALCIUM CHANNEL BETA SUBUNITS. E. Massa and M.D. Uhler* Graduate Program in Neuroscience, Mental Health Research Institute and Department of Biochemistry, Mental Health Research Institute, Ann Arbor, MI 48105.

The dihydropyridine-sensitive calcium channels in rabbit skeletal muscle consist of α_1, α_2, β, and γ subunits. The cDNAs of these skeletal muscle subunits have been cloned. The β subunit has been further characterized and shown to have two genes that encode for homologous proteins. β_1 is expressed in skeletal muscle and brain. In contrast, β_2 is expressed in brain, heart and lung. In order to isolate splice variants of the β_2 subunit, we screened a mouse brain cDNA library with a 900 bp DNA fragment corresponding to the first 900 nucleotides of the rabbit skeletal muscle β subunit coding region. Preliminary characterization of the isolated clones suggests that multiple splice variants of the β_2 exist in brain. Sequencing of one clone demonstrated the existence of a previously undescribed splice variant. The predicted amino terminal sequence of this splice variant was found to diverge from the published β_2 subunit sequence at amino acid 17. Initial characterization of the other clones suggests that additional splice variants of the β_1 subunit exist in brain. In the future, coexpression of these novel β_1 subunit splice variants with mouse neuronal α_1 subunits, currently being cloned in our laboratory, will be performed.

410.11

ISOLATION AND CHARACTERIZATION OF THE P-TYPE CALCIUM CHANNEL FROM MAMMALIAN MUSCLE. B. Cherksey*, M. Sugimori and R. Llinás. Dept. Physiol. & Biophys., NYU Med. Ctr. New York, N.Y. 10016

P-type calcium channels, which are specifically blocked by the polyamine channel blocker "synthetic FTX", have been shown, both functionally (Llinas, et al PNAS, 1989) and immunohistochemically(Hillman, et al, PNAS, 1991), to be present in diverse regions of the CNS. However, little is known about their distribution outside of the brain.

The polyamine, 1-arginyl N,N',-Bis(3-aminopropyl)-1,4-butane-diamine, which we have previously termed synthetic FTX(3:4) was coupled to Sepharose 4B CL via a 1,4-Butanediol Diglycidol ether linkage. This affinity gel allowed the extraction of a protein from rat solubilized skeletal muscle homogenate. The functional activity of the resulting protein solution was assessed using the "tip-dip" bilayer technique. The electrical activity was measured in solutions containing: 80 mM BaCl2, 10 mM HEPES, pH 7.4 in the pipette; 120 mM CsCl, 10 mM HEPES, pH 7.4 in the bathe. Single channel activity was recorded under these conditions was characterized by a slope conductance of 15±5 pS. The i-V curves exhibited some rectification. Erev was at +100±20 mV. The channel was rarely open at potentials more negative than -70 mV and opened with increasing frequency as the potential was made more positive. Single channels were blocked by the addition of Cd or Co at concentrations below 100 μM and by both the polyamine blocker synthetic FTX and by natural FTX. Ensemble averages of 60 mV steps from -90 to -30 mV showed that the channel activity did not inactivate during the 200 msec pulse duration.

Analysis of the protein solution isolated using the polyamine affinity gel was performed using SDS Polyacrylamine Gel Electrophorsesis. Under reducing conditions, only a single band (a doublet structure) at a molecular weight of 80±5 kDaltons was seen when gels were silver stained.

The results we have obtained suggest that the protein isolated from mammalian skeletal muscle using an sFTX affinity gel is a P-channel of similar structure and behavior to that found in CNS.

Supported by NIH grants NS13742 and AG09480

410.12

CHARACTERIZATION OF P-TYPE CALCIUM CHANNELS IN CEREBELLAR PURKINJE CELLS. M.M. Usowicz*, M. Sugimori, B. Cherksey & R. Llinás Dept. of Physiology and Biophysics, NYU Medical Center, 550 First Avenue, New York, NY 10016

There is good agreement between the pharmacological profiles of P-type Ca channels in cerebellar Purkinje cells, Ca channels expressed in *Xenopus* oocytes from brain mRNA, and the cloned BI brain Ca channel (see Tsien *et al*, TIPS 12, 349). However, it has been difficult to compare the biophysical properties of these channels, because the currents have not been recorded with the same Ba concentrations. Therefore, we have recorded P-type Ca channel currents carried by Ba ions ranging in concentration from 1mM to 110mM, and also by 2.4mM Ca. Cell-attached patch recordings were made at 22°C from the somata of adult Purkinje cells in thin cerebellar slices of the guinea-pig (Edwards *et al*, Pflugers Archiv 414:600).

The threshold of activation varied linearly with the log of $[Ba]_o$; it was shifted towards positive potentials as $[Ba]_o$ was increased, presumably due to screening of the surface potential by Ba ions. For instance, it was -48mV with 2mM Ba, -41mV with 5mM Ba and -23mV with 40mM Ba. Currents carried by 2.4mM Ca activated at about -41 mV. Moreover, we found little difference in single-channel conductances measured with 10-110mM Ba; channel openings were to three conductance levels of 10pS, 14-16pS and 19-21pS (slope conductances). As $[Ba]_o$ was reduced below 10mM, the unitary currents became progressively smaller and multiple levels could not be resolved. Single-channel currents carried by 2.4mM Ca were barely resolvable.

Supported by NINCDS grant NS 13742 and an SERC/NATO Fellowship (MMU)

410.13

P-CHANNEL IS RESPONSIBLE FOR HIGH-THRESHOLD DENDRITIC ACTION POTENTIAL IN INFERIOR OLIVE NEURONS, AN FTX STUDY. A. Manfridi*, B. Cherksey, M. Sugimori and R. Llinás. Dept. Physiology and Biophysics, NYU Medical Center, 550 First Avenue, NY, NY 10016.

Inferior olive cells have been shown to exhibit high-threshold and low-threshold calcium spikes (Llinás & Yarom J. Physio. 315:549,1981). The low-threshold spike is generated by a conductance consistent with the presence of T-type voltage-dependent calcium channels. The type of channel resonsible for the high-threshold spike has not been determined. A histochemical study of the distribution of P-channels in the CNS using P-channel specific antibodies indicated that the inferior olivary neurons express P-channels in abundance (Hillman et al. PNAS 88:7076,1991).

Since a polyamine purified from *Agelenopsis Aperta* (FTX) has been shown to block P-channels (Llinás et al. PNAS 86:1689,1989) the effect of HPLC-purified FTX as well as whole venom of *Agelenopsis Aperta* was tested in intracellularly recorded inferior olive neurons in guinea pig brainstem slices. Both of these substances produced a clear block of the high-threshold calcium spiking without affecting either the sodium action potential, the low-threshold calcium spike or calcium-dependent potassium conductance that generates the afterhyperpolarization. By contrast, the large high-threshold calcium dependent action potential and the afterhyperpolarization that follow were blocked. In addition, when the cell was depolarized, repetitive high-frequency sodium-dependent action potentials could be obtained. The results indicate at least two calcium conductances are found in the inferior olive, a T-type conductance responsible for the low-threshold spike and the P-type channel responsible for most of the high-threshold spike. (Supported by NIH grants AG09480 and NS13742)

410.14

IMMUNOLOGIC AND KINETIC CHARACTERIZATION OF A Na^+/Ca^{2+} EXCHANGER IN PRIMARY NEURONAL CELL CULTURES. **G. Hoel*, R. Pal, M. Hurlbert, J. Walsh, and M.L. Michaelis. Dept. Pharmacology and Toxicology, and Center for Biomedical Research, University of Kansas, Lawrence, KS 66045.**

The plasma membrane Na/Ca exchanger is believed to play a role in regulation of Ca fluxes in neurons. We recently reported the development of Ab's against a 36 kDa synaptic membrane protein which immunoprecipitated exchanger activity from solubilized membranes (J. Neurochem. 58:147, 1992). We have now used those Ab's to label primary neurons in culture with avidin-biotin conjugates to peroxidase. The antibodies produced significant labelling of axons, dendrites and cell bodies in neuronal cultures prepared from 18-day embryonic rat brain. Given the substantial amount of Ab labelling of the cells, it was of interest to see whether these cells exhibited Na /Ca exchanger activity with properties similar to those reported for adult brain synaptic membrane preparations. Points of contact between neurons stained quite heavily. Sodium-dependent Ca^{2+} transport was measured in washed homogenates of primary cortical neurons maintained for 3 -6 days in serum-free, defined culture medium. Kinetic determinations of transport activity revealed that Ca^{2+} uptake was linear for at least 30 seconds at 23° C. Uptake was measured across Ca^{2+} concentrations using 20 sec incubations and the kinetic constants were determined to be: K_{act} for Ca = ~ 39 uM and the V_{max} = ~ 0.55 nmol/mg protein/sec. These values are quite close to those reported for other mammalian brain preparations. (Supported by grant AA04732 and MMD Sci. Educ. Partnership).

ACETYLCHOLINE: CNS I

411.1

AFFERENTS TO THE MESOPONTINE CHOLINERGIC NUCLEI FROM THE PERIAQUEDUCTAL GRAY IN THE RAT

T.L. Steininger* and B.H. Wainer, Comm. on Neurobiology, and Dept. Pharm. and Phys. Sci., The University of Chicago, Chicago, IL 60637.

A large body of evidence suggests that the mesopontine cholinergic groups, the pedunculopontine tegmental (PPT) and laterodorsal tegmental (LDT) nuclei, participate in mechanisms of behavioral state control; specifically in arousal and the generation of rapid eye movement sleep. Previous retrograde tracing studies have identified putative afferents to the PPT from numerous regions, including the midbrain and brainstem reticular formation, lateral hypothalamic area, dorsal raphe nucleus, and the periaqueductal gray (PAG), particularly the ventrolateral region (Steininger et al., J. Comp. Neurol., in press). In the present study, we have utilized anterograde tracing with PHA-L combined with choline acetyltransferase immunohistochemistry to examine the PAG innervation of the mesopontine cholinergic neurons. At the light microscopic level, a dense innervation of PHA-L labeled fibers was observed in the region of the PPT and LDT. At higher magnification, numerous putative contacts were observed between anterogradely-labeled boutons and cholinergic neurons. The functional implications of these connections are unclear, but may be involved in mediating the physiological and behavioral correlates of arousal that are features of the 'defense reaction' elicited experimentally by ventrolateral PAG stimulation. (Supported by NS 17661 and MH 09919)

411.2

MEDIAL PREFRONTAL CORTEX PROJECTS DIRECTLY TO CHOLINERGIC NEURONS OF THE MESO-PONTINE TEGMENTUM R.S. Revay* and S.J. Grant Depts. of Biological Sciences, Psychology and Program in Neuroscience, University of Delaware, Newark, DE 19716

Stimulation of the medial pre-frontal cortex (MPFC) reliably activates neurons in meso-pontine nuclei including the laterodorsal tegmental nucleus (LDT) and rostral locus coeruleus (rLC). Although anatomical studies report direct projections from the MPFC to the LDT it is not clear if these cortical afferents directly and/or selectively contact the cholinergic neurons in the LDT. In order to resolve this question we used Phaseolus leucoagglutinin (PhaL) injected into the rat MPFC to label afferent fibers, and NADPH-diaphorase histochemistry to identify cholinergic neurons .

Both cholinergic and non-cholinergic neurons in the LDT were contacted by numerous branched and varicose afferent fibers. The fibers were denser at rostral levels and formed a continuous plexus that extended from the LDT to the adjacent dorsal raphe (DR), but avoided the intervening dorsal tegmental nucleus of Gudden. Some fibers swept laterally into the dorsolateral portion of the pedunculo-pontine nucleus. Fibers also extended ventrally into the reticular formation. Laterally, fibers generally spared Barringtons nucleus, but PhaL positive fibers occasionally entered the rLC. Fibers in the LC appeared thicker than fibers in other areas. The relative number of PhaL labeled fibers in the LDT and LC was related to the dorso-ventral positioning of the injection.

This study is consistent with our stimulation and pharmacologic studies suggesting a monosynaptic excitatory amino acid projection from the MPFC to the LDT. Studies combining retrograde tracers with glutamate immunohistochemistry are in progress to verify the cortical transmitter.

Supported by NIMH, the State of Delaware, and ICI Pharma.

411.3

ACTIONS OF CARBACHOL ON SYNAPTIC TRANSMISSION IN THE RAT NUCLEUS RAPHE DORSALIS (DR) IN VITRO. S. Birnstiel*, R.W. McCarley and R.W. Greene. Neuroscience Lab., V.A. Medical Center, Brockton, MA 02401.

The cholinergic agonist carbachol has been shown to induce a REM-sleep like state in vivo, whereas the serotonergic neurons in the DR have been postulated to inhibit REM. In order to investigate possible interactions between cholinergic and serotonergic systems, recordings were obtained from coronal slices of the rat brainstem with the electrical stimulation placed ventrolaterally to the DR.

Carbachol (1-10 μM) depolarized 23 out of 58 cells tested by about 4 mV with no significant change in membrane resistance. Carbachol had no effect on the membrane potential in 30 neurons and caused a hyperpolarization with a decrease in input resistance in 5 cells. The hyperpolarization, but not the depolarization persisted in the presence of TTX. Carbachol (10 μM) decreased the stimulus-evoked biphasic postsynaptic potentials (PSPs; recorded with electrodes filled with 2 M K-Methylsulfate). PSPs remaining in the presence of 50 μM bicuculline as well as those remaining in the presence of 2 mM kynurenate were equally reduced by carbachol. Preliminary results indicate that this action is mediated by muscarinic receptors, since it is blocked by atropine and not mimicked by the nicotinic agonist 1,1-Dimethyl-4-Phenyl-Piperazinium Iodide.

The present results suggest a presynaptic muscarinic inhibiton of evoked excitatory and inhibitory amino-acid mediated neurotransmission in the DR. (Supported by the DFG and NIMH grant MH39683.)

411.4

CHOLINERGIC MESOPONTINE TEGMENTAL NEURONS WITH BILATERAL PROJECTIONS TO THE PONTOMEDULLARY BRAIN STEM: A FLUORESCENT TRIPLE LABELING STUDY IN THE RAT M. Yanagihara*, K. Ito, L. Dauphin, & R. W. McCarley. Lab. Neurosci.,Dept.Psych.,Harvard Med.Sch./VAMC, Brockton Ma 02401.

The descending cholinergic projection from the laterodorsal tegmental (LDT) and pedunculopontine tegmental (PPT) nuclei to the pontomedullary reticular formation is thought to be implicated in the regulation of the rapid-eye-movement (REM) sleep phase. The fluorescent triple labeling methodology combined choline acetyltransferase (ChAT) labeling by fluorescent (AMCA) immunohistochemistry with the use of two different fluorescent retrograde tracers, fluorescein- and rhodamine-conjugated latex beads. The retrograde tracers were injected into the each side of the pontomedullary reticular formation and adjacent structures in 13 rats. In each case, triple-labeled neurons were found in the LDT and PPT nucleus, indicating ChAT immunopositive neurons with bifurcating axons. Overall, there was a contralateral projection in about 22% of all ipsilaterally projecting neurons in the pons and 15% in the medulla for the PPT. Furthermore, for contralaterally projecting PPT neurons, there were ipsilateral branches in 38% in the pons and 22% in the medulla. LDT percentages were less in both cases. Quantitative volumetric measurements indicated that all injections of retrograde label occupied less than 3% of the reticular nuclei; despite this small volume of injection, bilaterally projecting cholinergic neurons constituted 1.1% of PPT cholinergic neurons, and 0.35% of LDT cholinergic neurons. Such bilaterally projecting neurons appear suited for global regulation of excitability throughout the pontomedullary reticular formation and adjacent cholinoceptive structures.

411.5

Carbachol Induced Hyperpolarization of Neurons in the Rat Laterodorsal Tegmental Nucleus *in vitro*. Luebke, J.I.*, McCarley, R.W., & Greene, R. W. Neuroscience Lab, Dept. Psychiatry, Harvard Medical School, Brockton V.A.M.C, Brockton, MA 02401

Intracellular recordings were made from laterodorsal tegmental nucleus neurons in the *in vitro* brainstem slice preparation. Recordings were obtained from 95 cells which had mean input resistances of 160 (+/- 45) MOhms and action potential overshoots of at least 10mV. Virtually all cells, regardless of their cholinergic or non-cholinergic nature, responded to the application of carbachol (1-10 μM) with a membrane hyperpolarization and associated decrease in input resistance. Under voltage clamp conditions this response was shown to be due to an outward current that was strongly inwardly rectifying and had a reversal potential near the equilibrium potential for potassium. The carbachol response was partially blocked by cesium (2 mM) and fully blocked by barium (100 μM)-providing additional evidence for the activation of an inwardly rectifying potassium conductance by carbachol. The carbachol effect was not blocked by low concentrations of the M1 antagonist pirenzipine, indicating that the response was due to the activation of non-M1 acetylcholine receptors.

Supported by the Dept. of Veteran's Affairs and N.I.M.H. grant MH39683.

411.6

NORADRENALINE HYPERPOLARIZES CHOLINERGIC NEURONS IN RAT LATERODORSAL TEGMENTUM *IN VITRO*. J.A. Williams* and P.B. Reiner Kinsmen Laboratory of Neurological Research, Dept. of Psychiatry, University of British Columbia, Vancouver, B.C. Canada, V6T 1Z3.

Inhibition of brainstem cholinergic neurons by noradrenergic neurons of the locus ceruleus has long been suggested as one mechanism of behavioral state control. While there is anatomical evidence for a noradrenergic innervation of cholinergic neurons in the rat laterodorsal tegmentum (LDT), the direct inhibitory influence of noradrenaline upon cholinergic neurons has never been demonstrated. The purpose of the present study was to characterize the effect of noradrenaline upon cholinergic neurons in the rat LDT. Using whole-cell patch clamp recordings in slices, 26 cells were studied during bath application of 50 μM noradrenaline (NA). Cholinergic neurons were positively identified by intracellular labelling with biocytin and subsequent NADPH-diaphorase staining. 93% (13/14) of identified cholinergic neurons hyperpolarized with an increase in conductance in response to noradrenaline. In contrast, non-cholinergic neurons exhibited mixed responses to NA (25% [3/12] hyperpolarized, 33% [4/12] depolarized, 42% [5/12] no response). Bath application of 1 μM idazoxan, an α_2 adrenergic antagonist, blocked the hyperpolarizing effect of NA on cholinergic cells. To test whether the response to NA was a direct effect, some slices were bathed in low Ca^{2+}, high Mg^{2+} ACSF solution. The responses of cholinergic cells to NA in this condition were identical to those in normal ACSF. These results demonstrate for the first time the direct hyperpolarization of cholinergic neurons by NA, and that this is an α_2-mediated effect.

supported by I.O.D.E. and B.C.H.C.R.F.

411.7

EFFECTS OF SEROTONIN UPON BASAL FOREBRAIN NEURONS *IN VITRO*. Natalia A. Gorelova* and Peter B. Reiner, Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, BC V6T 1Z3 Canada.

Neurons of the basal forebrain project to the hippocampus and cerebral cortex and are thought to be intimately involved in the control of cortical arousal. Anatomical studies have demonstrated the existence of serotonergic afferents to the basal forebrain. Moreover, there is considerable evidence that serotonergic inputs to the medial septum are involved in generation of some types of hippocampal theta rhythm.

In order to understand the role of serotonergic afferents in control of cortical arousal, we studied the responses of basal forebrain neurons (medial septum, diagonal band) to serotonin using whole-cell patch clamp recordings in an *in vitro* slice preparation. Basal forebrain neurons exhibited heteregenous physiological properties, with a mean resting membrane potential of -52 ± 4.8 mV and input resistance of 350 ± 152 MΩ. In accord with the variable electrophysiological properties of basal forebrain neurons, responses to serotonin also varied. Of twelve cells studied, 6 cells depolarized, 2 cells hyperpolarized and 4 cells showed no response. Current efforts are directed towards correlating transmitter status with responses to serotonin.

[Supported by the Medical Research Council of Canada]

411.8

NUCLEUS BASALIS STIMULATION ELICITS NEOCORTICAL ACTIVATION AND FACILITATES THALAMOCORTICAL SYNAPTIC TRANSMISSION: INTRACELLULAR AND EXTRACELLULAR RECORDINGS IN RAT AUDITORY CORTEX Raju Metherate* and John H. Ashe Departments of Neuroscience and Psychology, University of California, Riverside CA 92521

Neocortical activation (EEG desynchronization) reflects a state of behavior and cortical information processing. Through largely undetermined cellular mechanisms, the neurotransmitter acetylcholine (ACh) is thought to be involved in neocortical activation. Since nucleus basalis (NB) neurons are a primary source of cortical ACh, these cells may mediate EEG activation. We have examined this hypothesis, and the implications of cortical activation for thalamocortical transmission, by stimulating the NB and the auditory thalamus (medial geniculate, MG) during intracellular and extracellular recordings in the middle to deep layers of auditory cortex in urethane-anesthetized rats.

Electrical or chemical stimulation of the caudal NB desynchronized the local EEG recorded in auditory cortex; this action was blocked by cortical application of the muscarinic receptor antagonist atropine. Simultaneously with its effect on the EEG, NB stimulation elicited depolarization (1-10 mV) of cortical neurons and altered subthreshold membrane potential fluctuations from large amplitude, slow (1-5 Hz) oscillations to low amplitude, fast (25-40 Hz) oscillations. MG stimulation elicited in auditory cortex an afferent volley (peak latency ca. 2 ms) followed at short latency (ca. 3 ms onset, 7 ms peak) by a negative-going field potential, or intracellularly, an excitatory postsynaptic potential (EPSP). NB stimulation did not alter the thalamocortical afferent volley, but facilitated the slope and amplitude of the negative-going field potential via a muscarinic action. NB stimulation also enhanced MG-evoked EPSPs, producing a higher probability of evoked spike discharge.

These data suggest that one function of ACh-mediated neocortical activation is to facilitate thalamocortical transmission.

Supported by the NSF.

411.9

Ca^{2+}/CALMODULIN KINASE MEDIATES MUSCARINIC BLOCKADE OF $g_{K(Ca)}$ AND ENHANCEMENT OF Ca^{2+} CHANGES IN HIPPOCAMPAL CA3 PYRAMIDAL NEURONS W. Müller#*, J.J. Petrozzino*, L. Griffith*, W. Danho§ and J.A. Connor *Dept. of Neurosciences, Roche Institute of Molecular Biology, Roche Research Center, Nutley NJ 07110 §Peptide Research Department, Hoffmann-LaRoche, Inc., Roche Research Center, Nutley NJ 07110 #Max-Planck-Institut für biophys. Chemie, W-3400 Göttingen, FRG.

An important intrinsic control of excitability in pyramidal neurons of the hippocampus is represented by adaptation of discharge during constant stimulation and a slow afterhyperpolarization following repetitive discharge. This negative feedback is mediated by Ca^{2+} dependent K-conductances $g_{K(Ca)}$ and is subject to down-modulation by muscarinic, serotonergic and glutamatergic inputs. Muscarinic modulation is of particular interest for its important role in synaptic plasticity and learning. This input uncouples intra-dendritic Ca^{2+} from $g_{K(Ca)}$ activation and thereby potentiates intradendritic Ca accumulations, a factor that possibly underlies facilitation of synaptic plasticity during muscarinic activation.

Using new peptide inhibitors of Ca^{2+}/calmodulin dependent kinase (CaM kinase) and PKC we found that muscarinic modification of $g_{K(Ca)}$ and Ca^{2+}-changes relies on CaM kinase activation, but the converging effects of serotonergic and glutamatergic agonists are mediated either through other kinases or by entirely different processes. We surmise that these in large part separate pathways include also divergent branches that are relevant for distinct functions of these neurotransmitters.

W.M. was supported in part by a Helmholtz-stipend from the BMFT.

411.10

MUSCARINIC AGONISTS AND PHORBOL ESTERS FACILITATE GLUTAMATE-MEDIATED RESPONSES IN RAT AUDITORY CORTEX *IN VITRO*. C.L. Cox*, J.H. Ashe and S. Haeri, Departments of Neuroscience and Psychology, University of California, Riverside, CA 92521.

The actions of muscarinic agonists, methacholine (MCh) and oxotremorine-M (Oxo-M), and the phorbol ester, phorbol 12,13 dibutyrate (PDBu), were tested upon glutamate-mediated membrane depolarizations. Glutamate, iontophoretically applied, (1M, 15-100 nA) produced a membrane depolarization associated with a decrease in membrane resistance. Iontophoretically applied acetylcholine (ACh)(1 M, 25-100 nA) or MCh (1 M, 25-100 nA) produced no or little (<2 mV) change in membrane potential, a slight increase in membrane resistance and a decrease in spike frequency accommodation. However, ACh or MCh facilitated the amplitudes of the glutamate-mediated membrane depolarizations that persisted for several minutes following the offset of the cholinergic agonists. The muscarinic antagonists, atropine (1 μM) and pirenzepine (1 μM), suppressed the facilitation. Oxo-M (100 μM), also produced a lasting facilitation of the glutamate depolarizations. These data suggest that activation of muscarinic receptors can produce a facilitation of glutamate-mediated responses.

Muscarinic receptor activation has been associated with an increase in phosphatidyl inositol turnover and activation of protein kinase C (PKC). We therefore tested the action of a PKC activator, PDBu, upon the glutamate depolarizations. PDBu (50-100μM), depolarized the cortical neurons and also produced a facilitation of the glutamate-mediated membrane depolarizations. The facilitation of the glutamate depolarizations outlasted the membrane depolarization produced by the phorbol ester. Furthermore, the facilitation of the glutamate depolarization persisted during clamping of the membrane potential at pre-drug levels, suggesting the facilitation is independent of the membrane depolarization produced by PDBu.

These data suggest that 1) activation of muscarinic receptors can facilitate the amplitude of glutamate-mediated depolarizations, 2) PKC activation facilitates glutamate-mediated depolarizations and 3) these actions occurs independent of a change in membrane potential suggesting a potential neuromodulatory action Supported by NSF BNS 9008818.

411.11

CHOLINERGIC SLOW SYNAPTIC ACTIONS ON RAT NEOCORTICAL NEURONS *IN VITRO*. J.H. Ashe*, C.L. Cox, R. Metherate and E. Jobst. Depts. of Neuroscience and Psychology, Univ. of Calif., Riverside, CA 92521.

Acetylcholine (ACh), exogenously applied, can produce lasting modifications of functional characteristics of cortical neurons. However, the actions of synaptically released ACh upon neuronal excitability and synaptic transmission in neocortex is not clearly understood. These experiments investigated the action of cholinergic agonists and endogenous ACh upon auditory cortical neurons.

Tetanic stimulation of deep cortical gray matter (10-100 μA; 8-40 pulses, 20-80 Hz) elicited a voltage-dependent long-lasting slow membrane depolarization (tens of seconds to many minutes). The slow depolarization persisted in the presence of amino acid antagonists (DNQX, APV, picrotoxin). Tetanic stimulation also produced voltage dependent 1) decreased spike frequency accommodation, 2) decreased slow afterhyperpolarization and 3) facilitated afterdepolarization. The effects of the tetanic stimulation were mimicked by exogenously applied cholinergic agonists, ACh and methacholine (MCh), enhanced by the anticholinesterase eserine, and attenuated by the muscarinic antagonists atropine or pirenzepine. These data suggest that synaptically released ACh produces multiple voltage-dependent actions via muscarinic receptors.

A subpopulation of neurons has been identified as "rhythmic bursting" neurons due to their low frequency rhythmic (5 Hz) bursting discharge to a depolarizing current pulse. These cells have been histologically identified as layer 5 pyramidal neurons, following biocytin injection. In these cells, tetanic stimulation reduced the number of bursts and increased the number of higher frequency (10-15 Hz) single spike discharge in response to a depolarizing current pulse, independently of membrane depolarization. This effect was enhanced by eserine, blocked by atropine and mimicked by muscarinic agonists, MCh and oxotremorine-M.

These data suggest that synaptically released ACh, acting via muscarinic receptors, can modulate the excitability of neocortical neurons. Modulation of the excitability of these neurons may influence the neurons response to subsequent afferent synaptic information and may serve as a potential mechanism of synaptic plasticity. Supported by NSF BNS 9008818.

411.12

CHOLINERGIC AND NORADRENERGIC MODULATION OF A SLOW OSCILLATION IN CAT NEOCORTICAL CELLS. F. Amzica*, A. Nuñez and M. Steriade, Lab. of Neurophysiology, Laval Univ. Sch. of Med., Quebec, Canada G1K 7P4.

Neocortical cells recorded from cat association areas 5 & 7 display an oscillation around 0.3 Hz, consisting of depolarizing and/or hyperpolarizing sequences, time-locked with a similar EEG rhythm (Steriade et al., this meeting). Here we report the modulation of this novel type of slow neuronal oscillation by shifting the EEG synchronization (high-amplitude and slow waves) to activated patterns. We used intracellular recordings of cortical cells under urethane anesthesia and changed the EEG state by a brief stimulation (pulse-trains at 30 Hz lasting for 1 s) of pedunculopontine tegmental (PPT) and locus coeruleus (LC) nuclei.

In most cells, PPT stimulation induced a depolarization (3-5 mV) associated with tonically increased firing and lasting for 10-20 s. The slow oscillation was blocked for 5-10 s and resumed thereafter. This cellular effect was voltage-dependent, as it could not be obtained at a membrane potential more negative than -85 mV. In some neurons, in which the oscillation mainly consisted of rhythmic hyperpolarizations sculpturing the background firing, PPT stimulation interrupted the oscillation by selectively suppressing the hyperpolarizing episodes. The time-course of the PPT-induced blockage of slow oscillation was similar to that of EEG activation. The PPT effect was suppressed by systemic administration of a muscarinic blocker, scopolamine. LC stimulation produced qualitatively similar effects on cells and EEG activity, but less pronounced and durable. After clonidine administration, the effects of LC stimulation were no longer observed. The similarity between the cholinergic and noradrenergic effects suggests that the major mechanism underlying the blockade of the slow cortical oscillation, and especially of the rhythmic hyperpolarization, is the reduction or suppression of potassium conductances. Supported by MRC of Canada (grant MT-3689).

411.13

SERIAL SECTION ANALYSIS OF THE ACETYLCHOLINE (ACh) INNERVATION IN ADULT RAT PARIETAL CORTEX. D. Umbriaco*, K.C. Watkins, L. Descarries, C. Cozzari and B.K. Hartman. CRSN, (Départements de pathologie et de physiologie), Université de Montréal, Montréal, Qué., CANADA; Istituto Biologia Cellulare CNR, Roma (ITALY), and Department of Psychiatry, University of Minnesota, Minneapolis (MN).

We have now completed the electron microscopic examination in serial thin sections of 785 ACh axon terminals (varicosities) from adult rat parietal cortex (Par1), immunostained with monoclonal antibodies against purified rat brain choline acetyltransferase (*Soc. Neurosci. Abstr.* 16: 200, 1990). This material from 4 rats was initially fixed by perfusion of a paraformaldehyde (2.5%)-glutaraldehyde (0.1%) mixture, and processed with the ABC method. 140 varicosities of layer I, 123 of layers II-III; 147 of layer IV; 159 of layer V and 216 of layer VI were photographed from end to end and examined at a final magnification of X 36 000 (average of 11 pictures per varicosity). The mean maximal transverse diameter of these varicosities was similar in every layer (0.49 ± 0.15 μm s.d.). Both large and small varicosities could be observed on the same fibers. In each layer, a relatively small proportion of the varicosities exhibited a synaptic membrane differentiation (9.3% in layer I; 13.8% in II-III; 11.6% in IV; 22% in V; 14.8% in VI), for a I-VI average of 14.5%. These 118 synaptic junctions were almost invariably symmetrical (98.2%). A majority were found on dendritic branches (80.7%), some on spines (22.8%) and none on cell bodies. Junctional and non junctional varicosities were present on the same fibers. There were only 4 varicosities with dual junctions and the 2 asymmetrical junctions were on spines in layers I and II-III. These data indicate that the ACh innervation in every layer of adult rat parietal cortex is mostly non junctional, with a slightly higher proportion of synaptic varicosities in layer V. Identification of receptive elements will be needed to determine the functional targets of such an innervation. [Supported by the FCAR and grants MT-3544 (MRC) and NS 12311].

411.14

CHOLINERGIC INNERVATION OF THE HUMAN AMYGDALOID COMPLEX. M. Emre, C. Geula, M-M. Mesulam* Harvard Medical School, Boston, MA 02215

The cholinergic innervation of the human amygdaloid complex was studied using choline acetyltransferase (ChAT) immunohistochemistry. ChAT-positive fibers and varicosities were observed throughout the amygdaloid complex. In parts of the amygdala the density of this cholinergic innervation was higher than in any part of the cerebral cortex. The highest level of ChAT-positive varicosities was seen in the basolateral nucleus and the lateral part of central nucleus. The basomedial, accessory basal and cortical nuclei, the amygdalo-hippocampal and cortico-amygdaloid transition areas and the anterior amygdaloid area showed a lower density of ChAT-positive varicosities and fibers. The lateral nucleus displayed an even lower density of innervation and there were only rare ChAT-positive fibers in the medial nucleus. Although the lateral nucleus displayed a low level of innervation in comparison to the other amygdaloid nuclei, the level of this innervation was approximately equivalent to that of entorhinal cortex, which receives one of the heaviest cholinergic innervations in the cerebral cortex. The pattern of differential staining was maintained throughout the anteroposterior extent of the amygdaloid complex. The distribution of ChAT-positive fibers and varicosities within the various nuclei and their subdivisions was by and large homogenous at a given level except for some patchiness in the central nucleus. The distribution of the cholinergic innervation as studied by ChAT immunohistochemistry was comparable to that observed with AChE histochemistry. These results confirm that, as part of the limbic system, the human amygdaloid complex receives a dense and differentially distributed cholinergic innervation.

411.15

ULTRASTRUCTURAL EVIDENCE FOR DISTINCT CHOLINERGIC AND VIP-ERGIC MODULATION OF INTRACORTICAL BLOOD VESSELS.
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Acetylcholine (ACh) and vasoactive intestinal polypeptide (VIP) exert potent vasoactive effects within the cerebral cortex. The relationships of ACh and VIP terminals with cortical microvessels were evaluated at the ultrastructural level after immunocytochemical labeling for choline acetyltransferase (ChAT; MCAT-1C, Cozzari et al., Soc. Neurosci. Abstr. 16: 200, 1990) or VIP (Peninsula). Rats were perfused with 4% paraformaldehyde (PF) and 0.025% glutaraldehyde followed by PF alone. Vibratome sections (60 μm) were immunostained for ChAT or VIP by the ABC method with DAB-nickel as the chromogen. An equal number (> 80) of ChAT and VIP terminals located close (0-3 μm) to blood vessels were photographed and analyzed in single thin sections (Bioquant II). These terminals were respectively located at an average distance of 0.62 $\pm$ 0.05 and 0.75 $\pm$ 0.07 μm from the vessel wall (capillaries or arterioles). Of these, 26% (ChAT) and 20% (VIP) were separated from to the basal lamina by a thin glial leaflet only. Irrespective of the distance, VIP (0.56 $\pm$ 0.05 μm^2) were significantly larger than ChAT terminals (0.34 $\pm$ 0.03 μm^2; $p < 0.001$). Membrane specializations were never observed at the neuro-vascular interface even in serially-sectioned ChAT (n=20) and VIP (n=5) terminals. Together with our previous data showing that 59% of cortical neurons stain for VIP, 28% for ChAT and 13% only colocalize both transmitters, the present results strongly suggest that intracortical vascular functions are primarily influenced by distinct ACh and VIP nerves. Supported by the MRC of Canada.

EXCITATORY AMINO ACIDS: RECEPTORS V

412.1

THE GLUTAMATE RECEPTOR ANTAGONIST NBQX DOES NOT AFFECT ACTIVITY IN NORMAL RATS AND RHESUS MONKEYS.
C. Kearns,[1] Z. Zhang,[1] J.T. Greenamyre[2] and D.M. Gash.*[1] Departments of Neurobiology and Anatomy[1] and Neurology,[2] University of Rochester, Rochester, NY 14642.

NBQX [2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f) quinoxaline], a selective antagonist of the AMPA subtype of glutamate receptor, has antiparkinsonian effects in monoamine-depleted rats and MPTP-treated primates (*Ann. Neurol.* 1991; 30:717-723). In the present study, NBQX was administered to normal rats and primates to determine if it produced any apparent side effects. In rats, various doses of NBQX (0, 5, 10, 20, 30 mg/kg; i.p.) were evaluated (n=9/dose). Each rat was tested measuring horizontal activity and stereotypy. There were no apparent side effects in rats treated with 0, 5, 10, 20 mg/kg NBQX. However, there was a slight but significant ($p>0.05$) narcotic effect observed in rats treated with 30 mg/kg NBQX. In the primate testing, 3 normal older (approximately 12-16 years old) female rhesus monkeys were administered doses of 0 and 1 mg/kg (i.m.) NBQX on alternate days. Each primate was subjected to 1 week of testing. Data were gathered by videotaping and computerized cage activity monitoring in which the number of times an animal crossed an infrared beam was recorded. These data were analyzed using a Clinical Rating Scale consisting of 4 measures of parkinsonian features (posture, gait, bradykinesia and balance), 3 measures of drug-related side effects (dyskinesias, vomiting and psychological disturbance) and 1 measure of overall level of activity. There were no apparent NBQX related side effects seen in any of the primates.

Supported by a grant from the Markey Foundation.

412.2

THE EFFECT OF THE SPECIFIC AMPA RECEPTOR ANTAGONIST NBQX UPON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT.
P.D. Suzdak* and M.J. Sheardown. Novo Nordisk A/S, CNS Division, DK-2760 Maaloev, Denmark.

Excitatory amino acid receptors can be classified by pharmacological and electrophysiological selectivity for the ligands NMDA, AMPA, kainic acid and t-ACPD. The present report characterizes the effect of antagonism of the AMPA receptor, with NBQX, on local cerebral glucose utilization (LCGU). NBQX has been previously shown to have neuroprotective effects. NBQX was administered at doses of 3 to 90 mg/kg i.p. 30 minutes prior to the administration of 50 uCi ^{14}C-2-deoxyglucose i.v. in conscious rats. NBQX (3 - 30 mg/kg) selectively increased LCGU in the lateral hebenula, raphe, superior and inferior colliculus and vestibular nucleus. NBQX at 60 mg/kg decreased LCGU in 47 of the 62 brain regions examined. NBQX at 90 mg/kg decreased LCGU in 60 of the 62 brain areas examined.

NBQX did not increase LCGU in limbic brain areas at any of the doses examined. In fact, NBQX at ≥60 mg/kg decreased LCGU in limbic areas. These data suggest that antagonism of the AMPA receptor in vivo with NBQX results in changes in LCGU different from that previously reported for non-competitive antagonists (i.e. PCP, MK 801), further suggesting that NBQX may not be associated with PCP-like effects.

412.3

KAINATE-INDUCED EPILEPSY LEADS TO AN INCREASE IN GLUTAMATE-STIMULATED PHOSPHOINOSITIDE METABOLISM IN THE HIPPOCAMPUS.
E.Mayat,[1]M.Lerner-Natoli,[1]G.Rondouin and M.Récasens*. INSERM U254,Hôpital St-Charles,[1]INSERM U249 & CNRS UPR 8402, 34000 Montpellier, FRANCE.

Intra-amygdala injections of kainate (KA) ultimately induce status epilepticus associated with neuronal loss in local and distant structures. In the hippocampus, a mossy fiber sprouting occurs in consequence of this seizure-related damage. Since glutamate metabotropic receptors (mGLU-R) may be involved in synaptic plasticity, we examined the agonist-induced inositol phosphate (IP) formation in hippocampal slices of rats subjected to an intra-amygdala KA injection (2.5 nmol in 1 ul). After 24 hours, no significant effect of KA application on IP accumulation was observed. However, 1 week following injection, significant increases of the metabotropic responses mediated by quisqualate (QA), ibotenate (IBO) and trans-aminocyclopentane-dicarboxylate (t-ACPD) were noted in hippocampal slices. In control animals, the IP formation ($\bar{X}\pm$SEM) for buffer, QA, IBO, t-ACPD and carbachol were 2,806±96; 4,145±109; 5,493±14; 7,506±754 and 4,164±757 dpm/mg protein respectively whereas in KA treated animals they were 3,123±629; 11,071±1054; 13,196±2,208; 19,119±1,377 and 5,662±1,119 dpm/mg protein.Significant increases were also obtained 2 and 6 weeks after injection. For carbachol, there was no significant increase at all times except after 6 weeks following KA treatment. This specific dramatic increase of the mGLU-R response in the hippocampus following seizure-related damage suggests that mGLU-R may play a role in the molecular mechanisms leading to brain plastic changes.

412.4

NMDA AND KAINATE/AMPA RECEPTOR GENE EXPRESSION IN HIPPOCAMPUS FOLLOWING KAINATE-INDUCED SEIZURES IN MATURE AND IMMATURE RATS.
L.K. Friedman*, D.E. Pellegrini, E.F. Sperber, S.L. Moshe, M.V.L. Bennett, R.S. Zukin. Dept. of Neuroscience, Albert Einstein Coll. of Med. Bronx, NY.

Glutamate receptors (GluR) are thought to play a role in epilepsy. We examined GluR gene expression in adult and 15 d rat brains after induction of status epilepticus by kainate (15 or 4.5 mg/kg i.p.). *In situ* hybridization was used to measure levels of NMDAR1, GluR1, GluR2, and GluR3 mRNAs in coronal sections from control and KA-injected animals 6 to 144 h after seizure onset. In adults, damage to amygdala, entorhinal cortex, and selective thalamic nuclei was evident at 6-12 h, while CA3/4 selective cell loss was not evident until 48 h. Autoradiography of adult brain sections indicated significant increases in GluR2 and GluR3 expression (50-100%) in the dentate gyrus (DG) and subiculum (35%) and a concomitant decrease (40-60%) in CA3/CA4 hippocampal subfields at 24 h. GluR1 expression was unchanged. Emulsion-dipped sections revealed that the observed changes were in mRNA content per neuron. After 12 h adult NMDAR1 mRNA levels were greatly reduced in CA1 (60-80 %) but unchanged in DG suggesting a specific role for genomic expression of glutamate receptor subtypes in seizure activity. In 15 d pups,an age when KA does not induce neurodegeneration in limbic structures, GluR1 and GluR2 dentate mRNA levels were elevated in DG by 50-100% 24 h after seizure onset with no changes in GluR3 mRNA. The three transcripts were unchanged in CA3/CA4. In conclusion, changes in adult mRNA expression occur prior to obvious CA3/4 pyramidal cell loss, but after afferent denervation from limbic structures. There are maturational differences in GluR mRNA expression that may be explained by the presence or absence of associated structural lesions.

412.5

CELL-ATTACHED RECORDINGS OF NMDA CHANNELS IN CHRONICALLY EPILEPTIC (KINDLED) NEURONS. G. Köhr*[1] and I. Mody[2]. [1]Center for Molecular Biology, Univ. of Heidelberg, F.R. Germany and [2]Dept. of Neurology & Neurol. Sciences, Stanford Univ. Sch. of Med., Stanford, CA.

Our studies in hippocampal slices and acutely dissociated neurons have shown that kindling-induced epilepsy increases synaptic activation of NMDA receptors, modifies whole-cell NMDA responses and alters the activation/inactivation kinetics of HVA Ca^{2+} currents. We have now examined if long-lasting changes can be detected at the level of single NMDA channels.

Cell-attached recordings were obtained in acutely dissociated control and kindled dentate gyrus granule cells in Mg^{2+}-free medium containing 3 μM glycine using NMDA as an agonist (1, 5 and 10 μM). Single open times and the conductance of single channels were comparable in control and kindled neurons. Kindled channels differed from controls by having a longer burst duration and a higher p_{open} at low NMDA concentrations (1 or 5 μM). Following kindling, the combined effect of the longer burst duration and the higher p_{open} was a 23-fold and a 3.6-fold increase in the charge carried through NMDA channels at 1 and 5 μM NMDA respectively. As a result of a reduced p_{open} at 10 μM NMDA the charge through kindled channels was 57% of that recorded in control cells. On-cell dose-response studies, according to a double-fill method (A. Auerbach, Biophys. J., 60: 660, 1991), were done by allowing NMDA (25 μM) to gradually diffuse to the channels over a period of 15-30 min. These experiments confirmed the higher affinity and augmented desensitization at high NMDA concentrations of kindled channels.

The chronically enhanced NMDA receptor/channel function may result from a covalent modification during kindling; the outcome for neuronal excitability conceivably contributes to epileptogenesis.

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412.6

CHRONIC ANTIDEPRESSANT TREATMENT DESENSITIZES THE NMDA RECEPTOR COMPLEX. G. Nowak, P. Skolnick* and I.A. Paul. Lab. Neuroscience. NIDDK, NIH, Bethesda, MD 20892.

Functional antagonists at the NMDA receptor complex, such as 1-aminocyclopropanecarboxylic acid (ACPC) and MK-801, possess antidepressant-like activity in animal models (Trullas, R. and Skolnick, P., *Eur. J. Pharmacol.*, 185:1, 1990) and down-regulate ß-adrenoceptors after chronic treatment (Paul, I.A. *et al.*, *Psychopharmacology*, 106:285, 1992). These findings indicate that NMDA receptors may be involved in antidepressant activity. To test this hypothesis, we examined the effect of treatment with ACPC and several "classical" antidepressant drugs on [^{3}H]5,7-dichlorokynurenic acid (DCKA) binding to strychnine-insensitive glycine modulatory sites and on [^{3}H]CGP-39653 binding to glutamate sites in mouse cortex. Chronic, but not acute treatment with ACPC, imipramine and amitriptyline reduced the affinity of glycine to inhibit [^{3}H]5,7-DCKA binding ($p < 0.05$). Chronic citalopram treatment also produced a marginal reduction in the affinity of glycine. [^{3}H]CGP-39653 binding is displaced by glycine with both high ($\approx$70%, $IC_{50} \approx$ 600 nM) and low ($\approx$30%, $IC_{50} \approx$ 3 mM) affinity components. Chronic ACPC and imipramine treatment significantly increased the proportion of low affinity sites ($p < 0.05$) and reduced the proportion of high affinity glycine-displaceable [^{3}H]CGP-39653 binding sites. These data indicate that the NMDA receptor complex may be involved in the neural adaptation produced by chronic antidepressant treatment.

412.7

DELAYED PHENCYCLIDINE EFFECTS ON NMDA SENSITIVE ^{3}H-GLUTAMATE BINDING X.-M. Gao,* T.Kakigi, C.A. Tamminga University of Maryland, MPRC, P.O. 21247, Baltimore, MD 21228

Phencyclidine (PCP) is a psychoactive drug which has prolonged psychotomimetic effects in humans, hence has been used as psychosis model. We have previously demonstrated that single doses of PCP in rat produces an initial increase then a prolonged decrease in regional cerebral glucose metabolism (rCMRglu) lasting longer than 24 hours. This effect is prominent in rat limbic structures. Moreover, schizophrenia studies have shown in vivo structural and metabolic changes in limbic structures and postmortem pathologic changes in limbic and paralimbic regions. Therefore, we have begun to explore the neurochemical correlates of this delayed PCP action, especially in the limbic system. Here, we report the 24 hour effect of PCP (8.6 mg/kg) on the NMDA sensitive ^{3}H-glutamate binding sites and on other glutamate receptors. NMDA and AMPA glutamate receptors were quantified according to standard technique, using ^{3}H-glutamate and ^{3}H-AMPA, respectively. Our results show an increase of more than 40% in NMDA binding in limbic structures like hippocampus, and in limbic related neocortex, like dorsolateral frontal, medial prefrontal, and posterior cingulate cortex, 24 hours after a single dose of PCP. This effect is significant in CA1 region of the hippocampus (dorsal: PCP=92.6±22.9, control=64.6±13.9 fmol/mg tissue; ventral: PCP= 86.0±15.5, control=62.8±11.8, Mean±SD, n=6,P<0.05), and dorsolateral prefrontal cortex (PCP=52.0±16.3, control=26.8±5.6, P<0.01). No change in AMPA receptor density was found. These data suggest that the psychotomimetic effects of PCP, particularly of those delayed effects may be mediated by limbic glutamatergic synapses selective for NMDA. These observations may suggest directions of study in schizophrenia.

412.8

EFFECTS OF POSTWEANING LEAD (Pb) EXPOSURE ON [^{3}H]DIZOCILPINE (MK-801) BINDING IN RAT BRAIN. S.C.Johnson*[1,2], J.T.Greenamyre[3], and D.A.Cory-Slechta[1]. Environ. Health Sci. Center[1], Program in Neuroscience[2],Dept. of Neurology[3], School of Med. and Dent., Univ. of Rochester, Rochester, NY 14642.

Pb has been shown to affect a number of neurotransmitter systems both in vivo and in vitro. Recently evidence has shown that in vitro Pb acutely inhibits LTP formation and that it inhibits the binding of MK-801, a noncompetitive antagonist of the NMDA subtype of glutamate receptors. However, there is still little data on the effects of long-term in vivo exposure to Pb on the neurochemistry of excitatory amino acid systems. We used quantitative autoradiography to assess [^{3}H]MK-801 binding sites in brains of rats exposed to Pb for 60 days using a Pb exposure regimen known to produce behavioral alterations. Rats were exposed to either 0, 50, or 2000 parts-per-million (ppm) Pb in their drinking water from 21 to 81 days of age. At that time, blood samples were taken for blood lead analysis via anodic stripping voltammetry, and the brain was taken for determination of [^{3}H]MK-801 binding using quantitative autoradiography. Control rats (n=8) had no measurable blood-Pb levels, while the 50 ppm exposure group (n=8) had a mean blood-Pb level of 15 ug/dl and the 2000 ppm exposure group (n=8) had a mean blood-Pb level of 82 ug/dl. Autoradiographic analyses revealed no significant effect of Pb exposure upon MK-801 binding in the striatum in either of the Pb exposure groups compared to controls. In the dentate gyrus of hippocampus, binding in the 50 ppm exposure group was significantly reduced in relation to the control and the 2000 ppm exposure group, whereas binding of the 2000 ppm group was elevated in comparison to both the control and 50 ppm groups. The 50 ppm Pb exposure likewise resulted in a reduction in binding in the CA1 region of hippocampus relative to the 2000 ppm, while the 2000 showed no difference relative to control. These findings suggest that there are significant changes in MK-801 binding in rats chronically exposed to Pb after weaning (day 21) and that the dose-response relationship of Pb exposure and MK-801 binding sites may reflect a U-shaped function. In addition, there are also regional differences in the Pb induced changes in MK-801 binding.ES05017,ES05093.

412.9

Interactions of EtOH and Glycine with the NMDA Receptor-Linked Ion Channel Complex in EtOH Withdrawal Seizure-Prone and -Resistant Mice. A. Janowsky*, L. Carter, and J. C. Crabbe, VAMC and Oregon Health Sciences Univ., Portland, OR 97201

We have (re)characterized the assay conditions that are required for equilibrium binding of [^{3}H]MK-801 to the NMDA receptor-linked ion channel complex. Results of our experiments indicate that radioligand binding to a well washed tissue preparation that includes glycine (10μM) and NMDA (10μM) requires 24 hrs to reach equilibrium. In addition, the dose response curve for the glycine-induced increase in the affinity of the binding site is optimal after 24 hrs incubation at 25°C, with Hill coefficients increasing over time.

Under these conditions, EtOH (3 - 100mM) has no effect on the characteristics of radioligand binding, or on the glycine-induced increase in affinity for radioligand binding. In addition, there appears to be no difference in the density of [^{3}H]MK-801 binding sites in cortex or hippocampus of naive EtOH-withdrawal seizure-prone (WSP) and -resistant (WSR) mice, which differ in handling-induced convulsion severity. Acute administration (24 hr inhalation) of EtOH did not alter the density or affinity of binding sites in either mouse line. Thus, under well defined equilibrium binding conditions, EtOH does not directly interact with either the glycine or [^{3}H]MK-801 binding sites on the NMDA receptor-linked ion channel complex. Differences between the effects of EtOH on radioligand binding under equilibrium and non-equilibrium conditions, and on EtOH-induced behaviors will be discussed. *This work was supported by NIAAA and the V.A.*

412.10

INCREASED NMDA RECEPTOR FUNCTION IN CEREBELLAR GRANULE CELLS EXPOSED CHRONICALLY TO ETHANOL. **K. Iorio, P.L. Hoffman, B. Tabakoff, K. N. Kumar and E. Michaelis*. Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80220, and Univ. Kansas, Lawrence, KS 66047.**

Acutely, ethanol is a selective inhibitor of the function of NMDA/glutamate receptors. To assess changes in NMDA receptor function after chronic ethanol exposure, we measured NMDA-stimulated increases in intracellular Ca^{2+} in primary cultures of cerebellar granule cells. The cells were obtained from 8-day old rats and exposed in vitro to 100 mM ethanol for two or more days or 20 mM ethanol for three or more days. In the ethanol-treated cells there was an increased maximal response to NMDA (in the presence of glycine) and glycine (in the presence of NMDA), with no change in the EC_{50} value for either compound. There was no change in inhibition of the NMDA response by competitive or non-competitive antagonists or by ethanol added acutely. Chronic ethanol exposure apparently produced an increase in the number of NMDA receptors, with no change in their properties. Western blot analysis of membrane proteins using an antibody raised against the recently-cloned 70 kDa glutamate-binding protein, believed to represent a subunit of an NMDA receptor, revealed a 60% increase in the amount of this protein in cells exposed to 100 mM ethanol for 2 days and a 100% increase for cells exposed for 4 days. These results are compatible with an ethanol-induced increase in synthesis, or decrease in degradation, of the glutamate binding subunit of an NMDA receptor. [Supported by grants AA 9005 and AA 4732 from NIAAA]

412.11

GLUTAMATE RECEPTOR SUBUNITS EXPRESSED IN *XENOPUS* OOCYTES DEMONSTRATE DIFFERING SENSITIVITIES TO ETHANOL. J. E. Dildy-Mayfield*, M. L. Robinson, and R. A. Harris. Univ. of CO Hlth. Sci. Ctr. & Denver VAMC, Denver, CO 80262.

Xenopus laevis oocytes were injected with either GluR1, R3, R1+3, R2+3 cRNA or GluR6 cDNA and ethanol's action on minimum and maximum kainate (KA)-induced currents was studied electrophysiologically. In GluR3-injected oocytes, both low (12.5 μM) and high (400 μM) KA responses were inhibited approximately 35 and 50% by 50 and 100 mM ethanol, respectively, with KA responses in GluR1-injected oocytes being slightly less sensitive to inhibition by ethanol. In comparison to GluR1 and R3, maximum KA responses in GluR6 expressing oocytes occurred in the presence of 10 μM KA and were less sensitive to ethanol in that 50 and 100 mM ethanol produced only 15 and 28% inhibition, respectively. Moreover, in GluR6-injected oocytes the ethanol sensitivity was dependent on the KA concentration with minimum KA responses (0.2 μM) being more sensitive to ethanol (e.g., 47% inhibition by 100 mM ethanol) which has also been observed in rat hippocampal or cerebellar mRNA injected oocytes (J. Neurochem. 58: 1569-1572, 1992 and unpublished observations). In addition, when GluR2+3 subunits were co-expressed, low KA responses were more sensitive to ethanol inhibition compared to high KA responses whereas the ethanol sensitivity of low and high KA responses in GluR1+3 expressing oocytes was similar to that of GluR3 alone. Thus, expression of GluR subunits in oocytes provides evidence for homomeric and heteromeric KA channels with differing sensitivities to KA and ethanol. GluR clones were kindly provided by J. Boulter and S. Heinemann and the R6 expression construct was provided by K. Cauley, Salk Institute.

412.12

3H-MK801 BINDING IN LONG-SLEEP (LS) AND SHORT-SLEEP (SS) MICE: DIFFERENCES FOUND IN EIGHT BRAIN REGIONS. W.R. Wilson* and A. C. Collins†. School of Pharmacy* and Inst. for Behavioral Genetics†, Univ. of Colorado, Boulder, CO 80309.

Previous studies have shown that LS and SS lines of mice, selectively bred for differences in hypnotic sensitivity to acutely administered ethanol, differ in stimulated glutamate (GLU) release from slices of cortex, striatum and hippocampus. Also, SS whole brain vesicles take up more 3H-GLU and contain more endogenous GLU than LS vesicles. Behavioral tests have shown that hypnotic sensitivity to ethanol is decreased by central administration of NMDA receptor agonists and increased by administration of NMDA receptor antagonists in both LS and SS lines of mice. These findings suggest that differences in NMDA receptors exist in LS and SS lines of mice. NMDA receptors were, therefore examined by measuring 3H-MK801 binding to the NMDA receptor ion channel, under saturating conditions of the agonist GLU, the coagonist glycine and the positive modulator spermidine. Binding was examined in hypothalamus, cerebellum, hindbrain, colliculi, hippocampus, midbrain, striatum and cortex. 3H-MK801 binding differed between the LS and SS lines in cerebellum, hippocampus and striatum. SS mice displayed greater levels of binding in hippocampus and striatum while LS mice displayed greater levels of binding in cerebellum. Binding affinity did not differ between LS and SS mice within a brain region, however, affinities varied between regions, which, suggests heterogeneity of NMDA receptors. The data support the hypothesis that NMDA receptor mediated events may play a role in genetic differences in hypnotic sensitivity to ethanol.
Supported by grants AA-06391 and DA-00116.

412.13

EXCITATORY AMINO ACID RECEPTORS IN SCHIZOPHRENIA: SELECTIVE PLASTIC RESPONSES IN THE HIPPOCAMPUS OF SOME SCHIZOPHRENIC INDIVIDUALS. J. Ulas*, L.C. Brunner, L. Nguyen and C.W. Cotman. Irvine Research Unit in Brain Aging, University of California, Irvine, CA 92717, USA.

There is growing evidence for interactions between central dopaminergic and glutamatergic systems. It has been suggested that hyperactivation of the dopaminergic system observed in schizophrenia may be due to a disturbance of the glutamatergic system. In addition, neuroanatomical studies suggest aberrant anatomical changes in the temporal lobe of schizophrenic patients. Since most of the neurons in this brain region utilize glutamate as a neurotransmitter we compared the distribution of NMDA receptors (L-[^{3}H]glutamate and [^{3}H]CPP binding sites) and non-NMDA receptors ([^{3}H]KA and [^{3}H]AMPA binding sites) in the hippocampus and parahippocampal gyrus of schizophrenic and normal, control brains. *In vitro* quantitative autoradiography studies were performed on 4 clinically assessed schizophrenic individuals and 12 age-matched control subjects. Ligand binding experiments did not reveal any changes in levels of L-[^{3}H]glutamate and [^{3}H]CPP binding to NMDA receptors in the schizophrenia *vs* control group. [^{3}H]KA and [^{3}H]AMPA binding levels were also largely maintained. Although there were no striking alterations in binding to NMDA and non-NMDA receptors between the schizophrenic and control groups, an analysis of binding to non-NMDA receptors in individual schizophrenic patients revealed different patterns of receptor changes in various individuals. For example, in one schizophrenic subject, a pronounced 37% increase in [^{3}H]KA binding was found in the outer 2/3 of the dentate gyrus molecular layer. This increase in [^{3}H]KA binding was accompanied by a widening of the zone occupied by KA receptors. In addition, the same individual exhibited a 51% increase in [^{3}H]AMPA binding in the infragranular layer. A similar response of KA and AMPA receptors was noted previously in the hippocampus of some Alzheimer's patients. This suggests that plastic responses, similar to those occuring in Alzheimer's disease, may have a common basis across several disease states.

412.14

SUBSTANTIA NIGRA NMDA AND AMPA RECEPTOR LOSSES IN A MOUSE MODEL OF PARKINSON'S DISEASE. U. Wüllner*, O. Isacson, J.B. Penney and A. B. Young Neurology Service, Massachusetts General Hospital, Boston, MA 02114

Exposure to the selective neurotoxin MPTP causes degeneration of mesencephalic dopaminergic neurons projecting to the striatum and parkinsonian symptoms in humans and various animal species. To investigate whether MPTP treatment elicits receptor changes resembling those found in Parkinson's disease we used quantitative receptor autoradiography in a mouse model of Parkinson's disease.

Mice (c57b) were treated with MPTP, 2x40mg/kg sc and sacrificed two and eight weeks after the onset of treatment, respectively. Striatal dopamine terminals measured with [^{3}H]mazindol binding to the dopamine uptake site were reduced to 17% of control at two weeks and to 38% at eight weeks (control: 1.73±0.12 pmol/mg protein, mean±sem; 2wk: 0.29±0.04; 8wk: 0.65±0.07) indicating recovery from the initial lesion. At two weeks, substantia nigra NMDA-sensitive [^{3}H]glutamate binding was reduced to 52% (control: 0.19±0.02; MPTP: 0.10±0.02; $p<0.05$). [^{3}H]AMPA binding was reduced to 76% (control: 0.64±0.07; MPTP: 0.44±0.06; $p<0.05$). No significant changes could be detected at eight weeks.

The decreases in NMDA and AMPA binding could be caused by decreased receptor synthesis in dopaminergic compacta cells or by down-regulation of receptor binding sites in pars reticulata due to increased glutamatergic activity of subthalamic projection neurons. Although, the impairment of the nigrostriatal dopaminergic system may not have been sufficient to elicit changes in subthalamic nucleus neurons. Supported by USPHS grant NS19613

412.15

DEVELOPMENTAL CHANGE OF INHIBITION BY LEAD OF *N*-METHYL-D-ASPARTATE-INDUCED CURRENTS IN CULTURED HIPPOCAMPAL NEURONS. H. Ujihara*, M. Alkondon and E.X. Albuquerque. Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

The ontogeny of the NMDA subtype of glutamatergic receptor/ion channel and developmental changes in inhibition of these currents by lead were studied by examining whole cell currents evoked by NMDA in cultured hippocampal neurons 1-30 days after plating of cells from fetuses obtained from 18-20-days gestation rats. We observed a maturation-dependent increase in conductance, indicating an increased density of NMDA receptors. The whole cell currents evoked by NMDA (10-100 μM) in the presence of glycine (1-100 μM) were composed of a rapidly decaying and a slowly decaying current, the ratio of these currents depending upon glycine concentration. The EC_{50} values for glycine were 1.8 μM and 0.3 μM, for the fast and slow components, respectively. The slow component became more predominant and the fast component less predominant along with cell maturation in culture. Pb^{2+} selectively inhibited NMDA currents recorded from young cultured neurons, exhibiting a noncompetitive antagonism against NMDA and glycine. The fast component, predominant at early states of neuronal development in culture that has the low affinity glycine site, was most affected by Pb^{2+}. These findings on Pb^{2+} actions at the NMDA receptor may explain the selective toxicity of this heavy metal on the immature brain. (Support: USPHS Grants NS25296 & NS05730)

412.16

THE SPASTIC HAN-WISTAR RAT AS A MODEL OF GLUTAMATE EXCITOTOXICITY: ELECTROPHYSIOLOGICAL ANALYSIS. R.W. Cohen*, J.E. Margulies, J.B. Watson, N.A. Buchwald, and M.S. Levine. Mental Retardation Res. Center, UCLA School of Medicine, Los Angeles, CA 90024.

Our laboratory has been studying a mutant strain of the Han-Wistar (HW) rat which carries an autosomal, recessive gene resulting in spastic paresis characterized by ataxia, tremor, and hind limb rigidity. Morphologically, starting about 30 days postnatally, progressive cell death occurs in the Purkinje cell layer of the cerebellum and the CA3 layer of the hippocampus. Currently, we are investigating the hypothesis that the cerebellar and hippocampal degeneration may be induced by glutamate (GLU) excitotoxicity. Evidence was first obtained from Xenopus oocytes expressing HW rat glutamate receptors following injection with mRNA from mutant or wildtype (control) cerebellum. Oocytes injected with mutant HW cerebellar mRNA (45-50 days of age) displayed significantly larger current responses to GLU and kainate (KA) than controls. The development of this disorder was studied by comparing the responses of oocytes injected with 30-35, 40-45, and 50-55 day old cerebellar mRNA from mutants or controls. Results indicated that GLU and KA evoked significantly larger inward currents in oocytes injected with mutant cerebellar mRNA after 40 days of age. GLU- and KA-dose response curves were also altered in oocytes injected with mutant mRNA (after 40 days). These curves exhibited reduced EC_{50}s, but displayed similar threshold dosages. To assess in situ electrophysiological alterations, field potentials were recorded in the Purkinje cell layer following stimulation of the molecular layer of mutant or control cerebellar slices. The results showed a 40% increase in the maximum amplitude of the response in the mutant cerebellum. There was no difference in the threshold voltage needed to evoke a response. These findings provide more evidence for abnormal, glutamate neurotransmission in the cerebellum of HW rats which may underlie the cellular degeneration observed in this mutation.

412.17

THE SPASTIC HAN-WISTAR RAT AS A MODEL OF GLUTAMATE EXCITOTOXICITY: MOLECULAR APPROACHES. J.E. Margulies*, R.W. Cohen, M.S. Levine and J.B. Watson. Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

We are studying a mutant strain of Han-Wistar (HW) rat which may provide information about genetically-induced alterations in excitatory amino acid receptor expression. Mutant HW rats carry an autosomal, recessive gene resulting in spastic paresis. Progressive degeneration of Purkinje cells in the cerebellum and CA3 pyramidal cells in the hippocampus occurs in mutant animals beginning around postnatal day 30 (P30), and marked cell loss is observed in both regions by P50. Voltage-clamped Xenopus oocytes injected with mutant HW cerebellar mRNA (50-55 days old) exhibit enhanced current responses to glutamate and kainate relative to oocytes injected with wildtype cerebellar mRNA. These data suggest that changes occur in the functional expression of glutamate receptors at an age corresponding to cerebellar degeneration. These changes may reflect a "mutant" multimeric receptor with enhanced sensitivity to kainate which places the cell at risk for excitotoxicity. To test the hypothesis that the stoichiometry of glutamate receptor subunit assembly is altered in mutant HW rats, expression of glutamate receptor subtype mRNAs (GluR1-5) was determined by Northern and slot blot analyses of cerebellar and hippocampal mRNA. Analysis of RNA from two independent sets of animals revealed a 30% decrease in GluR2 mRNA expression at 30-35 days and an 80% increase in GluR4 mRNA expression at 50-55 days in mutant cerebellum. GluR2 mRNA expression was also decreased by 30% in the hippocampus of 30-35 day old mutants. The cellular basis for the altered GluR2 and GluR4 mRNA expression can be investigated using in situ hybridization histochemistry. The early change in GluR2 mRNA expression in both mutant brain regions showing degeneration suggests that glutamate receptor assembly may be altered in neuronal populations at risk for or undergoing cell death. Conceivably, other untested kainate sensitive receptor subunits (e.g., KA-1, KA-2, GluR6, GluR7) are altered significantly in their expression in degenerating neurons.

GABA RECEPTORS: FUNCTION III

413.1

DEPOLARIZATION OF CULTURED EMBRYONIC RAT SPINAL CORD NEURONS BY GABA-A RECEPTOR ACTIVATION: MAGNITUDE AND IONIC BASIS. A.Kyrozis*, D.B.Reichling and A.B.MacDermott. Dept. Physiology & Cellular Biophysics and Center for Neurobiology and Behavior, Columbia Univ., New York, NY 10032.

GABA-A receptors activate Ca^{2+} entry into cultured spinal cord neurons via voltage-gated Ca^{2+} channels (Wang et al., Neu..Abs. 17:797, 1991). Patch techniques were used to test directly if the activation of the GABA-A receptor depolarizes embryonic dorsal horn neurons and by what ionic mechanism. Membrane voltage responses to 10 uM muscimol, a GABA-A receptor agonist, were recorded in current-clamp mode using perforated patch recording. Pipettes contained (mM): K_2SO_4 75, KCl 10, HEPES 10 and 100 ug/ml gramicidin or amphotericin B. Bath was NaCl-based (Cl^-=159mM). Of 65 neurons tested during the first week in culture, 10 depolarized to between -30 and -40 mV, 26 to between -40 and -50mV and 24 to less positive than -50mV. 5 cells were hyperpolarized. To test the ionic basis of the depolarization, cells with depolarizing responses were identified, the perforated patch electrode was pulled off, then whole cell recording was established using a second pipette to control $[Cl^-]_i$. Lowering $[Cl^-]_e$ to 49mM led to a depolarizing shift of +25 ± 2 mV (mean ± SEM; n=5) in the measured reversal potential. A +30 mV shift is predicted if Cl^- is the only permeating extracellular ion. Reducing $[Na^+]_e$ to 0 or 40 mM led to slightly depolarized reversal potentials (n=3) suggesting that Na^+ is not responsible for the depolarizing responses. These results demonstrate that GABA-A receptor activation can significantly depolarize a high percentage of embryonic cultured dorsal horn neurons and that an anionic conductance is mainly responsible for this effect, possibly due to a high $[Cl^-]_i$. (supported by EJKF and NIH)

413.2

GABA-INDUCED Ca^{2+} TRANSIENTS IN TYPE 1 ASTROCYTES IN PRIMARY CULTURES. Michael Nilsson[1], Peter S. Eriksson[1], Lars Rönnbäck[1,2] and Elisabeth Hansson*[1], Institute of Neurobiology[1] and Department of Neurology[2] University of Göteborg, Göteborg, Sweden.

By using the Ca^{2+}-sensitive indicator fura-2/AM, the cytosolic Ca^{2+} levels $[Ca^{2+}]_i$ were measured in type 1 astrocytes in rat cortical astroglial primary cultures, after stimulation with GABA, muscimol ($GABA_A$-agonist) or baclofen ($GABA_B$-agonist). We report that stimulation of both $GABA_A$ and $GABA_B$ receptors evokes Ca^{2+} transients in type 1 astrocytes. In some cells, the responses after GABA stimulation were blocked to baseline levels after exposure to bicuculline ($GABA_A$-antagonist). In other cells, bicuculline only slightly reduced the GABA-evoked responses, and the addition of phaclofen ($GABA_B$-antagonist) did not amplify this inhibition. However, the muscimol-evoked rises in $[Ca^{2+}]_i$ were completely inhibited after exposure to bicuculline, while the responses after baclofen could only be partly blocked by phaclofen. GABA evoked rises in $[Ca^{2+}]_i$ which alternatively were inhibited (mostly) or persisted in Ca^{2+}-free buffer. The rises in $[Ca^{2+}]_i$ persisted, but were reduced, in Ca^{2+}-free buffer after stimulation with muscimol, but were on the contrary inhibited after baclofen stimulation. The results suggest that type 1 astrocytes in primary culture express GABA receptors which can elevate $[Ca^{2+}]_i$ directly or indirectly via Ca^{2+} channels and/or via release from internal Ca^{2+} stores.

413.3

THE EFFECT OF FLUPIRTINE ON CULTURED NEURONS: A PATCH-CLAMP STUDY M. Wienrich*§, T. Weiser§, I. Szelényi† Battelle PR&E§, ASTA Medica AG† Frankfurt/M., Germany

In order to investigate the mechanism of action of Flupirtine, we tested its effect on cultured neurons from rat brain using the "whole-cell" configuration of the patch-clamp technique. Under symmetrical chloride-concentrations, Flupirtine induced a dose-dependent, reversible depolarization. This was suppressed in the presence of bicuculline indicating that Flupirtine has $GABA_A$-agonistic properties. With threshold concentrations of Flupirtine, the GABA-effect was enhanced in an over-additive manner. Experiments performed at voltage-clamped neurons demonstrated, that the action of Flupirtine is dependent on the membrane potential. At potentials more negative than -50 mV, the drug caused a monophasic chloride inward current. At more positive membrane potentials, the application of Flupirtine induced an additional transient outward current. Probing the effect with voltage-jump protocols showed, that this initial current is not carried by chloride, and thus is not mediated via the $GABA_A$-receptor.

413.4

COMPARISON OF DIAZEPAM EFFECTS ON FREE CALCIUM LEVELS IN SYNAPTOSOMES AND SYNAPTONEUROSOMES FROM RAT BRAIN. J.V. Martin*, G. Thorp, L.B. LaCorte and H. Lee. Biology Dept., Rutgers Univ., Camden, NJ 08102.

In previous work, stimulatory effects of diazepam (DZP) on free Ca^{2+} were demonstrated using the membrane-permeant acetoxymethyl ester of the fluorescent dye fura-2 (fura-2/AM) in rat brain synaptosomes (Martin *et al.*, 1991, Brain Res. 548:222). As an approach to evaluate the relative presynaptic and postsynaptic contributions to the observed effects of DZP on Ca^{2+} metabolism, we compared the effects of DZP on Ca^{2+} in synaptosomes and synaptoneurosomes from rat brain. A freshly dissected brain (without brainstem or cerebellum) was divided equally as starting material for the two preparations. Synaptosomes were made by the Percoll step gradient method of Dunkley, *et al.* (1988, Brain Res., 441:59) and synaptoneurosomes were made according to Hollingsworth, *et al.* (1985, J.Neurosci. 5:2240). Both preparations were maintained in buffered salt solution in which Na^+ was replaced by isomolar choline (136 mM choline chloride, 5.6 mM KCl, 1.3 mM $MgCl_2$, 11 mM glucose, pH 7.4) and incubated with fura-2/AM for 55 min, followed by extensive washing, so the only fluorescent response was due to fura-2 generated inside the organelles by endogenous esterases. In all cases, depolarization with 45 mM added KCl including 1.2 mM $CaCl_2$ increased the level of cytoplasmic free Ca^{2+}. While DZP increased the levels of synaptosomal Ca^{2+} in a dose-dependent fashion which was synergistic with the effects of depolarization (as before), there was no effect of doses of DZP as high as 100 μM on synaptoneurosomal Ca^{2+}. Since synaptoneurosomal membranes are primarily post-synaptic in origin, these findings support the idea that effects of DZP on Ca^{2+} may be selectively mediated through a presynaptic mechanism. Supported by the Busch Fund and Rutgers University Research Council.

413.5

EXCITOTOXIC LESIONS IN NEOSTRIATUM/GLOBUS PALLIDUS CAUSE AN INCREASE IN IRON STAINING AND GLIAL CELL NUMBERS IN GLOBUS PALLIDUS AND SUBSTANTIA NIGRA. S. Sastry, G.W. Arendash, R. Richmond*. Dept. of Biology and Institute for Biomolecular Science, University of South Florida, Tampa, FL 33620.

The substantia nigra (SN) and globus pallidus, two iron-rich brain areas, receive a dense GABAergic innervation. This anatomic relationship suggests that GABA is important in regulating brain iron. Therefore, we investigated the effects of denervation of striatal/pallidal GABA inputs to globus pallidus/SN on iron staining and correlative histology; apomorphine induced rotation behavior was also assessed. Adult male Sprague-Dawley rats were divided into three groups. The first group received four ibotenic acid infusions (5 µg/µl PBS each) throughout the neostriatum and globus pallidus (NG); the second and third groups received two infusions of ibotenic acid each, into either the anterior neostriatum (AN) or the posterior neostriatum/globus pallidus (PG) regions. Animals were sacrificed at 1 month following surgery. Alternate brain sections were stained with thionine or for iron. All lesions were limited to the neostriatum/globus pallidus; NG lesions involved this entire region, whereas AN and PG lesions destroyed either the rostral or caudal halves, respectively. In the NG group, an increase in iron staining and gliosis occurred throughout zona reticularis of the SN (SNr). These changes were accompanied by a decrease in overall volume of the SN. Similar results were obtained for the PG group, with the gliosis in SNr being relatively localized. The AN group showed only a minimal increase in SNr iron staining with a localized gliosis. Also evident in the AN group was a marked gliosis and increased iron staining within globus pallidus. Only NG and PG groups showed substantial ipsilateral rotation through 1 month after lesions. These results indicate that discrete excitotoxic lesions compromising GABAergic innervation to SN and globus pallidus alter iron levels in these areas, supporting a regulatory influence of GABA on brain iron.

413.6

EFFECTS OF EXCITATORY AND INHIBITORY NEUROTRANSMITTERS ON WHOLE CELL RECORDINGS IN GUSTATORY ZONE OF NTS. L. Wang, M. S. King* and R. M. Bradley, Dept. of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48109.

The gustatory zone of the NTS contains fibers and terminals immunoreactive for substance P (SP) as well as GABA-immunoreactive interneurons. Therefore, SP and GABA may have important influences on rostral NTS neurons. We investigated the effects of these transmitters on neurons in the gustatory zone of the NTS using whole cell recording in slices of the rat medulla. Superfusion of μM concentrations of SP transiently depolarized 54 of 88 neurons (61%) and increased input resistance, while GABA (10^{-3}M) hyperpolarized 105 of 156 neurons (67%) and decreased input resistance. Since they could be elicited when synaptic transmission was blocked, these effects were due to direct postsynaptic action on the recorded neurons. GABA acted on both $GABA_A$ and $GABA_B$ receptors because both muscimol (10^{-3}M; $GABA_A$ agonist) and baclophen (10^{-4}M; $GABA_B$ agonist) mimicked the effects of GABA. In addition, the $GABA_A$ antagonist bicuculline (10^{-4}M) and the $GABA_B$ antagonist phaclophen (10^{-3}M) strongly suppressed the neuronal responses to GABA. Bicuculline also depressed synaptically induced IPSPs evoked by electrical stimulation of the solitary tract. Of 34 neurons tested, 15 (44%) responded to both SP and GABA, suggesting inhibitory and excitatory modulation of single rostral NTS neurons. The integration of these excitatory and inhibitory influences may be important in processing gustatory information. This possibility is currently being investigated through the analysis of inhibitory and excitatory potentials, evoked by electrical stimulation of the solitary tract, in neurons in the gustatory zone of the NTS. [Supported by NIH DC00288].

413.7

GABA-INDUCED MEMBRANE CURRENTS IN DISSOCIATED MEDIAL SEPTUM/DIAGONAL BAND (MS/nDB) NEURONS. G.D. Frye* and W.H. Griffith. Dept. of Med. Pharmacol. & Toxicol., Texas A&M Univ. Coll. of Medicine, College Station, TX 77843-1114

Anatomical and electrophysiological data indicate that cells in MS/nDB receive major GABAergic innervation. Virtually all cells in these regions appear to receive GABAergic synaptic contacts. In the present study a significant fraction of spontaneous postsynaptic potentials recorded intracellularly in MS/nBD brain slices were blocked by picrotoxin (30µM). Whole-cell patch clamp recordings in acutely dissociated, adult MS/nDB neurons, identified GABA-induced membrane currents in all cells examined (rats, n=10; Guinea pigs, n=7). Rapid application of GABA (30sec) by a large bore pipette induced concentration-dependent currents from a holding potential of -60mV that reversed near 0mV. Responses to GABA (3µM) showed little desensitization unlike larger responses to GABA (10µM) which swiftly decreased an average of 42.3±1.3% during the application. The effects of combined application of GABA with pentobarbital, midazolam or ethanol are currently being investigated. Supported by AG07805 (WHG); AA06322 and RSDA AA00101 (GDF)

413.8

DIRECT ACTIVATION OF THE $GABA_A$ RECEPTOR BY ISOFLURANE AND EVIDENCE FOR SUBUNIT SPECIFICITY. T.G. Hales, N.L. Harrison, S. Glusman* and M.V. Jones. Dept. Anesthesiology, UCLA, LA. CA 90024 and Dept. of Anesthesia and Critical Care, University of Chicago, Chicago, Il 60637.

Intravenous (IV) and volatile general anesthetics (GAs) potentiate neuronal responses to GABA. In addition, IV GAs directly activate $GABA_A$ receptors at anesthetic doses. We have investigated direct activation and modulation of $GABA_A$ receptors by isoflurane (ISO). Whole-cell recordings were made from cultured hippocampal neurons (HPC) from day 20 embryonic rats and immortalized hypothalamic (GT1-7) neurons, using 145 mM CsCl or K-gluconate based electrode solutions. GT1-7 neurons have been shown to contain mRNA for α1, β1 and β3 $GABA_A$ receptor polypeptides, but do not express γ subunits (Hales et al., Mol. Pharm. in press). Bath applied ISO (1-3 MAC 0.51-1.53 mM) modulated currents evoked by GABA (50-100 µM) in HPC and GT1-7 cells, under voltage-clamp. ISO increased the time for half-decay of GABA-evoked currents, in GT1-7 cells (by 299 ± 74% SEM) and HPC (by 152 ± 41%). Bath application of ISO (0.17-3 MAC) to HPC also directly activated an outward current, with K-gluconate in the electrode and 145 mM $[Cl^-]_o$, at -40 mV. Pressure application of ISO (< 4 mM) to HPC activated inward currents at negative potentials, which reversed at +3 ± 1 mV with 145 mM Cl- in the electrode and extracellular solutions. Reducing extracellular $[Cl^-]$ to 30 mM shifted the reversal potential of the ISO activated current to +38 ± 7 mV. GABA responses reversed at similar potentials. Bicuculline methiodide (BMI) (10 µM) and picrotoxin (5 - 10 µM) inhibited ISO-evoked currents by 78 ± 8% and 40 - 80 %, respectively. In the absence of ISO, BMI (20 µM) had no effect on the conductance of HPC. These data are consistent with ISO directly activating the $GABA_A$ receptor. ISO (<4 mM) did not directly activate currents in GT1-7 cells. One explanation for these data is that direct activation of the $GABA_A$ receptor by ISO is subunit specific. $GABA_A$ receptor activation by ISO may require subunit(s) not expressed by GT1-7 cells.

413.9

NMDA-RECEPTOR CHANNELS ARE SPECIFIC SOURCES IN THE SUPPRESSION OF $GABA_A$ RECEPTOR FUNCTION BY INTRACELLULAR CALCIUM H. Shi* and A. Stelzer. Dep. of Pharmacology, SUNY Brooklyn, Brooklyn, NY 11203.

Elevation of intracellular calcium $[Ca^{2+}]_i$ leads to a suppression of $GABA_A$ whole-cell currents in acutely dissociated CA1 pyramidal cells (cf. Chen et al., 1990, J. Physiol.). In the same preparation, we examined the contribution of various sources of $[Ca^{2+}]_i$ elevating systems in the regulation of $GABA_A$ receptors. Under whole-cell clamp conditions, voltage activated calcium currents were elicited by voltage commands from -70 to +10 mV followed in short intervals by GABA-induced chloride currents. Alternatively, NMDA currents were elicited by pressure application followed at similar intervals by GABA chloride currents. GABA-mediated outward currents were measured at +10 mV under both experimental conditions. Ca^{2+} influxes through NMDA-receptor channels produced a marked suppression of $GABA_A$ peak-current amplitudes. Recovery from reductions of $GABA_A$ responses generated by Ca^{2+} influxes through NMDA-receptor channels were absent in 47 out of 69 cells and small and incomplete in the rest. Changes of $GABA_A$ responses were not observed when NMDA currents were triggered in the absence of extracellular Ca^{2+}. In addition, voltage-activated calcium currents of any size and interval preceeding GABA currents did not affect $GABA_A$ responses. We assessed the amount of $[Ca^{2+}]_i$ elevations by calculating the sum of integrated individual currents through NMDA and voltage gated channels, resp. Elevations of $[Ca^{2+}]_i$ through NMDA-receptor channels which lead to a significant reduction of $GABA_A$ responses represent only a small fraction (less than 20%) of (ineffective) $[Ca^{2+}]_i$ rises through voltage-activated calcium channels. These data demonstrate that rises of $[Ca^{2+}]_i$ through NMDA receptor channels represent a specific source in the long-term suppression of $GABA_A$-receptor function.

413.10

$[^3H]$Ro15-1788 IS NOT A NONSELECTIVE BENZODIAZEPINE RECEPTOR RADIOLIGAND. P.A. Maguire*, M.F. Davies and G.H. Loew. Molecular Research Institute, Palo Alto, CA 94304.

With the emergence of multiple $GABA_A$ receptor subunit variants and many possible combinations in the brain, it has become a challenge to determine, from receptor binding studies, the number and pharmacological characteristics of the sites present. A major weakness has been the use of fairly nonselective radioligands. Critical to the determination of receptor heterogeneity using curve-fitting programs such as LIGAND, is that the K_d of the radioligand must be determined at each putative site present, as the K_i of each competing ligand is dependent on this value. We have found that $[^3H]$Ro15-1788 is not sufficiently selective to clearly distinguish different receptor types using LIGAND, however, its absolute nonselectivity cannot be assumed. We have been exploring a powerful new method to determine the K_d and receptor density of this ligand in several rat brain regions. This method, a Fourier-derived affinity spectrum analysis (FASA), (Anal. Biochem. 157:221, 1986), transforms receptor binding data (free and bound ligand conc.) into a probability-density function and solves it using Fourier analysis, resulting in an "affinity spectrum" of density as a function of affinity. No assumptions are made as to the number of binding populations. We have used this method to investigate the affinity of $[^3H]$Ro15-1788 at BDZ sites in several tissues, including the rat cerebellum and olfactory bulb. In each tissue, the radioligand binds to multiple sites with K_ds varying 10 to 20-fold. This information will greatly help the analysis of competitive binding data using LIGAND, by the determination of the number of binding sites, and by providing initial estimates for the subsequent calculation. The use of the FASA method, along with data analysis using LIGAND, should enhance our understanding of BDZ binding sites in the rat brain.

413.11

EFFECT OF A BENZODIAZEPINE AGONIST AND INVERSE AGONIST AND ZINC ON GABA-MEDIATED CHLORIDE INFLUX INTO CEREBELLAR AND CORTICAL MICROSACS M.F. Davies*, P.A. Maguire and G.H. Loew. Molecular Research Institute, Palo Alto, CA 94304.

Modulation of GABA-mediated ^{36}Cl influx into microsacs by ligands binding to the benzodiazepine (BDZ) site on $GABA_A$ receptors is a potentially useful discriminant of agonist and inverse agonist actions. To develop reliable conditions for assessing such actions, we have studied the effects of a prototypical agonist flunitrazepam (FLU 1μM) and inverse agonist DMCM (1μM) in rat cerebellar and cortical microsacs. In both tissues, most of GABA mediated ^{36}Cl flux occurred in the first 3 of 5s. Significant enhancement of this effect by FLU and reduction of it by DMCM was achieved in both tissues when the drugs were preincubated with the microsacs with ^{36}Cl and GABA for 3s. The DMCM effect was in part due to a reduction of flux in the nominal absence of GABA. Although reproducible, the effects of these BDZ ligands are modest (<50%), and may be due to not all GABA mediated Cl ion channels being sensitive to BDZs. To approach this problem, we have preformed these studies in the presence of zinc (100μM), known to preferentially inhibit GABA receptors that are insensitive to BDZs because they lack a γ subunit. The presence of Zn substantially reduced GABA (100μM) mediated ^{36}Cl flux in cortex (61±6%) and in cerebellum (51±8%) suggesting a significant proportion of the Cl channels are likely insensitive to BDZs. Under these conditions FLU increased dramatically to 273% from 42% without Zn. These results indicate that optimum conditions for measuring the effects of BDZ agonists and inverse agonists on GABA mediated ^{36}Cl flux involves measuring the effect within 3s of addition of GABA, but with the ligands in prior contact with the GABA receptor. Addition of Zn appears to enhance the effect of these ligands by allowing the measurement of Cl flux only through BDZ sensitive GABA channels.

413.12

BIOCHEMICAL MARKERS OF GABAERGIC AND DOPAMINERGIC TRANSMISSION IN THE CNS OF ROMAN HIGH-AVOIDANCE AND ROMAN LOW-AVOIDANCE RATS.

O. Giorgi*, M. Orlandi, D. Lecca, M.G. Pibiri, M.R. Murgia, A. Fernandez-Teruel (1), R.M. Escorihuela (1), P. Driscoll (2) and M.G. Corda.

Dept. of Exp. Biology, Neurosci. Section, Univ. of Cagliari, Italy. (1) Medical Psychol. Unit, School of Med., Autonomous Univ. of Barcelona, Spain. (2) Verhaltensbiologie Laborder ETHZ, Zurich, Switzerland.

A range of biochemical markers of GABAergic and dopaminergic (DAergic) neurotransmission were examined in the CNS of Roman high-avoidance (RHA/Verh) and Roman low-avoidance (RLA/Verh) rats, two psychogenetically selected lines which differ in their level of emotionality. The stimulatory effect of GABA (10 μM-100 μM) on ^{36}Cl-uptake was reduced in the cerebral cortex of RLA/Verh rats as compared with RHA/Verh rats. In addition, the increase of the GABA-dependent ^{36}Cl- uptake produced by diazepam (1 nM-100 nM) was larger in RLA/Verh rats than in their RHA/Verh counterparts. By contrast, no strain-related changes were detected in ^{3}H-GABA, ^{3}H-Flunitrazepam and ^{35}S-TBPS binding in the cerebral cortex. On the other hand, the density of D1 DA receptors labeled with ^{3}H-SCH 23390 was decreased in the nucleus accumbens of RLA/Verh rats as compared with RHA/Verh rats, whereas the binding parameters of these receptors were not different in the amygdala, prefrontal cortex and striatum of the two strains. It is proposed that these biochemical differences may play a role in the distinct emotional responsiveness and stress reactivity of RHA/Verh and RLA/Verh rats.

413.13

MDL 26479 MODULATES $GABA_A$ RECEPTOR ACTIVATED SINGLE CHANNEL CHLORIDE CURRENT FROM CULTURED RAT HIPPOCAMPAL NEURONS. C.J. ROGERS* and A.M. OGDEN, Marion Merrell Dow Research Institute, Cincinnati, OH 45215.

$GABA_A$ receptor complex chloride current is gated through the integral ion channel by $GABA_A$ receptor activation and modulated via allosteric receptor interactions. MDL 26479, may act via a benzodiazepine-like binding site as an inverse agonist. In vivo, MDL 26479 displaced [^{3}H] Ro 15-1788 binding consistent with a benzodiazepine-like interaction. Extracellular recordings from the hippocampus revealed increased excitability with MDL 26479. [^{3}H]-Hemicholinium-3 binding was increased with MDL 26479 suggesting a benzodiazepine inverse agonist-like action. Behavioral studies indicate MDL 26479 enhances cognition. To determine whether MDL 26479 modulates GABA-activated Cl^- current and the mechanism by which it may do so, we used the single channel patch clamp recording technique. Single channel recordings were obtained from cultured rat hippocampal neurons in response to the pressure application, from 10-25 micron diameter micropipettes, of GABA (2μM) alone or GABA (2μM) plus MDL 26479 (20, 100 or 300nM). Single channel currents were pooled from up to 80 different recordings for each of the different conditions tested.

MDL 26479 modulated GABA-activated Cl^- current in a concentration dependent manner. Total Cl^- current, comparing GABA (2μM) alone versus GABA (2μM) plus MDL 26479 (20nM) was decreased by 38%, decreased by 69% at 100nM, but increased by 134% at 300nM. The mean open duration of the channel for the ~30 pS conductance state was concentration dependent: 2.42 msec, GABA (2μM); 6.1 msec, GABA (2μM) plus MDL 26479 (20nM); 3.34 msec, GABA (2μM) plus MDL 26479 (100nM) and 1.67 msec, for GABA (2μM) plus MDL 26479 (300nM). Further investigations are underway to better understand this concentration dependent response and to determine whether this compound selectively activates specific receptor subunit configurations.

413.14

EFFECTS OF MDL 26,479 ON MUSCIMOL STIMULATED ^{36}CHLORIDE INFLUX INTO BRAIN MEMBRANE VESICLES. J.A. MILLER*, D.L. Braun and P.A. Chmielewski, Marion Merrell Dow Research Institute, Cincinnati, OH, 45215.

The cognition enhancer MDL 26,479 possesses benzodiazepine (BZD) inverse agonist activity. However, MDL 26,479 does not bind to known sites on the $GABA_A$ receptor complex or other receptors. We examined the effects of MDL 26,479 on muscimol stimulated ^{36}Cl influx into brain membrane vesicles. Membranes were prepared by the method of Harris and Allan (Science 228: 1108, 1985).

MDL 26,479 at low (< 100 nM) and high (> 1 μM) concentrations inhibited muscimol stimulated ^{36}Cl influx. At intermediate concentrations (150 nM to 1 μM) MDL 26,479 enhanced the muscimol stimulated ^{36}Cl influx. In contrast the BZD inverse agonists DMCM and β-CCM (10 nM to 10 μM) had only inhibitory effects. When MDL 26,479 was combined with DMCM at concentrations ranging from 10 nM to 10 μM these two were additive in effect even at supramaximal concentrations. In contrast, when β-CCM and DMCM were combined they were additive only at threshold concentrations. The BZD antagonists flumazenil (10 μM) enhanced the inhibitory effect and blocked the stimulating effect of MDL 26,479 on ^{36}Cl flux whereas it antagonized the inhibitory effect of DMCM. These data suggest that MDL 26,479 acts as a combined positive and negative modulator at the $GABA_A$ chloride ion channel at a site which is distinct from the BZD site.

413.15

A COMPARISON OF THE ACTIONS OF THE BENZODIAZEPINE (BZ) PARTIAL AGONIST, Ro16-6028, WITH "FULL AGONIST" BZs ON THE $GABA_A$ RECEPTOR COMPLEX (GRC). D.A. Finn* and K.W. Gee. Dept. Pharmacology, College of Medicine, Univ. of California, Irvine, CA 92717.

Ro16-6028 (Bretazenil) is a relatively new imidazo-diazepinone derivative with a pharmacological profile characteristic of a BZ partial agonist. Ro16-6028 exhibits potent anticonflict and anticonvulsant effects with minimal psychomotor impairment or development of physical dependence. The present study utilized modulation of [^{35}S]*t*-butylbicyclophosphorothionate (TBPS) binding and enhancement of GABA-stimulated ^{36}Cl- (chloride) uptake as measures of GRC function to further assess Ro16-6028's partial agonist profile. Ro16-6028 was the most potent BZ examined, exhibiting an IC_{50} (concentration at which half-maximal inhibition of specific [^{35}S]TBPS binding occurs) of 1.1nM, compared to 21.7nM for diazepam (DZP), 4.3nM for clonazepam (CLON) and 4.7nM for flunitrazepam (FLU). However, Ro16-6028 was less efficacious in that it produced 27% inhibition of specific [^{35}S]TBPS binding, compared to DZP (49%), CLON (34%) or FLU (41%). In the presence of 10μM DZP, Ro16-6028 antagonized the inhibition of [^{35}S]TBPS binding due to DZP alone. These results provide further support that Ro16-6028 is acting as a partial agonist at the BZ receptor in modulating [^{35}S]TBPS binding. (Supported by NIH grants NS25986 and NS24645).

413.16

REDUCTION OF CALCIUM INFLUX BLOCKS HALOTHANE EFFECT ON $^{36}Cl^-$ EFFLUX RATE IN RAT BRAIN CORTICAL SLICES. B. Longoni*, G.C. Demontis and R.W. Olsen. Department of Pharmacology, School of Medicine, University of California, Los Angeles, CA 90024.

Halothane (0.56-1.7 mM) increases muscimol (1-10 μM) stimulated $^{36}Cl^-$ efflux in rat brain cortical slices. This halothane action is accounted for by an increase in [^{3}H]muscimol binding affinity for GABA-A receptors (Longoni and Olsen, 1991 *Abstr. Soc. Neurosci.* 17: 1341). Additionally, following four minutes exposure to muscimol plus halothane, removal of both drugs produces a large increase in $^{36}Cl^-$ efflux lasting for several minutes. Halothane's action on GABA-A receptors has been suggested to involve an increase in intracellular Ca^{2+} (Mody et al., 1991). We find that incubation conditions which reduce intracellular Ca^{2+} prevent the increased $^{36}Cl^-$ flux seen following exposure to and removal of halothane plus muscimol. $CoCl_2$ (200 μM), or Ca^{2+}-free medium, or BAPTA-AM (5 μM, a membrane permeant Ca^{2+} chelator) in the medium, all block the effect. The time course, halothane dose-dependence, and calcium-dependence of this effect will be compared to the direct effects of halothane on GABA-A receptors.

Supported by NIH Grant AA07680 to RWO.

413.17

Bretazenil, unlike CL-218,872 or abecarnil, is a partial agonist in electrophysiological assays of benzodiazepine (BZ) receptor activity.
C. M. Wang* and R. F. Cox, Burroughs Wellcome Co., Research Triangle Park, NC 27709.

Novel BZ receptor ligands have been described as anxiolytic without sedation and myorelaxation. The basis for anxioselectivity is unknown but has been attributed to either "partial" BZ receptor agonism (i.e., low efficacy) or subtype selectivity. We examined the effects of bretazenil (Ro 16-6028, a benzodiazepine), CL-218,872 (a triazolopyridazine) and abecarnil (a β-carboline), all so-called BZ partial agonists, on GABA-Cl channel activity of cultured hippocampal neurons in vitro and on firing rates of substantia nigra pars reticulata (SNR) neurons in vivo.

Clonazepam and diazepam, known BZ receptor agonists, potentiated GABA-Cl channel activity in vitro (EC50s ~0.1 uM) and inhibited SNR firing by up to 90% (ED50s 0.1 and 0.6 mg/kg i.v., respectively). Abecarnil, bretazenil and CL-218872 were all potent in vitro (EC50s 0.1, 0.2 and 0.5 uM, respectively). Abecarnil inhibited SNR firing by 86% at 4 mg/kg i.v. (ED50 0.3 mg/kg) and CL-218,872 by 77% at 8 mg/kg i.v. (ED50 1 mg/kg). The inhibitory effect of bretazenil plateaued at 50% after 4 to 8 mg/kg. Furthermore, bretazenil partially reversed or blocked clonazepam-induced inhibition of SNR firing.

These results suggest that bretazenil is a partial BZ receptor agonist relative to the full agonists diazepam and clonazepam. Both abecarnil and CL-218,872 have high efficacy, and their anxioselective effects may be due to factors other than partial agonism (e.g., BZ receptor subtype selectivity).

413.18

GABA CONCENTRATIONS CAN INFLUENCE THE PHARMACOLOGICAL PROFILE OF GABAA RECEPTOR ACTIVATED CURRENTS. G. White*and P. Ross. Neurogen Corp., Branford CT, 06405.

In general, the pharmacological profile of a ligand-gated channel is independent of ligand concentration, within the confines of agonist/agonist or agonist/antagonist interactions which can be predicted from simple pharmacological models. Holland et al. (Mol. Pharm. 39, 1991) reported that effects of some butyrolactones on GABA activated currents depended upon the concentration of GABA employed. This finding represents a significant departure from simple pharmacological models. We report additional evidence that the pharmacological profile of GABAA gated currents can depend upon the concentration of GABA employed. Adult rat dorsal root ganglion (DRG) neurons were isolated and recorded from using the whole-cell variant of the patch-clamp technique as described previously (White, J. Neurophys. 64, 1990). Oocytes were prepared and recorded from in a manner similar to that described by Malherbe et al. (Mol. Brain Res. 8, 1990). In oocytes injected with RNA from adult rat cortex and in DRG neurons, the anesthetic agent trichloroethanol (TCEt, 10 mM) potentiated current amplitudes at 5 μM GABA (835±183%, n=5 and 656±127%,n=5), but not at ~EC50 (100 μM GABA, n=6) concentration of GABA in oocytes (-3±9%, n=5). 5 mM TCEt actually reduced current amplitude by 26±6% (n=6) at concentrations of GABA > EC50 in DRG neurons (100 μM GABA employed, EC50=51 μM, n=17). In addition, 40 sec pre-application of 5 μM GABA often resulted in a reduced potentiation by TCEt, suggesting a time dependent as well as concentration dependent phenomenon. In DRG neurons, current activated by 5 μM GABA was potentiated 144±21% (n=7) in the presence of 25 nM alprazolam, whereas current activated by 50 μM GABA was not potentiated (-6±3%, n=7). A parallel shift in the GABA concentration/response curve should have resulted in a 40% potentiation at 50 μM GABA. A parallel shift in the concentration response curve for GABA in the presence of 25 nM alprazolam was observed in 2 of 2 oocytes examined. We conclude that the pharmacological properties of the GABAA receptor ionophore complex are varied and intricate.

PEPTIDES: BIOSYNTHESIS AND METABOLISM II

414.1

A *cis* ELEMENT IN THE NEUROPEPTIDE Y GENE MEDIATES STIMULATION BY NERVE GROWTH FACTOR G.L. Yount*, R.A. Maikis and J.D. White. Div. Endocrinology and Dept Neurobiology & Behavior, SUNY Stony Brook, Stony Brook, NY 11794

Neuropeptide Y (NPY) gene expression, in rat PC12 cells, is stimulated by nerve growth factor (NGF) at the level of transcription. To investigate the mechanism by which NGF regulates rat NPY expression, we have transfected PC12 cells with vectors containing parts of the NPY gene promoter linked to a reporter gene in a modified pUC18 plasmid. The first vector contains 702 bp of the NPY 5' flanking sequence that includes a partial consensus AP1 sequence which is completely conserved in the human NPY gene (TGACTGC). A 81 bp deletion subclone of the first vector generated a second vector lacking the putative AP1 binding site. One day after electroporation, the cells were grown in the presence or absence of NGF (75ng/ml) for one day. mRNA levels of the reporter gene, the constitutive gene cyclophilin and the endogenous preproNPY were measured from total RNA. NGF induced a 2.5-fold increase in reporter mRNA levels in PC12 cells transfected with the vector containing the full length promoter region. Identical transfection experiments with the deletion vector demonstrated a loss of NGF stimulation. In all the transfection experiments, NGF treatment induced preproNPY mRNA levels an average of 6-fold above controls while cyclophilin remained unchanged. These data reveal a *cis*-acting element between -176 bp and -95 bp upstream of the transcription start of the rat NPY gene that mediates transcriptional activation seen with NGF treatment.

414.2

TRANSCRIPTIONAL CONTROL OF HIPPOCAMPAL NEUROPEPTIDE Y EXPRESSION. J.B. McCarthy, G.L. Yount, P. Camp, and J.D. White*. Div. Endocrinology and Dept. Neurobiology & Behavior, SUNY Stony Brook, Stony Brook, NY 11794.

Hippocampal neuropeptide Y (NPY) expression is predominatly localized to neurons in the dentate gyrus hilus and stratum oriens. In the current study NPY expression was analyzed following pentylenetetrazole (PTZ) induced seizure. Male Sprague-Dawley rats (200-225gm) were injected ip with 57mg PTZ/kg bw or vehicle. At 3, 6, 12, 18, and 24 hr post injection, animals were terminated and hippocampi removed. Total RNA was isolated from hippocampal extracts and preproNPY mRNA content was measured by nuclease protection analysis. PreproNPY mRNA levels increased 4.5-fold by 6 hr relative to control values, before returning to normal by 24 hr post-injection. *In situ* hybridization analysis of preproNPY mRNA levels in control and 6hr post-PTZ animals revealed that the change in total hippocampal preproNPY mRNA content was due to the apparent induction of NPY expression in dentate gyrus granule cells. Nuclear run-on analysis of control and 6 hr post-PTZ hippocampal extracts demonstrated a 2.5-fold increase in total hipocampal NPY transcription in PTZ-injected animals. The possible requirement for protein synthesis for increased NPY expression was investigated by anisomycin treatment prior to and throughout the 6 hr post-seizure interval. *In situ* hybridization analysis demonstrated a block of preproNPY mRNA induction in granule cells, suggesting a role for prior gene induction in activity-dependent NPY transcriptional activation. Thus, the simplest explanation for these data is that increased neural activity induced by convulsant treatment results in increased NPY transcription in granule cells that may be mediated by one or more cellular immediate early genes.

414.3

SECOND MESSENGER AND RECEPTOR-MEDIATED REGULATION OF SUPERIOR CERVICAL GANGLION NEUROPEPTIDE Y (NPY) EXPRESSION AND SECRETION. V. May* and K.M. Braas. Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

Superior cervical ganglion (SCG) neurons synthesize and secrete catecholamines and bioactive neuropeptides in response to a variety of stimuli. We have examined key regulators of neuropeptide Y (NPY) expression and secretion in primary rat SCG neuronal cell cultures. Enzymatically dissociated SCG cells from newborn rats were treated with 10 μM cytosine arabinoside to remove nonneuronal cells. Enriched SCG neuronal cells were subsequently cultured in serum-free defined medium containing 32 ng/ml NGF. The basal NPY secretory rate was less than 1% of the cell NPY content/h. To examine the regulation of SCG NPY expression and secretion, SCG neuronal cultures were treated with a variety of second messenger system activators and specific receptor ligands for 0 to 96 h. Dibutyryl cAMP elicited dose-dependent changes in NPY secretion and cell content. Sustained SCG neuronal NPY secretion stimulation of 8- to 10-fold was observed with dibutyryl cAMP treatment; other second messenger system activators, including the calcium ionophore A23187 or phorbol myristate acetate, elicited smaller reponses. Treatment of SCG cultures with pituitary adenylate cyclase activating polypeptide (PACAP) increased NPY secretion over 10-fold, in a dose-dependent manner; approximately 100-fold greater levels of the related peptide, VIP, were required to elicit similar half maximal stimulation of NPY secretion. (Supported by HD-27468 to VM)

414.4

TRANSCRIPTIONAL ACTIVATION OF NEUROPEPTIDE Y (NPY) PRECURSOR GENE BY MEMBRANE DEPOLARIZATION. H. Higuchi*, K. Nakano, A. Iwasa, and N. Miki. Dept. of Pharmacology I, Osaka Univ. Sch. of Med., Osaka 565, Japan.

Gene expression of neuropeptide Y (NPY), an important cotransmitter of catecholaminergic neurons, is regulated by neural activity via transsynaptic control. To investigate how neural activity changes its expression, changes in NPY mRNA abundance and transcriptional activity of the gene were studied in NG108-15 and PC12 cells. In these cells the membrane depolarization stimuli (high potassium and veratridine) increased NPY mRNA abundance time-dependently. The effect of veratridine was blocked completely by tetrodotoxin, while that of potassium was hardly suppressed. Voltage-dependent Ca channel blockers inhibited the increase in NPY gene expression by membrane depolarization completely. Transient assay using the CAT reporter genes containing the rat NPY gene promoter indicated that veratridine stimulates transcriptional activity of NPY gene. These findings suggested that membrane depolarization increases the NPY gene expression through transcriptional activation following Ca entry.

414.5

NEUROPEPTIDE Y EXPRESSION IN TARGET SPECIFIC NEURONS IN THE RAT SUPERIOR CERVICAL GANGLION. A.F. Elshaar*, K. Hentschel, and L.L. Wright, Dept. Anatomy/Neurobiology, BUSM, Boston, MA 02118.

This study was to compare the pattern of neuropeptide Y-like immunoreactivity (NPY-li) in adult and developing rat sympathetic superior cervical ganglion (SCG) neurons projecting to purely vascular targets, and those projecting to "mixed" vascular and glandular targets. Adult and postnatal day 5 (P5), P15, P25 male rats were used. The retrograde tracer Flurogold (FG) was injected into the temporalis muscle or frontal cortex to label SCG neurons projecting to a "purely vascular" target, and into the submandibular gland, to label neurons projecting to a "mixed" end organ. Immunofluorescence was utilized to compare the relative proportions of each population of SCG neurons that contained NPY-li.

Analysis of the results showed that nearly all (98%) of temporalis projecting neurons contain NPY, most (76%) of cerebral blood vessels projecting neurons contain NPY, and few (9%) of submandibular projecting neurons contain NPY. The results were similar at P5, P15, and P25, in that most of the SCG neurons projecting to the cerebral blood vessels and temporalis muscle contained NPY-li, while significantly few of those projecting to the submandibular gland contained NPY-li. From these data, we conclude that the population of neurons projecting to these target organs differ significantly in the percentage of neurons displaying NPY-li in adult and that this pattern is present after the fifth postnatal day of development. This work has been supported by a B.U. GSRA to AFE.

414.6

COMPARTMENTALIZATION OF VASOPRESSIN mRNA WITHIN HYPOTHALAMIC MAGNOCELLULAR PERIKARYA AND THEIR PROJECTIONS TO THE MEDIAN EMINENCE: IN SITU HYBRIDIZATION AT THE ULTRASTRUCTURAL LEVEL. A. Trembleau, M. Morales*, and F. E. Bloom, Neuropharmacolgy, The Scripps Res. Inst., La Jolla, CA 92037.

The neuronal localization of vasopressin (VP) mRNA was studied using a digoxigenin-labeled oligonucleotide complementary to nucleotides 908-943 of the VP cDNA. Vibratome sections of rat brain fixed by perfusion with 4% paraformaldehyde and 0.1% glutaraldehyde were permeabilized either by freezing or with triton X-100 and hybridized with the digoxigenin-labeled probe. Following the washing steps, the oligonucleotide was detected using an anti-digoxigenin antibody and the ABC-peroxidase method. The peroxidase was revealed with DAB enhanced with $NiCl_2$, the sections were postfixed with osmium tetroxide and embedded in epoxy resin. This method allowed us to label specifically VP magnocellular neurons of the rat hypothalamus. In addition, its high sensitivity was demonstrated by the labeling of VP neurons of the suprachiasmatic nucleus, where VP mRNA concentration is known to be low. At the electron microscope level, the well preserved morphology allowed for precise subcellular localization of VP mRNA: 1. MAGNOCELLULAR PERIKARYA. In the perikarya, the label was mainly associated with the membrane of the rough endoplasmic reticulum (RER), but not its lumen. This labeling was localized to discrete areas along the RER, suggesting a possible compartmentalization of VP mRNA within the RER. The Golgi apparatus, as well as neurosecretory granules appeared to be devoid of label. 2. AXONAL COMPARTMENT. Some axonal swellings in the median eminence, containing secretory granules, were highly labeled. These positive swellings were more abundant in salt-loaded rats. Some positive swellings were also observed in lactating rats and much more rarely in control animals. Within these swellings, the labeling was diffuse and therefore the precise localization of VP mRNA could not be ascertained. This limitation may be overcome through the use of colloidal gold probes.

414.7

ARGININE VASOPRESSIN (AVP) GENE EXPRESSION IN DISCRETE AREAS OF THE RAT BRAIN FOLLOWING PRE-TREATMENT WITH AVP. **P. Poulin*, P. Szot[1], O.J. Pittman and D.M. Dorsa[1]. Neuroscience Research Group, University of Calgary, Calgary, Alberta, CANADA and [1]GRECC, VA Medical Center, Seattle, WA 98108.**

It has been shown that AVP enhances its own release and enhances (sensitizes) the responses of the rat brain to subsequent AVP exposure. One possible mechanism for these effects may be by AVP-induced alteration in AVP gene expression. The level of cytoplasmic AVP mRNA was quantified autoradiographically by in situ hybridization in discrete areas of male Sprague Dawley rat brain 24 hrs following AVP (100 pmol icv) or vehicle (physiological saline icv) pre-treatment. Following decapitation, brains were frozen and 20 µm sections were hybridized with a ^{35}S-labeled probe for AVP mRNA, emulsion coated, developed and analyzed for the number of labeled cells and/or for densitometry. Preliminary results show that while AVP pre-treatment enhanced the number of hybridization positive cells in the Bed nucleus of the stria terminalis increases from 26 (control) to 35 (AVP pre-treated), the number of AVP mRNA expressing cells in the medial amygdala (16 control; 18 AVP pre-treated) and the paraventricular nucleus (rod .284 control; .283 AVP pre-treated) was not enhanced by AVP pre-treatment. These results suggest that pre-exposure of the rat brain to AVP results in discrete alterations of the expression of AVP message. Studies are now in progress to evaluate AVP mRNA levels in discrete areas of rat brain 1, 3, 6, and 12 hrs following AVP (100 pmol icv) or vehicle (physiological saline) pre-treatment. This work was supported by Savoy Foundation (PP) MRC (QJP) and VA medical center and NS 20311 (PS and DMD).

414.8

CCK mRNA EXPRESSION, PRO-CCK PROCESSING AND REGULATED SECRETION OF CCK IMMUNOREACTIVE PEPTIDES BY THREE ENDOCRINE TUMOR CELLS IN CULTURE: A VALID MODEL FOR BRAIN CCK? M.C.Beinfeld*, Dept. Pharm. & Physiol. Sci., St. Louis Univ. School of Medicine, St. Louis MO 63104.

Subclones of three common endocrine cell lines At-T20, rat insulinoma (RIN 5F), and a thyroid medullary carinoma (WE) express a single CCK mRNA species the same size as that found in rat brain. The WE cells contained levels of immunoreactive CCK comparable to rat cerebral cortex (about 4 ng/ mg protein) while the other cells lines contained significantly less CCK. Like the rat brain, they were able to correctly process pro-CCK to a form which co-eluted with CCK 8 on Sephadex and HPLC. All three cell lines secreted CCK 8 as well as some CCK 33, and this secretion was significantly enhanced by cAMP, but not by tumor-promoting phorbol esters.

When transfected with a eukaryotic expression vector containing the rat CCK cDNA behind the CMV promoter, the At-T20 cells expressed about 500 times as much CCK as the wild type. These cells still correctly processed pro-CCK to CCK 8, which suggests that either the expression of the processing enzymes is coordinately regulated with expression of the prohorme or that the enzymes are present in a considerable excess.

In summary, these cells lines appear to be a good model for studying pro-CCK processing in the brain and should facilitate future studies of CCK expression, biosynthesis, processing, and secretion. Supported by NIH 18667.

414.9

HALOPERIDOL-INDUCED NEUROTENSIN GENE EXPRESSION IN STRIATAL NEURONS IS REDUCED BY CHRONIC TREATMENT. K.M. Merchant*, D.J. Dobie and D.M. Dorsa. GRECC, Seattle VA Medical Center; Depts. of Psychiatry & Behavioral Sciences and Pharmacol., Univ. of Washington, Seattle, WA 98195.

Expression of neurotensin/neuromedin N (NT/N) gene in dorsolateral striatal (DLSt) neurons appears to be under the regulation of dopamine D_2 receptors such that acute blockade of D_2 receptors by neuroleptics (e.g., haloperidol) raises the level of NT/N mRNA by 1000%. The present study investigated the responses of this NT system to chronic haloperidol administration, a treatment which has been shown to increase the expression of D_2 receptors. Male Sprague-Dawley rats were treated s.c. with saline or haloperidol (1 mg/kg/day) using osmotic minipumps and sacrificed on day 28. A separate group of rats was treated similarly followed by removal of the pumps on day 28 and was challenged 24 hr later with a single dose of saline or haloperidol. Expression of NT/N mRNA was examined by *in situ* hybridization. Densito-metric analysis of film autoradiograms revealed that NT/N mRNA expression in DLSt was significantly lower in rats which received chronic haloperidol when compared to those which received acute haloperidol following chronic saline treatment. The tolerance in NT/N mRNA expression was not modified by an acute haloperidol challenge to rats chronically treated with haloperidol. It is likely that the decreased sensitivity of DLSt NT neurons to chronic haloperidol reflects a compensatory postsynaptic response to upregulation of D_2 receptors. (Supported by Scottish Rite Schizophrenia Foundation, the VA, and NS 20311)

414.10

REGULATION OF SOMATOSTATIN GENE EXPRESSION IN DISSOCIATED CULTURES FROM CEREBRAL CORTEX. G.T.Capone*, and C.B.Choi Dept of Pediatrics, The Kennedy Krieger Institute and Johns Hopkins Medical Institutions, Baltimore MD 21205

Somatostatin (SOM) is a small peptide transmitter/modulator found in the cerebral cortex. The two biologically active forms SOM-14 and SOM-28 are both derived from the same inactive precursor molecule preprosomatostatin (ppSOM). Within the cortex, SOM is localized to a small subset of interneurons and comprise about 2% of the total neuronal population.These neurons have been implicated in the pathogenesis of several neurologic disorders (Alzheimer's disease, Down syndrome, Epilepsy and hypoxic-ischemic encephalopathy).

Previous studies have demonstrated that the ppSOM gene can be regulated by such divergent stimuli as second messengers (cAMP), neurotransmitters (acetlycholine, glutamate) or membrane depolarization. The purpose of our study is to characterize ppSOM gene expression in developing cortical neurons using agents which mimic the effects of cAMP. Primary cultures of dissociated cerebral cortex were made from E15 mouse brain and grown for 7 days in MEM, with 10%FCS/10%HS in 95% air and 5% CO_2 . Cultures were stimulated with N^6-2-O dibutyrl cAMP or 8-Bromo cAMP then total RNA was isolated at 4,8,12 and 24 hours. Changes in ppSOM mRNA abundance was measured using Northern blot analysis. Stimulation with dbcAMP resulted in a (10-fold) increase in ppSOM mRNA by 4 hours and a (>50-fold) increase by 8 hours. When cultures were stimulated with BrcAMP increased ppSOM mRNA was also seen at 4 hours (2-fold), 8 hours (6-fold), 12 hours (9-fold) and 24 hours (7-fold) compared to untreated controls. Our results support previous findings which demonstrate that ppSOM gene expression is regulated via cAMP mediated signal transduction pathways and extend the observation to neurons in the developing cerebral cortex.

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414.11

REGULATION OF SOMATOSTATIN GENE EXPRESSION BY CALCIUM AND TRIIODOTHYRONINE IN A RAT MEDULLARY THYROID CARCINOMA CELL LINE. L.J. Huffman and C.S. Whisnant*. Dept. of Physiology, West Virginia Univ. Hlth. Sci. Center, Morgantown, WV 26506.

Somatostatin (SRIF), a neuropeptide produced by C cells in the thyroid gland, has been shown to play a role in the modulation of thyroidal function. In the present study, we have assessed whether SRIF gene expression is regulated by calcium or triiodothyronine (T_3). Since cyclic AMP is a known regulator of SRIF gene transcription, we also evaluated the effect of forskolin or a cyclic AMP analog on SRIF production. Rat medullary thyroid carcinoma cells (MTC 6-23) were grown in DMEM and 15% horse serum. For experiments, cells were incubated in DMEM-0.05 mg/ml bacitracin. The media content of SRIF and calcitonin was determined by radioimmunoassay. Northern blot analysis (20 μg total RNA/lane) was used to assess SRIF mRNA levels. Forskolin (10^{-6}M) or the cyclic AMP analog (10^{-3}M) increased SRIF mRNA levels and SRIF in the media after four, eight, and 24 hours of stimulation ($p<.05$ vs control). A four hour exposure to calcium (3mM) was associated with two-fold increases in the media content of calcitonin and SRIF as well as intracellular SRIF mRNA levels ($p<.05$ vs. 0.5 mM calcium). However, T_3 (10^{-10}M; four hours) had no effect on SRIF mRNA level or media content. These results indicate that calcium acts to increase calcitonin release as well as cause increases in both SRIF secretion and steady state SRIF mRNA levels. Since SRIF has been shown to inhibit calcitonin secretion, SRIF may act in an autocrine fashion to limit calcitonin production in response to a calcium challenge. (Supported by WVU Med.Corp. and NIH BRG No.2 S07 RR05433-29).

414.12

EVALUATION OF A PUTATIVE CIS-ACTING ELEMENT IN THE 5' UPSTREAM REGION OF THE MURINE POMC GENE. J. F. Bishop* and M. M. Mouradian, Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

Transcription of the proopiomelanocortin (POMC) gene is inducible by phorbol esters and cAMP. We have previously identified two potential target sites for transacting factors between nucleotides -137 and -106 of the murine POMC gene that are 11 bases apart. One site is homologous to the consensus sequence for AP-1 binding (TCAGCCA) and the other for AP-2 (CCCCCCTCCC). To investigate the potential enhancer activity of this region, we have fused the -137 to -106 sequence upstream to the SV40 promoter in a chloramphenicol acetyltransferase (CAT) expression vector yielding pCAT-POMC-137/106. Transient transfection of AtT-20 cells with this construct revealed two fold induction of CAT expression compared with the enhancer-less vector. Alteration of this construct by point deletion of a single C from the AP-2-like sequence resulted in loss of this enhancer activity. No significant change in CAT expression was seen in response to forskolin and/or TPA treatment in pCAT-POMC-137/106 transfected cells, suggesting that the -137 to -106 region of the POMC gene is not by itself involved in AP-1 or AP-2 mediated transcription. Other factors that interact with this sequence to confer its enhancer activity remain to be explored.

414.13

DOPAMINERGIC REGULATION OF TRANSCRIPTIONAL ACTIVITY OF ENKEPHALIN GENE IN RAT BASAL GANGLIA. S. P. Sivam*and A.K. Chaudhary, Department of Pharmacology & Toxicology, Indiana University School of Medicine, 3400 Broadway, Gary IN 46408.

This study examined whether a deficiency of dopamine during neonatal developmental period will induce permanent alterations in the rate of transcription of enkephalin gene. Dopamine deficiency was induced in Sprague-Dawley rat pups, by intracisternal administration of 100 µg of free base of 6-hydroxydopamine (6-OHDA) on the third day after birth. The animals were sacrificed 60 days after the 6-OHDA lesion. Striatal tissues were used for the determination of preproenkephalin (PPE) mRNAs by Northern blot analysis, Met^5-enkephalin (ME) levels by radioimmunoassay and amines and metabolites by HPLC. The rate of transcription of PPE gene was determined by a nuclear run-on assay using nuclei isolated from fresh or frozen tissues; the ^{32}P-labelled run-on transcripts obtained from a run-on transcription reaction were hybridized to a slot-blotted nitrocellulose or nylon membrane containing a plasmid with rat cDNA for PPE. As expected, dopaminergic denervated animals exhibited a severe loss of dopamine (>90% of control), an increase in PPE-mRNA and an increase in ME levels. The rate of transcription of PPE gene as evidenced from the run-on transcription assay was increased in lesioned animals. The increase in the rate of PPE transcription gene coupled with an increase in PPE-mRNA and its translation product ME indicate an enhanced rate of biosynthesis of enkephalin in lesioned animals. The results suggest a crucial role for dopamine in the development and regulation of enkephalin neurons of the basal ganglia. Supported by USPHS grant NS26063.

414.14

GLUCOCORTICOIDS POTENTIATE FORSKOLIN STIMULATION OF ENKEPHALIN PRODUCTION IN BRAIN CELL CULTURES. **M. K. McMillian*, H. H. Suh, L. Thai, K. R. Pennypacker, P. M. Hudson and J. S. Hong. LMIN/NIEHS/NIH, MD14-06, Research Triangle Park, NC 27709**

Dexamethasone (DEX, 10^{-7} M) increased [Met^5]enkephalin (ME) secretion from cultured striatal and hippocampal cells 2-fold over basal release after 24 hrs and 4 days exposure, an effect comparable to stimulations with the adenylate cyclase activator forskolin (FSK,10^{-5} M) and the protein kinase C activator phorbol myristate acetate (PMA, 10^{-7} M). DEX strongly potentiated the stimulation by FSK (to > 8-fold over basal) at 4 days, but not after 24 hrs. DEX did not increase the response to PMA or to the Ca^{2+} ionophore ionomycin (10^{-6} M). The potentiation by DEX was mimicked by corticosterone and competitively blocked by the anti-glucocorticoid RU486. Surprisingly, progesterone pretreatment abolished the DEX effect. Stimulated ME release at 4 days reflected synthesis of peptide, being much greater than cell ME content, and increased preproenkephalin (PPE) mRNA was observed in in situ hybridization studies. In hippocampal cultures, PPE mRNA and ME and precursor peptides appeared restricted to a subpopulation of type-1 astrocytes. In striatal cultures, neurons may also contribute to the observed effects of DEX on ME.

414.15

LONG-TERM ACTIVATION OF NICOTINIC RECEPTORS IS REQUIRED FOR STIMULATION OF PROENKEPHALIN (PROENK) GENE EXPRESSION AND FOR LONG-TERM [MET^5]-ENKEPHALIN (ME) SECRETION IN BOVINE ADRENAL MEDULLARY (BAM) CELLS. H.H. Suh*P. M. Hudson, M. K. McMillian, D. Leszczyszyn and J-S. Hong, LMIN/NIEHS/NIH. RTP, NC 27709

We have previously reported that long-term exposure of BAM cells to nicotine increases both the secretion of ME and the expression of proENK gene. In order to elucidate the molecular mechanisms underlying these long-term effects of nicotine, studies were undertaken to determine whether continuous activation of nicotinic receptors is required for both the delayed increase in ME secretion and proENK mRNA. Cholinergic receptor antagonists, hexamethonium (1 mM) and atropine (1 µM), were added to the incubation media at different time points (0.5, 1, 3, 6, 9, 12, and 24 hr) after nicotine treatment and aliquots of incubation medium were taken at each time point for ME determination and the cells were harvested at 24 hr for proENK mRNA measurements. Nicotine (10 µM) stimulated two distinct phases of ME secretion; a short-term rapid phase, followed after 9 hr by a sustained increase in ME release. Hexamethonium and atropine added 0.5 to 6 hr after nicotine treatment significantly inhibited the long-term phase of ME secretion and the expression of the proENK mRNA levels measured after 24 hr of nicotine treatment. Surprisingly, these antagonists were ineffective in blocking the nicotine-induced responses when added 9 hr post-treatment. Nuclear run-on assays showed that the transcriptional rate for proENK mRNA was increased by nicotine for at least 9 hr after drug treatment. Our results suggest that the long-term (at least 6 hr) stimulation of BAM cells with nicotine is required for increases of proENK mRNA and the long-term secretion of ME. The increase in proENK mRNA levels induced by nicotine reflects increased transcription of the proENK gene. Currently, we are comparing these effects of nicotine with effects of this drug on the expression of tyrosine hydroxylase mRNA and the short- and long-term secretion of catecholamines.

414.16

NICOTINE INCREASES [MET^5]ENKEPHALIN (ME) IN RAT ADRENAL MEDULLA AND SPLEEN. **P.M.Hudson*, M.K.McMillian, K.R.Pennypacker and J.S.Hong, LMIN/NIEHS/NIH, MD14-06, Research Triangle Park, NC 27709.**

The promoter region of the preproenkephalin gene contains elements for regulation by a number of transcription factors and second messenger systems. In striatum and glia, cAMP and CREB appear important for regulation. In rat adrenal, there is evidence for AP-1 binding as a primary regulator, and in spleen NFκB has been reported to regulate enkephalin expression. Nicotine stimulates adrenal medulla and sympathetic and parasympathetic ganglia. Chronic nicotine (3mg/kg bidaily) was no more effective than saline injections at increasing adrenal ME, in agreement with reported findings. However, acute repeated nicotine injections (3mg/kg every 30 minutes for 3 hours) produced a significant 2-fold increase relative to saline injections when measured after 2 days. Adrenal medulla AP-1 binding was decreased by nicotine and the decrease was long lasting (12 hrs) after acute repeated injections. Adrenal NFκB binding was increased by nicotine. In spleen, repeated acute nicotine injections also increased ME by about 2-fold after 2 days. Both AP-1 binding and NFκB binding were increased in spleen by nicotine. Our data indicate differential regulation of ME in peripheral tissues by nicotine.

414.17

EFFECT OF NICOTINE ON RELEASE OF CALCITONIN GENE-RELATED PEPTIDE FROM THE RAT TRACHEA

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Calcitonin gene-related peptide (CGRP) exists in the peripheral terminals of sensory C-fibers which innervate the airway. Topical nicotine is known to stimulate there terminals[1]. Given this excitatory effect, the present study assessed whether nicotine could evoke the release of C-afferent CGRP from the rat trachea. The rat trachea (from larynx to carina) was dissected free and incubated in oxygenated Krebs solution kept at 37•C. After 30-min equilibrium time, the trachea was sequentially incubated in tubes containing 2ml of : 1) Krebs (baseline); 2) Krebs + drugs (nicotine or capsaicin); 3) Krebs (post) for 10-min. CGRP levels in each test tube was analyzed by RIA. Nicotine ($5x10^{-6}$ to $5x10^{-5}$M) caused a concentration-dependent increase in the levels of CGRP in the perfusate. Forty minutes after the first exposure to nicotine, a second challenge with nicotine revealed a concentration-dependent desensitization of CGRP release. The releasing effect of capsaicin (10^{-7}M) was unaffected by nicotine desensitization. Pretreatment, however, with capsaicin (10^{-5}M) blocked nicotine (10^{-5}M)-induced peptide release. The result suggests that nicotine activated the peripheral terminals of capsaicin-sensitive primary afferents in the trachea and released CGRP.

1. Am. Rev. Resp. Dis. 137: 1330-1335, 1988.

(This work is supported by Tobacco Related Disease Program, University of California. X.-Y. H.)

PEPTIDES: PHYSIOLOGICAL EFFECTS II

415.1

THE EFFECTS OF REPEATED INTRACEREBRAL ADMINISTRATION OF CORTICOTROPIN-RELEASING FACTOR (CRF) ON THE ELECTROPHYSIOLOGICAL RESPONSE OF THE LOCUS COERULEUS TO SUBSEQUENT CRF OR CLONIDINE. L.H. Conti* and S.L. Foote. Dept. of Psychiatry, School of Medicine, Univ California San Diego, La Jolla, CA 92093.

Male Sprague Dawley rats were handled daily for 1 week following surgical implantation of a guide cannula aimed at the lateral ventricle. For the next 9 days, rats were either handled only, or handled while receiving an infusion (icv) of either CRF (3 ug) or sterile saline once each day. Twenty-four hours after the last infusion, rats were anesthetized with halothane and LC multiple unit activity was recorded. After establishing stable baseline activity, CRF (3 ug in 1 ul) or clonidine (1 ug in 1 ul) was infused into the lateral ventricle. In rats that had received no previous infusions, acute CRF produced a long-lasting increase in LC activity: 15 min post infusion, mean multiple unit activity was 176 % of baseline. However, in rats repeatedly treated with CRF prior to this acute challenge, CRF did not increase multiple unit activity in the LC: mean activity 15 min post infusion was 89 % of baseline. Additionally, in rats repeatedly treated with saline (icv), CRF failed to increase LC activity: mean activity 15 min post infusion was 98 % of baseline. In rats which were repeatedly handled but which received no infusions, CRF resulted in increased LC activity comparable to that seen in naive controls. The inhibitory effect of clonidine on LC activity was also diminished following repeated CRF administration. It is possible that the stress of repeated infusions resulted in repeated release of endogenous CRF which produced a functional decrease in responsivity to exogenous CRF and clonidine. This possibility is currently being investigated.

415.2

CYSTEAMINE PRETREATMENT ENHANCES INHIBITORY ACTION OF SS_{28} IN THE DENTATE GYRUS. T.W.J. Watson*, L. Borg. Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

We have previously reported that both SS_{14} and SS_{28} exert an inhibitory action on the evoked ECFP pop spike recorded from the dentate gyrus (DG) and cause direct postsynaptic hyperpolarization of dentate granule cells. These experiments were designed to examine the effects of the somatostatin depleting agent cysteamine on responses to SS_{14}/SS_{28} in DG. 500 μm thick transverse rat hippocampal (HPC) slices were completely submerged and constantly perfused in a recording chamber with oxygenated pH buffered aCSF. Three to four week old Sprague Dawley rats were pretreated with a single injection of 200 mg/kg of cysteamine given 15 to 20 hours prior to preparation of brain slices. All recordings were carried out in 10^{-4} M bicuculline. Cysteamine pretreatment resulted in selective increase in the inhibitory action of SS_{28} on the DG ECFP. Threshold of inhibitory effect was noted at 0.1 μM with complete and reversible supression of evoked activity being observed at concentrations of 3 to 5 μM. Interestingly, inhibitory action of SS_{14} was reduced in the cysteamine pretreated slices. When cysteamine was added to the perfusate (100 μM) a gradual increase in amplitude and number of evoked pop spikes was observed and this increase in activity persisted during the wash. These findings indicate that cysteamine pretreatment results in selective enhancement of the response to SS_{28} with relative down regulation of SS_{14} response and suggests the possibility of a differential effect on receptor subtypes. The increased amplitude and repetitive firing observed during bath application of cysteamine supports the concept that somatostatinergic neurons in the DG play an inhibitory role in controlling the firing of DG granule cells and that loss of these neurons may be involved in the pathophysiology of certain seizure disorders.

415.3

NEUROPROTECTIVE AND LTP-ENHANCIMG EFFECTS OF CHOLECYSTOKININ IN HIPPOCAMPAL SLICES. K.Kawasaki ※ and M.Yasui, Div. of Pharmacol., Shionogi Res. Labs., Shionogi & Co., Ltd., Osaka 553, Japan.

Cholecystokinin octapeptide (CCK-8) is densely distributed in the hippocampus, however its functional significance in this area is still unclear. Here we demonstrate neuroprotective and long-term potentiation (LTP)-enhancing effects of CCK-8. CCK-8 dose-dependently enhanced the magnitude of LTP induced by tetanic stimulation of Schaffer collateral-commissural fibers in the CA1 region of guinea pig hippocampal slices. This enhancing effect was antagonized by concomitant application of a CCK_B-receptor antagonist. Furthermore, desulfated CCK-8 showed a similar action on the LTP. The late negative component of evoked response, which was sensitively reduced by a K^+ channel blocker and by elevation of extracellular [K^+], was reduced by CCK-8. In vitro ischemic insult (hypoxia & hypoglycemia) induced a transient disappearance of evoked response followed by a short hyperexcitable period and a long-lasting dysfunction of CA1 pyramidal neurons in hippocampal slices of stroke-prone spontaneously hypertensive rat. CCK-8 reduced spreading depression-like depolarization and shortened time for the early disappearance of evoked potential as well as that for recovery of population spike. This neuroprotective action was also mediated by CCK_B-receptors. These results suggest that CCK-8 plays a positive role in the memory system and shows neuro-protective effects possibly by closing K^+ channels via breakdown of phosphoinositide in the hippocampus.

415.4

VASOPRESSIN ELICITS A LASTING INCREASE IN EXCITATORY POSTSYNAPTIC POTENTIALS (EPSPs) IN VENTRAL HIPPOCAMPUS NEURONS. I.J.A. Urban and P.J. French Rudolf Magnus Institute, University of Utrecht, The Netherlands. (Spon: ENA)

Vasopressin (VP) is a neuropeptide which is synaptically released in several of brain structures, including the lateral septum (LS) and ventral hippocampus (VH). Small amounts of VP, applied centrally, alter acquisition and extinction of various conditioned behaviors as well as the activity of brain neurons. In LS slices, pM concentrations of VP for hours enhanced EPSPs in LS neurons, without affecting the resting membrane potential (RMP), input resistance (IR) and other properties of these neurons. We used conventional microelectrode (3M KAc) and studied in brain slices the effect of VP on the EPSPs in neurons in the CA1/subiculum region of VH, evoked by stimulation of the stratum radiatum. Application for 15 min with 10^{-10} M VP had little effect on either RMP (before VP: -62.6±7.5 mV; 35 min after VP: -60.7±5.3 mV), IR (before VP: 46.8±6.5 MΩ; 35 min after VP: 49.2±8.7 MΩ) or the threshold for action potential was little changed by VP (before VP: 50.51±3.4 mV; 35 min after VP: 48.7±4.9 mV). In 14 of 20 neurons studied, EPSPs commenced to increase during and after VP application. At 35 min after the peptide application the amplitude of the EPSPs attained on average 137.5% of the control values and remained at this level for the rest of the recording (maxim 2.5 hrs). In the remaining 6 neurons EPSPs either did not changed (n=4) or decreased (n=2) after the VP treatment. Thus, pM concentration of VP can for many hours enhance the efficacy of the excitatory, presumably glutamatergic, transmission on CA1/subiculum neurons of the VH presumably by augmenting the glutamate release and/or the sensitivity of postsynaptic glutamate receptors. This VP effect was presumably different from the short excitatory action on the hippocampus, LS and other brain neurons that required much higher VP concentrations, may be of physiological significance and could be related to the behavioral effects of this neuropeptide.

415.5

EFFECTS OF CEREBROLYSIN ON SYNAPTIC TRANSMISSION IN THE RAT HIPPOCAMPUS. J.M. Wojtowicz, S. Wang and A. Baskys*, Department of Physiology and Clarke Inst. of Psychiatry, Univ. Toronto, Toronto, Ont, M5S 1A8, Canada.

Cerebrolysin (CB) is a brain tissue-derived mixture of polypeptides and amino acids that is widely used in treatment of dementias. To understand how CB exerts its therapeutic actions we applied it acutely to the rat hippocampal slices from 3-5 week old Wistar rats. CB was dissolved in saline and applied to slices for 5-10 minutes. CB inhibited the evoked field potential (FP) responses in the CA1 region of the hippocampus. The threshold of the effect was observed at 2μL/ml and a maximal inhibition at 10-20μL/ml. At 10μL/ml FPs were inhibited by 59% (S.E.=9.5, n=11). In 5 out 11 experiments a long-lasting increase (28%, S.E.=12.7) of FPs followed the inhibition. The effects were not associated with changes in the presynaptic fibre volley or paired pulse facilitation. Addition of 10μM bicuculline did not affect the CB action on FP, suggesting independence of $GABA_A$ receptors. Similar effects were observed in CA3 area of the hippocampus but not in the dentate gyrus where smaller inhibition (16%, S.E.=1.5, n=4) followed by 18.5% increase was noted. Given complex, albeit unknown composition of CB, it appears to have remarkably specific action on synaptic circuits in the hippocampus. Further studies are needed in order to elucidate the active ingredient(s) of CB. Supported by EBEWE, Austria.

415.6

MODULATION OF PAIRED-PULSE STIMULATION IN THE RAT HIPPOCAMPAL SLICE BY CHOLECYSTOKININ OR BICUCULLINE. E. Rich-Bennett*, D. Dahl, and B. B. LeCompte III. The University of Texas at Dallas, Richardson, TX 75083.

Cholecystokinin (CCK) is an ubiquitous cortical neuropeptide, and has been found to be colocalized with GABA in the hippocampal dentate gyrus. Paired-pulse stimulation was used to examine the effects of CCK (8S) and bicuculline (BIC, a $GABA_A$ antagonist) on activation of granule cells by selective orthodromic stimulation of the medial (MPP) or lateral (LPP) perforant pathways. An index of paired-pulse inhibition (PPI) or facilitation (PPF) was obtained by an initial control stimulus (S1) followed 10 sec later by paired stimuli ($S2_A$ & $S2_B$) with interpulse intervals (IPI) in the range of 5 to 1000 ms. The indices were determined by the ratio $S2_B/S1$, and were used to obtain paired-pulse response curves. Each pathway condition consisted of a drug-free (ACSF) response curve, followed by a drug response curve obtained during perfusion with either 1 μM CCK-8S or 1 μM BIC.

At the granule cell layer, each pathway possessed similar ACSF response curves: an early PPI (< 40 ms IPI), an intermediate PPF (30-200 ms), and a late PPI (200-1000 ms). With the LPP, BIC attenuated the inhibition of the early PPI, as well as the facilitation of the intermediate PPF, but had little or no effect on the late PPI. CCK-8S had little effect on the early or late PPI, but greatly enhanced the intermediate PPF. With the MPP, BIC attenuated inhibition of both the early and the late PPIs, as well as the intermediate PPF. The results with CCK-8S were similar to those found with the LPP. In contrast to the cell layer, the molecular layer ACSF response curves clearly differentiated the LPP from the MPP: the LPP showed a PPF for 10-1000 ms IPIs, whereas the MPP showed either no change or a slight PPI. Neither BIC nor CCK-8S had a clear effect on the molecular layer responses. (Supported by the Whitehall Foundation.)

415.7

ACTIONS OF SOMATOSTATIN ON GABA-ERGIC SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS. Z. Xie* and B. R. Sastry, Neurosci. Lab., Dept. of Pharmacol. & Therap., Univ. of B. C., Vancouver, B. C., Canada, V6T 1Z3.

Since somatostatin (SS) and gamma-aminobutyric acid (GABA) are co-localized in some neurons, the interactions between the actions of SS and GABA-ergic inhibitory postsynaptic potentials (IPSPs) in the CA1 pyramidal neurons of guinea pig hippocampal slices, are investigated. SS (SS-14, 2 μM) induced a hyperpolarization of the CA1 neurons associated with a reduction in the input resistance of the cells (n=16). These effects were not blocked by picrotoxinin (20 μM) (n=24) or phaclofen (1 mM) (n=16). Chelation of intracellular Ca^{2+} (Ca^{2+}_i) with BAPTA (n=6) or inhibition of protein kinase C (PKC) with sphingosine (30 μM) (n=4) had no significant effects on the hyperpolarizing action of SS. The peptide suppressed the $GABA_A$ receptor-mediated fast IPSPs and the $GABA_B$ receptor-mediated slow IPSPs, but had no significant effect on the excitatory postsynaptic potentials (EPSPs) (n=16). SS-induced depression of the IPSPs was not due to the hyperpolarization of the neurons. SS-induced hyperpolarization of the CA1 neurons was greatly reduced in the presence of baclofen (20 μM, n=6), an effect that was not due to the hyperpolarization by baclofen. The presence of QX-314 in the CA1 neurons, prevented the hyperpolarization of the neurons by SS and baclofen (n=12). QX-314 blocked the depressant effect of the peptide on the fast IPSP but not of the depressant effects of baclofen on the EPSP and the fast IPSP (n=12).

These results indicate that SS depresses GABA-ergic IPSP without affecting the GABA receptors. The peptide suppresses the fast IPSP through a QX-314-sensitive postsynaptic mechanism. SS can modulate IPSPs if the peptide receptors and the postsynaptic $GABA_B$ receptors are coupled to the same channels or the peptide and the amino acid act through the same intracellular second messengers. (The authors are grateful to Astra Pharmaceuticals for the gift of QX-314.)

415.8

GALANIN INDUCED ACETYLCHOLINE RELEASE IN THE RAT STRIATUM; POSSIBLE MECHANISMS. A. Pramanik* and S.O. Ögren. Dept. of Histology and Neurobiology, Karolinska Institute, Box 60 400, S-104 01 Stockholm, Sweden.

Galanin (GAL), a biologically active neuropeptide, is widely distributed in the CNS. Available evidences suggest that GAL is an inhibitory modulator of several neurotransmitters at both the pre- and postsynaptic levels. Recently we have for the first time demonstrated that GAL also possesses a facilitatory modulation of acetylcholine (ACh) in the rat striatum (Ögren and Pramanik, Neurosci. Lett. 128 (1991) 253-256).

The effect of GAL on the basal and the muscarinic agonist/antagonist mediated release of ACh was investigated in the enflurane-anaesthetized rats (body wt. 260-280 g) using in vivo microdialysis and HPLC techniques. GAL (3 nmol/10 μl), applied in the lateral ventricle (i.c.v.) or perfused through the microdialysis membrane into the striatum, was found to enhance basal ACh release. The GAL evoked ACh release was completely prevented by bupivacaine (Na^+-channel blocker, 0.25%) when coperfused with GAL. This shows that the effect of GAL depends on neuronal activity and thus is ascribed to action potential-dependent processes. In addition, the putative GAL antagonist M15 (3 nmol/10 μl, i.c.v./intrastriatally) completely blocked the GAL evoked ACh release, suggesting that GAL stimulates the basal ACh release in the rat striatum by a direct action of the peptide on striatal GAL receptors. GAL ((3 nmol/10 μl, i.c.v. or intrastriatally) was able to reverse the oxotremorine (0.3 mg/kg, i.p.) or carbachol (0.1 mM, infusion) induced inihibition of ACh release but did not change the scopolamine (0.25 and 0.5 mg/kg, i.p.) or pirenzepine (1 μM, infusion) stimulated release of ACh. In contrast to the GAL evoked ACh release, the scopolamine (0.25 mg/kg, i.p.) stimulated ACh release was not blocked by M15, indicating that the mechanisms behind GAL and scopolamine evoked striatal ACh release differ. Our data indicate that stimulation of the basal ACh release by GAL in the striatum occurs in striatal interneurons. The implication of this finding for both the peptide functions and how peptide-receptor coregulations may occur in the CNS will be further discussed.

415.9

SYNTHESIS AND EVALUATION OF THE PHYSIOLOGICAL EFFECTS OF HUMAN GALANIN. T.P. Iismaa,* D.G.P. Carey, D.J. Chisholm, I. Ferguson, K.K.Y. Ho, E.W. Kraegen, N.D. Oakes, I.A. Rajkovic and I. Shine. Garvan Institute of Medical Research, St Vincent's Hospital, Darlinghurst 2010, Sydney, Australia.

Human galanin is a 30 amino acid peptide which differs significantly from porcine, bovine and rodent galanin both in regard to sequence in the carboxy-terminal segment of the peptide and in the absence of carboxy-terminal amidation. Synthetic human galanin, prepared using a solid phase peptide synthesiser and purified to homogeneity using high pressure liquid chromatography, was administered to rats and humans in order to assess the biological activity of human galanin. Intravenous bolus administration of synthetic human galanin to conscious rats during glucose infusion increased blood glucose elevation and reduced circulating insulin, consistent with established effects of native galanin in the rat. Synthetic human galanin was infused for 90 min into healthy human volunteers at low and high levels (33 and 132 pmol/kg/min, respectively) for 90 min or saline control (n=8). A 25g intravenous glucose bolus was administered 30 min into the galanin infusion. There was a small drop in systemic blood pressure and an increase in resting heart rate during the infusion. All subjects reported a metallic taste and hypersalivation. No significant effect of human galanin on plasma glucose, serum insulin (basal or stimulated) or C-peptide levels was observed relative to control. Plasma growth hormone levels rose even in the face of a glucose load in both low and high dose infusions. Thus, human galanin is an ineffective β-cell suppressant in humans, compared with rats, but significantly increases growth hormone secretion.

415.10

PASSIVE IMMUNIZATION TO GALANIN ALTERS BIOGENIC AMINE METABOLISM IN MALE RATS. S.M. Gabriel* and P.J. Knott, Dept. Psychiatry, Mount Sinai School of Medicine, New York, NY 10029 and Bronx VA Hospital, Bronx NY 10468.

Galanin is found in biogenic amine-containing neurons. In the present study, male rats were implanted with lateral ventricular cannulae. After surgical recovery, 2μl of preimmune or a specific anti-galanin serum were administered intraventricularly to freely-moving, conscious rats. Animals received two morning injections, 24 hours apart. Hypothalamic tissues were harvested 4 to 6 hours after the second injection for analysis of biogenic amine and metabolite concentrations by HPLC with electrochemical detection. Concentrations of the serotonin metabolite, 5-hydroxy-indol-acetic acid [5HIAA] were significantly elevated 33% and 39% in anti-galanin versus preimmune-treated rats in the medial basal and preoptic hypothalamus, respectively. Concentrations of 5HIAA were unchanged in the paraventricular and supraoptic hypothalamus. Presumably, passive immunization sequesters released peptide. Thus, these data suggest that galanin exerts an ongoing inhibitory influence on serotonergic neurotransmission, perhaps subsequent to increased serotonin release or monoamine oxidase activity. This indicates that a novel biological interaction exists between galanin and serotonin.

415.11

N-Acetyl-Aspartyl-Glutamate Potentiates Inward Currents in Rat Dorsal Lateral Geniculate Nucleus Neurons In Vitro. R.A. Brautigan and D.A. Eagles* Department of Biology, Georgetown University, Washington, DC 20057.

N-acetyl-aspartyl-glutamate (NAAG) is an endogenous dipeptide found in varying concentations throughout the brain and is proposed to have a neurotransmitter or neuromodulatory role in the central nervous system. In the visual system, high concentrations and a calcium-dependent release have been reported (Moffett et al., Brain Res 538:86-94, 1991). Among the highest concentrations noted was that of the dorsal lateral geniculate nucleus (dLGN). Since retinogeniculate transmission is thought to be mediated by glutamate, and NAAG has been reported to activate glutamate receptors, its effect was studied on dLGN neurons in the rat. In vitro slice preparations were used to do whole cell patch clamp electrophysiology on 300 micron thick thalamic slices containing the dLGN.

When NAAG was applied via a pressure pipette (9 mM in ACSF, pH 7.3) positioned upstream from the recording site, cells clamped at the resting membrane potential (typically -68 to -73 mv) exhibited fluxes of inward current (as much as -.14 pA) for varying periods of time before returning to baseline current values. Similar responses were obtained when glutamate (10 mM in ACSF, pH 7.3) was applied in the same manner, suggesting that responses to NAAG were mediated by glutamate receptors. These results suggest a role for NAAG as a neurotransmitter in the dLGN.

PEPTIDES: PHYSIOLOGICAL EFFECTS III

416.1

PARTICIPATION OF ENDOGENOUS NEUROPEPTIDE Y IN THE SUPPRESSION OF BARORECEPTOR REFLEX RESPONSE BY LOCUS COERULEUS IN THE RAT. J.Y.H. Chan*, C.D. Shih and S.H.H. Chan. Dept. of Med. Res., Vet. Gen. Hosp.-Taipei and Inst. of Pharmacol., Natl. Yang-Ming Med. Coll., Taipei, Taiwan, R.O.C.

We evaluated the role of endogenous neuropeptide Y (NPY) in the suppression of baroreceptor reflex (BRR) response by locus coeruleus (LC), using adult, male Sprague-Dawley rats anesthetized with pentobarbital sodium (40 mg/kg, i.p.). Under an electrical stimulation condition (10-s train of 1-ms rectangular pulses, at 10-20 μA and 10-20 Hz) that did not appreciably alter the basal systemic arterial pressure and heart rate, the LC significantly suppressed the BRR responses induced by an intravenous injection of phenylephrine (5 μg/kg). Bilateral microinjection of NPY (4.65 pmol/20 nl), or an antiserum against NPY (1:20, 20 nl), into the nucleus tractus solitarius (NTS), the terminal site of baroreceptor afferents, elicited respectively a reduction in, and an enhancement of, the BRR response. Direct application of the NPY antiserum into the NTS also attenuated the suppressive effect of the LC on the BRR response. Treatments with normal rabbit serum, and heat-inactived NPY or NPY antiserum, on the other hand, were ineffective. These results suggest that neurons in the brain that contain NPY may exert a tonic reduction in the BRR sensitivity. Furthermore, the endogenous NPY may participate in the modulation of the same reflex by the LC. Both these actions may possibly take place at the NTS.

416.2

EFFECT OF NEUROPEPTIDE-Y (NPY) ON BLOOD FLOW IN THE RAT TAIL AND FOOT. M. E. Heath and J. R. Thomas*, Navy Medical Research Institute, Bethesda, MD 20889.

The purpose of this study was to assess the effect of i.v. administered NPY on superficial cutaneous microcirculatory blood flow in the tail and foot (by laser doppler flowmetry), and on total blood flow in the tail (via venous occlusion plethysmography). Male 300 g Long-Evans rats, with jugular catheters, were anesthetized, placed in a tubular plexiglass holder, and allowed to equilibrate for 25-30 min before experiments. NPY, norepinephrine (NE), [Leu^{31}, Pro^{34}]NPY, NPY[13-36] or saline control was administered i.v. following 5 min of baseline data. Increasing doses of NPY, from 16 to 64 ug/kg, induced an immediate, marked, and long lasting decrease in superficial cutaneous microcirculatory blood flow in both foot (max=50%) and tail (max=25%). In contrast, total blood flow in the tail either showed no change or increased (max=25%). In addition, the volume of blood in the tail usually increased in response to NPY, thus suggesting relaxation of and reduced resistance in the larger tail vessels. NE and the specific Y_1 receptor agonist, [Leu^{31}, Pro^{34}]NPY, produced effects on superficial foot and tail blood flow and on total tail blood flow similar to that of NPY, although smaller in magnitude. NPY[13-36], a specific Y_2 receptor agonist, did not appear to modulate blood flow in either the foot or tail. These findings suggest that NPY Y_1 receptors do, and Y_2 receptors do not, participate in the cutaneous microvascular blood flow response of NPY.

416.3

VASCULAR EFFECTS OF SYSTEMIC IMMUNIZATION AGAINST VASOACTIVE INTESTINAL PEPTIDE AND NEUROPEPTIDE Y. M. Michalkiewicz*, L.J. Huffman, M. Dey and G.A. Hedge. Department of Physiology, West Virginia Univ. Hlth. Sci. Ctr., Morgantown, WV 26506

The regulatory peptides, VIP and NPY, are present in autonomic nerve fibers innervating blood vessels. Exogenous VIP exerts vasodilatory effects resulting in a decrease in blood pressure (BP), while NPY has vasoconstrictor effects resulting in increased BP. To study the role of endogenous VIP and NPY in the regulation of BP, we passively immunized rats against these peptides. Anti-VIP serum (AVS-1) and anti-NPY serum (ANS-1) were generated in rabbits against synthetic porcine VIP and NPY, respectively. VIP monoclonal antibody (VIP-1b) was supplied by J.C. Porter (Univ. of Texas). AVS-1 has a B_{max}=4.91 nmol/ml and Kd=0.83 nM for VIP and does not crossreact with any of 17 other peptides tested, including peptide histidine isoleucine. ANS-1 has a B_{max}= 1.99 nmol/ml and Kd=0.47 nM for NPY and does not crossreact significantly with rat peptide YY, rat pancreatic peptide or any of 8 other peptides tested. Arterial BP was continuously measured in anesthetized (ketamine & pentobarbital) Sprague-Dawley rats (males, 220-250g) for 45 minutes beginning 5 min before treatments. BP was not changed after ANS-1 administration (1 ml, iv) when compared to BP in control rats (saline or normal rabbit serum). In contrast, when AVS-1 (1 ml, iv) was given, BP increased (144%, $p<0.01$). Heart rate was not changed. Similarly, VIP-1b (2-10 mg IgG, iv) increased BP in a dose-dependent manner (up to 138%, $p<0.01$). These results suggest that VIP may function as a tonic vasodilator and be involved in the physiological regulation of BP in the rat, whereas the involvement of NPY in the maintenance of normal BP is not evident. (Supported by NSF DCB-8904470).

416.4

DIFFERENTIAL ACTION OF NEUROPEPTIDE Y AND SUBTYPE AGONISTS ON SINGLE UNIT ACTIVITY IN THE PARAVENTRICULAR HYPOTHALAMUS. V.B. Aramakis*, J.H. Ashe, J. Juranek, L.M. Lomeli, A. Taneja & B. G. Stanley. Depts. Neuroscience & Psychology, University of California, Riverside, CA 92521

Hypothalamic neuropeptide Y (NPY) has multiple behavioral and physiological functions and has been shown to produce both increases and decreases in neuronal activity. To determine the possible contributions of NPY receptor subtypes to these responses, the effects on neuronal activity of NPY and the presumed Y1 and Y2 agonists [Pro34]-NPY and C2-NPY were examined. Coronal slices of rat hypothalamus were maintained in vitro and extracellular single unit recordings have been obtained from 39 paraventricular nucleus (PVN) neurons and 7 perifornical area (PFH) neurons. Cells in the PVN had a spontaneous firing rate of 3.2 spikes/sec; spontaneous firing rate for cells in the PFH was 7.8 spikes/sec. In the PVN, microtopical application of NPY and [Pro34]-NPY produced a repeatable decrease (11/16 at 1.5 μM and 6/13 cells at 0.15 μM) on average, to 20% of spontaneous rates. The reduction lasted from 3 to 20 min. Similar responses to NPY were evident in the PFH. In contrast, C2-NPY (0.15 μM) application in the PVN predominantly increased firing rate an average of 79% in 5/9 cells. Excitation lasted an average of 4 min and most cells returned to their baseline firing rates. The present findings suggest that the NPY agonists have differential effects on neuronal discharge rate; perhaps via actions on different NPY receptors. Supported by NSF BNS9008818 and NIH NS24268.

416.5

BI-DIRECTIONAL REGULATION OF INTRACELLULAR CALCIUM CONCENTRATION BY NEUROPEPTIDE Y (NPY) RECEPTOR ACTIVATION IN PC12 CELLS. X. Chen and T.C. Westfall* Dept. Pharmacol. & Physiol. Sci., St. Louis Univ. Sch. of Med., St. Louis, MO 63104

We have previously demonstrated that catecholamine (CA) release from PC12 cells can be modulated by NPY receptor activation through a non-cyclic AMP pathway (Soc. Neurosci. Abstract 17:79.9, 1991). In the present study, we tested the hypothesis that NPY modulates CA release via its action on intracellular Ca^{++} concentration ($[Ca^{++}]_i$) in PC12 cells. PC12 cells were co-cultured with A10 cells (embryonic rat aorta smooth muscle cell) and differentiated with either nerve growth factor (NGF) or dexamethasone (DEX) for 5-7 days. Changes in $[Ca^{++}]_i$ in single PC12 cells were monitored by spectrofluorometry. NPY ($5x10^{-7}$ M) potentiated the K^+-induced increase in $[Ca^{++}]_i$ in the PC12 cells differentiated by NGF or DEX. In contrast, NPY^{13-36} (10^{-7} M and 10^{-6} M), which was shown to inhibit evoked CA release in PC12 cells through activation of Y_2 receptors, attenuated K^+-induced increase in $[Ca^{++}]_i$ in NGF-treated cells. The attenuation by NPY^{13-36} was not observed in DEX-treated cells, which is consistent with our previous results showing that DEX-treated PC12 cells have neither Y_2 binding sites nor Y_2 receptor-mediated modulation of CA release. The attenuation of K^+-evoked increases in $[Ca^{++}]_i$ induced by NPY^{13-36} was dependent on extracellular Ca^{++}, but the potentiation induced by NPY was not. NPY at a concentration of 10^{-7} M showed a potentiation of K^+-evoked increases in $[Ca^{++}]_i$ in Ca^{++}-free buffer, although it had no effect in Ca^{++}-containing buffer. The effect of NPY was attenuated by thapsigargin, an intracellular Ca^{++} depleting agent. PYX-2, a NPY receptor antagonist, significantly blocked NPY- and NPY^{13-36}-induced potentiation and attenuation, respectively. These results suggest that NPY^{13-36} attenuates CA release by inhibiting extracellular Ca^{++} influx through Y_2 receptor activation. (Supported by HL35202 and HL26319).

416.6

NEUROPEPTIDE Y MODULATION OF MELATONIN SECRETION FROM RAT PINEALOCYTES. V. Simonneaux*, S. Bichet and P. Pévet. Lab of Neurobiology of Rhythmic and Seasonal Functions, URA-CNRS 1332, 12, rue de l'Université, 67000 Strasbourg, France.

Pineal gland of mammals receives a noradrenergic input indirectly from the retina. The day/night rhythm of norepinerphrine (NE) release to the pineal induces a day/night rhythm of melatonin synthesis and release with high nightime values. Neuropeptide Y (NPY) is co-localized with NE in pineal sympathetic nerve endings. The studies of NPY physiological effect on pineal activity however have brought different results as the techniques used were different: organ culture, in vivo administration, cell culture. The aim of this work was to examine the effect of various concentrations of NPY (0.1 to 100 nM) on melatonin secretion by pinealocytes previously stimulated or not by the ß-adrenergic agonist isoproterenol (ISO). This study was performed on 2 day-old pinealocyte cultures of one month-old rats. NPY alone induces a small stimulation of melatonin secretion. Together with ISO, NPY displays a clear potentiatory effet on melatonin secretion especially at low ISO concentrations. The most efficient concentration of NPY in stimulating pineal activity is 10 nM.

In conclusion, this study demonstrates that NPY stimulates melatonin secretion at a postsynaptic level in the rat pineal gland.

416.7

EFFECTS OF LHRH FRAGMENTS ON THE ELECTROPHYSIOLOGICAL PROPERTIES OF CA1 NEURONS IN THE RAT HIPPOCAMPAL SLICE. Y.S. Chen, M. Wong, and R.L. Moss*. Dept. of Physiol., UT Southwestern Med. Center, Dallas, TX 75235.

The decapeptide, luteinizing hormone-releasing hormone (LHRH), exerts both behavioral and electrophysiological actions in extra-pituitary CNS sites. LHRH has been shown to be cleaved into specific LHRH fragments in brain tissue. Ac-$LHRH^{5-10}$, a synthetic fragment without LH-releasing activity, has been demonstrated to facilitate lordosis behavior (Dudley and Moss, 1988) and displace LHRH from its hippocampal membrane receptor (Thompson and Moss, 1992). In contrast, $LHRH^{1-6}$ has no effect on LH-release nor on lordosis. The present study investigated the effects of these LHRH fragments on the electrophysiological properties of CA1 neurons. Intracellular recordings were made from CA1 neurons in the hippocampal slice from ovariectomized female rats. The fragments were applied to neurons by addition to the superfusing medium. Ac-$LHRH^{5-10}$ and LHRH at 10^{-7}M had similar actions on CA1 neurons: a long-duration depolarization associated with increased input resistance, reduction in the slow afterhyperpolarization (AHP), and decrease in accommodation. However, at a concentration of 10^{-8}M, some cells were responsive to LHRH, but not responsive to Ac-$LHRH^{5-10}$, or had a stronger response to LHRH than to Ac-$LHRH^{5-10}$. This suggests that LHRH was a more potent modulator of CA1 neurons than the fragment. This result correlates with the relative binding affinities of LHRH and Ac-$LHRH^{5-10}$ in the hippocampus. $LHRH^{1-6}$ had no effect on CA1 neurons. These data suggest that Ac-$LHRH^{5-10}$, a behaviorally active fragment, might have a modulatory action on hippocampal neurons. Supported by NIH grant MH47418.

416.8

VIP INCREASES $[Ca^{++}]_i$ IN RAT PINEALOCYTES BY INCREASING PERMEABILITY ACROSS THE PLASMA MEMBRANE. N.C. Schaad*, J. Vanecek, D.C. Klein and J.T. Russell, Laboratory of Developmental Neurobiology, NICHD, NIH, Bethesda MD 20892.

VIP acts on many cell types trough mechanisms which are not fully understood. In the pineal gland, VIP increases cAMP accumulation and N-acetyltransferase (NAT) activity, the rate-controlling step in melatonin synthesis. It is known that stimulation of these parameters by norepinephrine (NE) is strongly dependent upon α_1-adrenergic elevation of $[Ca^{++}]_i$. Accordingly, in this investigation we determined whether $[Ca^{++}]_i$ is also elevated by VIP; this was examined using computer assisted single cell analysis of pinealocytes in primary culture.

VIP (1-100 nM) produces a rapid and sustained increase in $[Ca^{++}]_i$ in more than 85 % of the cells, as does NE. This indicates that both agonists act on the same population of pinealocytes. The VIP-induced increase in $[Ca^{++}]_i$ is absent when $[Ca^{++}]_o$ is less than 1 μM. In contrast, the NE response is reduced to a transient increase in these conditions, suggesting that VIP and NE may act in part trough different mechanisms. The VIP-induced increase in $[Ca^{++}]_i$ is not abolished by classical voltage-gated Ca^{++}-channel inhibitors, such as nicardipine, D-600 and omega-conotoxin. The effect of VIP was mimicked by PACAP 1-28.

These studies indicate that VIP can elevate $[Ca^{++}]_i$ and that it acts by opening plasma membrane channels which are distinct from classical voltage-gated channels.

416.9

VIP INFLUENCES ELECTROPHYSIOLOGICAL AND METABOLIC ACTIVITIES IN VIVO. A. BARDEA, R. GLAZER, G. LILING* AND I. GOZES, DEPT. CHEM. PATH. SACKLER MED. SCHOOL, TEL AVIV UNIV. TEL AVIV, ISRAEL.

VASOACTIVE INTESTINAL PEPTIDE (VIP) IS A NEUROMODULATOR, GROWTH REGULATOR AND SECRETAGOGUE FOR NEURONAL SURVIVAL FACTORS (GOZES AND BRENNEMAN, 1989, MOL. NEUROBIOL.3,1). TO MEASURE THE INFLUENCE OF VIP ON METABOLIC AND ELECTROPHYSIOLOGICAL ACTIVITIES IN VIVO, WE HAVE EMPLOYED A MULTIPARAMETER MONITORING SYSTEM LOCATED ON THE SURFACE OF THE BRAIN CORTEX OF THE LIVING RAT (MAYEVSKY ET AL., 1991, SPIE PROC.1431,303). WE MEASURED: LEVELS OF INTRACELLULAR NADH, CONTRACTION AND DILATION OF BLOOD VESSELS, VOLUME OF BLOOD, BLOOD FLOW, EEG, EXTRACELLULAR IONS (CA AND K) AND pH. THESE PARAMETERS WERE MEASURED AFTER STIMULATION WITH KCL WHICH PRODUCES A REGION OF DEPOLARIZATION AS FOLLOWS. THE ELECTROPHYSIOLOGICAL ACTIVITY IS CHARACTERIZED BY SPREADING DEPRESSION (SD) WHICH IS REFLECTED IN DEPRESSED EEG, AND ENHANCED IONIC WAVES INCLUDING HIGH AMPLITUDE K WAVES RESULTING FROM K LEAKS AT THE TIME OF DEPOLARIZATION. THE METABOLIC ACTIVITY IS CHARACTERIZED BY A DECREASE IN THE LEVEL OF NADH, AN INCREASE IN BLOOD FLOW AND IN VASODILATION LEADING TO ATP PRODUCTION NEEDED FOR ENERGY SUPPLY TO THE NA/K PUMP. AFTER INJECTION OF VIP INTO THE LATERAL VENTRICLE OF THE BRAIN (60-1400 ng) MULTIPLE CHANGES WERE OBSERVED IN THE K DEPOLARIZATION REGION, INCLUDING, A REDUCTION IN THE AMPLITUDE OF THE K WAVE AND A PROLONGATION OF THE DEPRESSED EEG. THE DEPOLARIZATION REGION AFTER TREATMENT WITH VIP WAS MORE SENSITIVE AND DISPLAYED A HIGHER WAVE FREQUENCY IN A DOSE DEPENDENT MANNER, IMPLYING A METABOLIC AND ELECTROPHYSIOLOGICAL ACTIVITY FOR VIP. FINALLY, THE PATTERNS OF VIP GENE EXPRESSION IN VIVO SUGGEST RECIPROCAL RELATIONSHIP BETWEEN ELECTROPHYSIOLOGICAL ACTIVITY AND VIP IN THE CORTEX, WHERE VIP GENE ACTIVITY REACHES A PEAK AT THE TIME OF BRAIN MATURATION (GOZES ET AL., 1987, MOL. BRAIN RES. 2,137.) AND IN THE SUPRACHIASMATIC NUCLEUS (THE BIOLOGICAL CLOCK), WHERE OUR RECENT RESULTS SUGGEST VIP AS A PART OF AN ENDOGENOUS OSCILLATOR.

416.10

ANTAGONISTIC PROPERTIES OF $CGRP_{8-37}$ ON NEUROCHEMICAL EFFECTS OF HUMAN CALCITONIN GENE-RELATED PEPTIDE. D. MENARD*, A.L. DRUMHELLER, A. FOURNIER, AND F.B. JOLICOEUR Departments of Psychiatry and Pharmacology, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4 INRS-Santé, Pointe Claire, Qc, Canada

Pre-administration of the C-terminal fragment $hCGRP_{8-37}$ antagonizes some but not all neurobehavioral effects of hCGRP (Menard et al., N.Sc. 1991). In the present study, we investigated the effects of CGRP administered alone or following pre-treatment with $hCGRP_{8-37}$ on brain regional concentrations of norepinephrine, (NE), dopamine (DA), DOPAC, HVA, as well as serotonin (5-HT) and 5-HIAA . Peptides were administered ICV in a dose of 20 µg and , 20 min later, brains were removed and dissected for neurochemical assays No neurochemical changes were found in striatum or septum. However, hCGRP reduced DA and increased HVA concentrations in the nucleus accumbens. Increased concentrations of all amines and metabolites were found in the globus pallidus, amygdala and frontal cortex. In the hippocampus, except for HVA, all neurochemical parameters were elevated by hCGRP . In the hypothalamus, hCGRP significantly increased and decreased DOPAC and HVA respectively without affecting other neurochemical parameters. In the substantia nigra, marked decreases in Da and HVA levels were the only significant changes found. Pre-treatment with $hCGRP_{8-37}$ either significantly attenuated or completely abolished all neurochemical effetcs of hCGRP in the substantia nigra, amygdala , nucleus accumbens and globus pallidus. In the frontal cortex , except for the increase in HVA concentrations, all neurochemical effects were blocked by $hCGRP_{8-37}$. CGRP-induced increases in hioppocampal DOPAC and 5-HIAA concentrations were also not affected by the fragment. Finally , $hCGRP_{8-37}$ did not alter any of the neurochemical actions of hCGRP in the hypothalamus. Together, these results reveal pervasive but heterogeneous neurochemical effects of hCGRP and point to the existence of pharmacologically distinct receptors mediating these effects .Supported by the Medical Research Council of Canada

416.11

PHARMACOLOGY OF THE EFFECTS OF BRADYKININ AND SEROTONIN ON THE RELEASE OF CGRP FROM THE RAT TRACHEA

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Depolarization of peripheral terminals of C-fiber afferents in the rat trachea can induce a local release of CGRP. In a previous study, application of bradykinin (BK) into the tracheal perfusate induced a dose-dependent increase in CGRP release, while serotonin (5-HT, 10^{-6}M) with no effect alone, facilitated CGRP release by capsaicin (caps 10^{-6}M)[1]. In the present study, we found that BK ($5x10^{-6}$M)-evoked CGRP release was significantly inhibited by a B2 antagonist: [D-Arg^0, Hyp^3, $Thi^{5,8}$, D-Phe^7]-BK $5x10^{-6}$M or indomethacin 10^{-5}M, but not by a B1 antagonist: [Des-Arg^9, Leu^8]-BK. The facilitatory effect of 5-HT was not markedly affected by 5-HT_1 or 5-HT_2 antagonists: s(-) propranolol or methysergide, but completely blocked by 5-HT_3 receptor antagonist ICS 205-930 10^{-6}M and also by indomethacin 10^{-5}M. PGE_1, E_2, F_{2a} as well as I_2 (10^{-8} to 10^{-6}M) were able to induce CGRP release from the rat trachea. None of them, however, have been found to potentiate caps-evoked peptide release. Thus, BK-induced CGRP release appears mediated by activation of the B2 receptor and subsequent PG formation. The sensitization effect of 5-HT on the releasing function of primary afferent terminal is apparently mediated by a 5-HT_3 receptor and prostanoid synthesis.

1. Society of Neuroscience Abstract 17: p398, 1991.

(This work is supported by Tobacco Related Disease Program, University of California. X.-Y. H.)

416.12

ANATOMICAL DELIMITATION OF A NEW ANIMAL MODEL OF DEPRESSION USING CALCITONIN. S. Souad and R. de Beaurepaire*, Laboratoire de Pharmacologie, CHRU Côte de Nacre, 14032 Caen, France.

Calcitonin is a peripheral peptide hormone which has several functional, behavioral, and hormonal effects when injected into the rat cerebral ventricles or into specific brain sites: calcitonin decreases food intake, locomotor activity, intestinal motility, produces sleep disorders, increases cortisol secretion, and inhibits sex hormones secretion and TRH-induced TSH secretion. On the whole, these calcitonin effects mimic the symptoms of the human depressive syndrome. Moreover, the anorectic effect of calcitonin can be reversed by tricyclic antidepressants. We therefore proposed the effects of calcitonin in the animal as a new model of depression. Previous experiments have shown that many of the effects of calcitonin listed above are related to an effect on the paraventricular nucleus of the hypothalamus. Calcitonin has also marked analgesic and hyperthermic effects which are not common symptoms in depression. To clarify possible anatomical grounds for our model, we studied the brain sites involved in the analgesic and hyperthermic effects of calcitonin.

We tested the effects of intracerebral injections of salmon calcitonin (15ng in 0.3µl) on pain sensitivity (hot plate test) and rectal temperature. The results show that the sites involved in calcitonin induced analgesia and hyperthermia are the preoptic area, the dorso-medial and posterior nucleus of the hypothalamus, the arquate nucleus, the centro-medial nucleus of the thalamus, and an area in the internal part of the zona incerta. The ventro-medial and the paraventricular nucleus of the hypothalamus are not involved in calcitonin-induced analgesia and hyperthermia.

We conclude that calcitonin-induced analgesia and hyperthermia can be excluded from our model of depression which may fit more specifically with a selective action of calcitonin on the paraventricular nucleus of the hypothalamus.

416.13

DYNORPHIN A (1-17) INDUCED DEPRESSION OF VENTRAL ROOT POTENTIALS IS MEDIATED THROUGH KAPPA OPIATE RECEPTORS. H. Ristic and L. Isaac*. Department of Pharmacology, University of Illinois College of Medicine at Chicago, Chicago, IL 60680.

Intrathecal application of dynorphin A (1-17) (DYN) results in a dose-dependent hindlimb paralysis of 30-60 min duration which may be mediated through kappa opiate receptor interaction. Additionally, repeated injections of DYN at 2 hr intervals results in desensitization to paralysis. Finally, direct application of DYN to the spinal cord results in a dose-dependent depression of the ventral root potentials (VRP) of 30-60 min duration. To investigate this apparent parallelism we determined whether the VRP depression is kappa receptor mediated and whether it is subject to desensitization.

Rats were surgically prepared, under urethane anesthesia, to record VRP monosynaptic and polysynaptic reflexes. The kappa opiate receptor antagonist nor-binaltorphimine (nor-BNI 20nmol) was applied directly to the spinal cord 60 min prior to DYN application and in separate experiments DYN (10nmol) was applied at 2 hr intervals to the spinal cord.

Nor-BNI resulted in a parallel shift of the dose-response curve for DYN-induced depression of the VRP. With nor-BNI the ED_{50} was 8 nmol and with saline it was 4 nmol. Repeated application of DYN resulted in desensitization to depression of the VRP of about 50%.

These data suggest that DYN-induced depression of the VRP is mediated through the kappa opiate receptor. Because both DYN-induced paralysis and depression of the VRP are mediated through kappa receptor interaction they may be causally related. Although the mechanism of these effects remain to be investigated they both involve desensitization. Supported by NSF BNS-8918963 and NIH NS-30295.

416.14

COMPARISON OF BEHAVIORAL EFFECTS OF DYNORPHIN A (1-17) WITH DYNORPHIN A (1-13) AFTER INTRATHECAL INJECTION IN THE RAT. **Z-X. Qu, T. J. Marczynski* and L. Isaac Department of Pharmacology, Univ. of Illinois College of Medicine at Chicago, Chicago, IL 60680**

Previously, we reported that dynorphin A (1-13) administered intrathecally (i.t.) to rats resulted in a reversible hindlimb paralysis and an irreversible inhibition of the tail-flick reflex[1,2]. These effects followed a dose-response relationship with identical ED_{50} values.

In the present investigation, we report the influence of dynorphin A (1-17), the peptide configuration of the endogenous substance, on these same behaviors after i.t. injection in rats. Injection of this substance results in a reversible hindlimb paralysis and an irreversible inhibition of the tail-flick reflex. These effects follow a dose-response relationship with an ED_{50} value for paralysis of 7 nmole and a value for inhibition of the tail-flick reflex of 18 nmole whereas the value for the (1-13) configuration is 10 nmole for both behaviors.

With (1-13), paralysis and inhibition of the tail-flick reflex always occur together; on the other hand with (1-17), some animals paralyze while their tail-flick reflex remains intact suggesting that different mechanisms may be involved with the different peptide configurations.

These data demonstrate a fundamental difference between dynorphin A (1-17) and dynorphin A (1-13). In conclusion, it appears that use of the (1-17) peptide to study the physiologic and pathophysiologic role of dynorphin in spinal cord function is preferred over the use of the (1-13) configuration.

1. Caudle, R.M. and Isaac, L.: Brain Res. 435:1, 1987.
2. Stewart, P. and Isaac, L.: Life Sci. 44:1505, 1989.
Supported by NSF BNS-8918963 and NIH NS-30295

416.15

EVIDENCE FOR A DIRECT ACTION OF MET-ENKEPHALIN ON GABAergic TERMINALS IN ORGANOTYPIC SLICE CULTURES OF THE RAT HIPPOCAMPUS. J.C. Rekling *. Institute of Neurophysiology, University of Copenhagen, DK-2200 N, Denmark.

Intracellular recordings from pyramidal cells in organotypic slice cultures of the hippocampus were performed with 1 M KCl containing electrodes. Spontaneous EPSPs were blocked by 20 µM NBQX, APV and synaptic transmitter release due to sodium-dependent action potentials was blocked by TTX. Under these circumstances a tonic bombardment of miniature IPSPs were seen. The IPSPs were blocked by 20 µM bicuculline and thus were GABAergic.

Application of 10-20 µM met-enkephalin reduced the frequency of spontaneous IPSPs by up to 50 % and this effect was reversed upon wash for 20 min.

These observations point to the conclusion that pyramidal cells in organotypic cultures of the hippocampus are exposed to tonic GABAergic inhibition, which is not the result of propagating sodium-dependent action potentials, and that this tonic inhibition can be reduced by met-enkephalin through a direct action of met-enkephalin on the presynaptic terminals.

416.16

EVIDENCE THAT PITUITARY ADENYLATE CYCLASE ACTIVATOR PEPTIDE (PACAP) IS A PRESYNAPTIC NEUROTRANSMITTER IN THE BOVINE ADRENAL MEDULLA. J.C. Waymire, V. Hemelt, and D. Marshak*. The Department of Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77030.

VIP has been shown to stimulate catecholamine (CA) synthesis in isolated bovine adrenal chromaffin cells. One of the peculiar aspects of this stimulation is the high concentration of peptide (µM) required, as if VIP is mimicking the action of other peptides that act endogenously at lower concentrations. Accordingly, we tested the effect of PACAP, a VIP-like peptide, on the CA synthetic pathway in bovine adrenal chromaffin cells. These studies revealed a potent interaction of either PACAP-27 or PACAP-38. At the short time points, 10 sec to 5 min, CA synthesis is stimulated 4-5 fold with a 1/2 max concentration of 20 nM. Tyrosine hydroxylase (TH), the rate limiting enzyme in CA synthesis, is phosphorylated selectively on serine 40 by both PACAPs. At longer time points, TH mRNA synthesis and levels are elevated, followed by increased TH protein and activity. Morphological studies using perfusion-fixed, intact bovine adrenal medulla showed PACAP-27 immunoreactive axons innervating BAC. Labeled axons and terminals were found throughout the medulla but were particularly dense near the adrenal cortex and around blood vessels. The labeling was unaffected by synthetic 1 µM synthetic VIP but completely abolished by synthetic PACAP-27. These results support the conclusion that PACAP is a presynaptic neurotransmitter in the bovine adrenal medulla and a promising candidate for the endogenous ligand of the "VIP receptor" described previously. Supported by USPHS grants NS11061 & EY06472.

416.17

PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) REGULATION OF AtT-20/D16v CORTICOTROPE CELL FUNCTION. K.M. Braas*, L.M. Konopka[1], C.A. Brandenburg and V. May. Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405, and [1]Department of Biological Psychiatry, Hines VA Medical Center, Hines, IL 60141.

The α-amidated hypothalamic peptides, pituitary adenylate cyclase activating polypeptides (PACAP38 and PACAP27), share considerable amino acid homology with vasoactive intestinal peptide; however, the physiological roles of the PACAP peptides remain unclear. PACAP alters the functions of anterior pituitary gonadotropes, somatotropes and a small population of corticotropes. To investigate the roles of PACAP in corticotrope function, we examined PACAP-mediated regulation of ACTH/endorphin secretion and membrane depolarization using AtT-20/D16v mouse pituitary corticotrope cells. The basal secretory rate for AtT-20 cell ACTH was approximately 3% of cellular hormone content/h. Treatment of AtT-20 cells with 100 nM PACAP38 for 3 to 6 h stimulated hormone release approximately 1.5- to 1.8-fold. PACAP27 elicited a similar stimulated secretory response. AtT-20 cells displayed spontaneous action potentials; occasionally these action potentials led to the initiation of sustained depolarizations (SD) (< 3 sec), followed by hyperpolarizations (< 1 sec). The input resistance significantly decreased during the plateau of the depolarizations. 100 nM PACAP38 increased the rate of SD approximately 2-fold, suggesting a possible correlation with increased hormone release. (Support: NSF grant 9010044 to KMB, HD-27468 to VM and VA RAG 0001 to LMK)

OPIOIDS: ANATOMY AND PHYSIOLOGY I

417.1

THE PENETRATION OF DYNORPHIN1-13 ACROSS THE BLOOD BRAIN BARRIER J.L. Browning*, T.D. Turner, M.A. Widmayer and D.S. Baskin Dept. of Neurosurgery, VAMC Houston and Baylor College of Medicine, Houston, TX 77030.

The opioid peptide dynorphin 1-13 has been shown to increase survival and decrease neurologic deficit and infarct size in a feline model of focal cerebral ischemia (FCI). The ability of ([^{3}H]-pro^{10})-dynorphin1-13 (DYN) to cross the blood brain barrier (BBB) was studied in rats, cats and cats with FCI. The brain uptake index (BUI) as measured by a modified Oldendorf technique showed that the BUI of DYN was not significantly different from sucrose (vascular space reference) in the rat. Peptidase inhibitors had no effect. In contrast, the BUI of DYN in the cat was significantly greater than that of sucrose in hippocampus, cortex and cerebellum. In addition, the BUI of DYN was even greater in cats with FCI, such that the BUI of DYN was significantly greater in hippocampus, cortex, brainstem and hypothalamus. These results suggest that DYN does cross the BBB of cats and may act centrally in ischemia to mediate its neuroprotective action.

417.2

LATERALIZATION OF PROOPIOMELANOCORTIN mRNA CONTENT IN THE RAT MEDIOBASOHYPOTHALAMUS. D.D. Rasmussen*. Dept. of Reprod. Med., Univ. Calif. at San Diego, La Jolla, CA 92093-0802.

We have previously demonstrated that β-endorphin (βEND) content is lateralized in the medial preoptic and caudal arcuate areas of the male rat brain, and that this lateralization is differentially modulated by left *vs* right hemicastration (Neuroendocrinol.Lett. 12:232). We have now initiated a series of studies to further investigate the mechanism of this apparently lateralized control. In this first study we investigated lateralization of proopiomelanocortin (POMC; precursor for βEND biosynthesis) mRNA content in the adult male rat caudal mediobasohypothalamus (MBH). 16 rats were sacrificed at 1400 h and the left and right half-MBHs were removed. Left *vs* right half-MBHs from sets of 2 rats were pooled, and cytoplasmic POMC mRNA contents were quantified by solution hybridization/RNase protection assay. POMC mRNA content was consistently (8/8) greater (20.9±1.2%, $p<0.01$) in the left caudal MBH relative to the right. This difference was similar regardless of whether the results were expressed as pg POMC mRNA, pg POMC mRNA/μg total RNA or fg POMC mRNA/pg cyclophilin (internal reference) mRNA. These results are consistent with our previous demonstration that left caudal MBH βEND content is greater than right, and suggest greater forebrain opiomelanocortinergic activity originating in the left *vs* right MBH. We have previously demonstrated that MBH POMC mRNA content is correlated with MBH tyrosine hydroxylase (TH) mRNA content, suggesting interaction between MBH opiomelanocortinergic and dopaminergic activity (Neuroendocrinology, in press). Consequently we are further investigating whether MBH TH mRNA is also lateralized and whether this lateralization is correlated with that of POMC mRNA.

417.3

TWO DIFFERENT OPIOID CELL GROUPS IN LAMINA X OF RAT LUMBOSACRAL SPINAL CORD. A.P. Nicholas, Z. Xu and T. Hökfelt*. Department of Histology and Neurobiology, Karolinska Institute, Stockholm, 10401, Sweden.

Using a tri-color immunofluoresence technique, the coexistence of enkephalin-like immunoreactivity (ENK-LI) with immunoreactivities for other peptides was examined in neurons of lamina X in the lumbosacral spinal cord of colchicine-treated rats. Briefly, animals were anesthetized, the lumbosacral laminae were removed, the dura mater excised and colchicine soaked gelfoam plugs were placed under the dorsal roots for 1-2 hrs and then removed. After a 48 hr survival time, the animals were perfused with formaldehyde/picric acid and the lumbosaral cords removed, frozen and cut (14 μm) on a cryostat. These sections were incubated overnight in 1:20 mouse anti-ENK, plus either a combination of 1:400 rabbit anti-cholecystokinin (CCK) and 1:200 guinea pig anti-galanin (GAL), or a combination of 1:400 rabbit anti-neurotensin (NT) and 1:400 goat anti-neuropeptide Y (NPY) antisera. Secondary antisera (1:40; Jackson Immuno Research) consisted of a combination of AMCA (blue) conjugated donkey anti-mouse and FITC (green) conjugated donkey anti-rabbit, plus Lissamine Rhodamine (red) conjugated either to goat anti-guinea pig or to donkey anti-goat antibodies. ENK was shown to coexistist with CCK and GAL in almost all large lamina X neurons from L1 to L5, while ENK coexisted with NPY in almost all smaller lamina X neurons from L6 to S2. In this caudal region, a few ENK/NPY/NT cells were also seen, but NT-positive cells were primarily a separate cell group. Thus, the present study showed that in lamina X of the lumbosacral spinal cord, there are at least two major populations of opioid neurons: a rostral group of large ENK cells that are CCK and GAL positive and and caudal group of smaller ENK cells that are primarily NPY (and sometimes NT) positive. Previous studies have shown that CCK/GAL and ENK cells in lamina X of the lumbosacral cord project to higher brain centers and it has been suggested that they are involved with pain transmission.

417.4

ULTRASTRUCTURAL LOCALIZATION OF ENKEPHALIN AND GABA IN RAT SUBFORNICAL ORGAN V.M. Pickel* and J. Chan. Division of Neurobiology, Department of Neurology and Neuroscience, Cornell University Medical Center, New York, N.Y. 10021

We examined the ultrastructural localization of Met^{5}-enkephalin (ME) and GABA in the rat subfornical organ (SFO). ME-like immunoperoxidase labeling was intensely localized to large dense core vesicles (DCVs) in axon terminals. These terminals formed symmetric synapses primarily on unlabeled dendrites and also were seen within perivascular spaces and in the parenchyma adjacent to fenestrated capillaries in the central and caudal portions of the SFO. GABA labeled soma, dendrites and terminals were numerous throughout the SFO. In dually labeled sections, immunoperoxidase labeling for ME was detected in a few terminals forming synapses with cells showing gold silver labeling for GABA, but most of the targets were without detectable immunoreactivity. Other types of associations between ME and GABA included: colocalization within single axon terminals, convergence of separately labeled terminals on common targets, and appositions between separately labeled terminals. Single as well as dually labeled terminals formed symmetric synapses with unlabeled dendrites throughout the SFO. These results suggest that in rat SFO, GABA and opioid peptides may be released from separate, or sometimes the same, axon terminals to inhibit (symmetric junctions) local neurons. Moreover, the release of one or both putative transmitters may be modulated by circulating hormones or axonal associations. The findings suggest that GABA may be involved in the inhibition of renal water, sodium and potassium secretion by opiates at the level of the SFO (Grant support: MH00078, DA04600, HL18974).

417.5

VENTRAL TEGMENTAL AREA NEURONS RECEIVE CONVERGENT GABAERGIC AND ENKEPHALINERGIC INPUT FROM THE SAME AND MORPHOLOGICALLY DISTINCT TERMINALS S.R. Sesack* and V.M. Pickel. Depts. Behavioral Neuroscience, Univ. Pittsburgh, PA 15260 and Neurology & Neuroscience, Cornell Univ. Med. Coll., NY, NY 10021.

We examined the ultrastructural basis for functional interactions between enkephalin (ENK) and γ-aminobutyric acid (GABA) in the rat ventral tegmental area (VTA), using dual immunoperoxidase-gold methods. Immunoreactive terminals were either singly labeled for ENK or GABA, or were dually labeled for both substances. Immunoreactivity for ENK was intensely associated with dense-cored vesicles localized along non-synaptic portions of the plasmalemmal surface, while GABA labeling was primarily associated with small clear vesicles, some of which aggregated at presynaptic specializations. GABA-labeled terminals, with or without ENK-immunoreactivity, formed symmetric synapses on unlabeled dendrites, while singly ENK-labeled terminals formed either symmetric or asymmetric synapses on unlabeled targets. Separately labeled GABA and ENK-immunoreactive terminals frequently converged on common dendrites, or were in direct apposition to one another. These results suggest that GABA and ENK (1) are colocalized in a subset of VTA terminals; (2) are differentially released from distinct vesicle populations, regardless of their co-distribution; and (3) may have opposing actions on common VTA neurons following their release from the same or separate terminals. Our findings suggest multiple sites through which GABA and opioid peptides may interact to modulate the activity of dopaminergic and non-dopaminergic VTA neurons. This work was supported by USPHS grants: NS08193, MH40342, MH00078, DA04600.

417.6

EXTRACELLULAR NUCLEUS ACCUMBENS DOPAMINE IS INCREASED BY MICROINJECTIONS OF SELECTIVE μ OPIOID ANTAGONISTS INTO THE VENTRAL TEGMENTAL AREA. D. P. Devine*, P. Leone, and R. A. Wise. Ctr. for Stud. Behav. Neurobiol., Dept. Psychol., Concordia Univ., Montreal, Canada H3G 1M8.

We have previously reported that ventral tegmental area (VTA) microinjections of the selective μ opioid receptor agonist DAGO produce increases in extracellular nucleus accumbens (NAS) dopamine (DA), DOPAC, and HVA, assayed with intracranial microdialysis and HPLC with electrochemical detection. Interestingly, VTA microinjections of the selective μ-antagonist CTOP also produce increases in NAS DA and metabolites. The dose-dependence of the CTOP-induced increases in NAS DA and metabolites was evaluated, and the μ-receptor selectivity of the response to CTOP (a substituted somatostatin analogue) was assessed by comparison with the response to a structurally dissimilar μ antagonist, ß-funaltrexamine (ß-FNA: a fumarate methyl ester derivative of naltrexone). VTA microinjections of CTOP in the dose range 0.03 - 3.00 nmoles/μl produced dose-dependent increases in NAS DA and metabolites. VTA ß-FNA (1.0 nmoles/μl) produced increases in NAS DA and metabolites which were roughly equivalent to the increases produced by an equimolar microinjection of CTOP. One possibility is that these effects of CTOP and ß-FNA may be mediated through actions at μ receptors located on GABAergic afferents to the VTA. Release of these afferents from opioid-inhibition could result in increased GABA-mediated inhibition of GABAergic interneurons intrinsic to the VTA. This increased inhibition of GABAergic interneurons would then produce a disinhibition of mesolimbic dopaminergic neurons, resulting in the observed increases in NAS DA and metabolite concentrations. Therefore, under physiological conditions, GABAergic afferents to the VTA and GABAergic interneurons within the VTA may interact in a complex manner to modulate mesolimbic dopaminergic activity.

417.7

INTERACTION OF DOPAMINE AND NMDA RECEPTORS IN THE REGULATION OF DYNOPRHIN AND c-FOS/FRA EXPRESSION. D. Bronstein *, H. Ye, W. Q. Zhang, K. Pennypacker, & J.-S. Hong. Lab. Mol. & Integr. Neurosci., Natl. Inst. Environ. Hlth. Sci., Research Triangle Park, NC 27709.

It is well established that, in the striatum, biosynthesis of the opioid peptide dynorphin (Dyn) increases following activation of D1 receptors and decreases following D1 receptor blockade. In the present experiments, we were interested in examining some of the molecular mechanisms which may mediate, or interact with, the dopaminergic regulation of Dyn biosynthesis in the striatum. Repeated treatment (twice daily for 7 days) with the dopaminergic agonist, apomorphine (APO; 5 mg/kg), caused a small but significant increase in Dyn-ir in the striatum; this increase was accompanied by increases in the immediate early gene products, c-fos and/or. fra. Pretreatment of animals with a low dose (.125 mg/kg) of MK-801, a non-competetive antagonist of NMDA receptors, blocked the increases in both Dyn and fos/fra immunoreactivity. In a subsequent experiment, animals received unilateral 6-hydroxydopamine lesions of the substantia nigra (destroying the dopaminergic innervation of Dyn-containing neurons in the striatum) and then were injected repeatedly with APO alone or together with MK-801. APO treatment alone caused a 4-fold increase in Dyn-ir levels as well as an intense stimulation of fos/fra peptide expression in the lesioned striatum. However, in contrast to its effects in intact animals, MK-801 did not appear to have any effect on the APO-induced increase in fos/fra expression in 6-OHDA lesioned rats although it did reverse the increase in Dyn. These data demonstrate that changes in fos/fra expression are sometimes dissociated from Dyn and suggest that other factors in addition to fos/fra may be involved in the nigrostriatal regulation of Dyn expression.

417.8

REGULATION OF C-FOS AND 28 K CALBINDIN mRNA IN RAT CEREBELLUM IN RESPONSE TO ACUTE AND CHRONIC MORPHINE. P.S. Tirumalai and R.D. Howells*. Department of Biochemistry and Molecular Biology, UMDNJ-New Jersey Medical School, Newark, NJ 07103

The cellular and biochemical adaptations which underlie the addictive state are poorly understood. Since it is likely that changes in gene expression accompany the development of addiction, we are examining changes in the expression of candidate genes. In this study, the effect of acute and chronic morphine administration on expression of the proto-oncogene transcription factor, c-*fos* and the 28 kDa calcium-binding protein, calbindin, was examined in rat cerebellum and rat brain (minus the cerebellum). Adult male Sprague-Dawley rats were injected with saline or with escalating doses of morphine sulfate twice daily for 15 days. Rats were sacrificed 45 min after the last injection, the cerebellum and remaining brain minus the cerebellum were removed, and total RNA was extracted using the acid guanidinium thiocyanate/phenol chloroform method. RNA levels were quantified by Northern blot analysis. In addition, other rats received a single injection of morphine (10 mg/kg) and were sacrificed after 45 min, 4 hr, or 24 hr later. The effect of naloxone-precipitated withdrawal on gene expression in morphine-addicted rats was also analyzed 45 min after naloxone (1 mg/kg ip). Levels of c-fos mRNA were increased 2.5-fold in cerebellum and brain 45 min after a single dose of morphine compared to saline-injected controls. Calbindin mRNA levels in cerebellum were decreased to 30%-40% control at 45 min and 4 h after a single morphine injection. Tolerance developed to these effects in that levels of c-fos and calbindin were not altered 45 min after morphine injection in morphine-addicted rats. Unlike the cerebellum, calbindin mRNA was increased 3-fold compared to controls 45 min after morphine injection in chronically injected animals in the remainder of the brain. Naloxone-precipitated withdrawal caused a 30% decrease in c-fos levels in the brain minus the cerebellum. Differential effects on c-fos and calbindin gene expression following either acute or chronic morphine administration may be important aspects of the adaptation of the nervous system to morphine. Supported by DA 05819.

417.9

C-FOS INDUCTION AND PROENKEPHALIN (Penk) GENE EXPRESSION CAN BE DISSOCIATED IN RAT ADRENAL (RA). Y.S. Zhu*, S.O. Franklin, M. Brodsky, T. Huang and C.E. Inturrisi. Dept. of Pharmacology, Cornell Univ. Med. College, New York, NY

Increases or decreases in transsynaptic activity in the RA activate c-fos and Penk gene expression in a sequential manner. We investigated whether c-fos induction and Penk gene expression can be dissociated in RA. Increases in transsynaptic activity by a neurogenic stressor [a metrazole (MTZ)-convulsion] produced a sequential elevation of c-fos and preproenkephalin (PPenk) mRNAs in RA. In hypophysectomized RA, the MTZ-induced increase in PPenk mRNA, but not c-fos mRNA was significantly reduced. In normal RA, ACTH treatment blocks the MTZ-induction of c-fos mRNA and Fos protein without a significant effect on the MTZ-induction of PPenk mRNA. Removal of transsynaptic activity by adrenal medullary explantation also produced a sequential induction of c-fos and PPenk mRNAs. The induction by explantation of PPenk mRNA, but not c-fos mRNA was blocked by cycloheximide (2 mg/ml). Forskolin (25 uM) had no effect on the explant-induced increase in c-fos mRNA at 1 hr, but reduced the explant-induced increase in PPenk mRNA at 24 hrs. These results demonstrate that although the Penk gene is an apparent target of Fos, c-fos induction does not always result in Penk gene expression and Penk gene expression can occur in the absence of c-fos induction. (Supported by DA01457 and DA07274.)

417.10

MU-OPIOID ENHANCEMENT OF NMDA AND AMPA RESPONSES IN HORIZONTAL VERSUS CORONAL SECTIONS OF RAT LOCUS COERULEUS. S. Oleskevich* and J.T. Williams. The Vollum Institute, Oregon Health Sciences University, Portland, Oregon, 97201.

The μ-opioid receptor agonist DAGO was recently reported to selectively enhance the NMDA component of the glutamate response in spinal trigeminal neurons via activation of protein kinase C (Huang, 1991). We have investigated this modulation by DAGO in locus coeruleus (LC) neurons where μ-opioids elicit inhibitory effects through *augmentation of a K+ conductance. Intracellular voltage clamp* recordings were performed on LC neurons (holding potential -60 mV) in horizontal or coronal brainstem slices. All drugs were applied by superfusion. Following pretreatment with idazoxan (1 μM) and picrotoxin (100 μM), the response to NMDA (30 μM) was increased by DAGO (1 μM) by a factor of 1.9 (-167 ± 12 versus -307 ± 30 pA). This enhancement was observed in horizontal sections of the LC (n=10) but not in coronal sections (n=6; factor of 1.1) nor in horizontal sections treated with 2 mM barium (n=3; factor of 1.1). The specificity of the DAGO-mediated enhancement was tested following application of AMPA (100-300 nM). The AMPA response (125 ± 39 pA) was potentiated by a factor of 2.3 by DAGO (275 ± 68 pA) in horizontal sections (n=4) but not in coronal sections of the LC (n=4; factor of 1.6). Current-voltage relationships from horizontal sections indicate a convergeance of the AMPA and AMPA/DAGO regression lines. A lack of voltage clamp control of the dendritic arborization may explain the enhanced responses to NMDA and AMPA following DAGO application. (Supported by FRSQ and NIH DA04523).

417.11

ACTIVATION OF LOCUS COERULEUS DURING CLONIDINE WITHDRAWAL: AN *IN VIVO* VOLTAMMETRIC STUDY. M. Hong*, S. Duggan, B. Milne and K. Jhamandas. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario K7L 3N6

In the locus coeruleus (LC), a major noradrenergic brain center, both α_2-adrenoceptor and opioid agonists inhibit neuronal firing. While it is known that the increase in LC firing during morphine withdrawal is suppressed by α_2-agents such as clonidine, it is not known whether increases in LC activity occur during clonidine withdrawal. To investigate this possibility, LC activity in the rat was measured using differential normal pulse voltammetry (DNPV). Clonidine (10 μg i.c.v.) significantly reduced LC activity to 54.4 ± 3.1% of baseline 45 min following injection. A subsequent injection of yohimbine (0.5 mg/kg i.v.) or the more selective α_2-antagonist atipamezole (0.2 mg/kg i.v.) resulted in a reversal of the depressant effects of clonidine and a significant rebound increase in LC activity: peak increases of 135.0 ± 1.7% above baseline 45 min following yohimbine and 131.6 ± 1.8% above baseline 45 min following atipamezole. Yohimbine, but not atipamezole, produced a significant increase in LC activity in saline treated animals, peak increase 136.6 ± 6.8% above baseline 45 min following yohimbine. These data suggest that activation of LC occurs during acute clonidine withdrawal.

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417.12

A SODIUM-DEPENDENT COMPONENT OF THE OPIOID-INDUCED OUTWARD CURRENT IN LOCUS COERULEUS (LC) NEURONS

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Opioids hyperpolarize LC neurons by opening K^+ channels. However, the opioid-induced current often does not reverse at K^+ reversal potentials in these neurons. We provide evidence to suggest that this is in part due to opioids turning off an additional current - a resting Na^+-dependent conductance.

Bath-applied met-enkephalin (200 μM) produced an outward current at -60 mV in rat LC neurons in brain slices. An 80% substitution of Na^+ in the ACSF by choline/atropine or TRIS produced a persistent outward current (choline - 144.3 ± 18.5 pA, TRIS - 136 ± 25.6 pA, n=12) with a decrease in conductance, indicating the presence of a resting Na^+-dependent current in LC neurons. Both treatments produced ~ 50% decrease in the opioid-induced-outward current and altered its reversal potential, bringing it closer to the K^+ reversal potential. $BaCl_2$ (100 μM), a K^+ channel blocker, also decreased the amplitude of the agonist-induced outward current by about 50% and together with choline or TRIS, it almost completely abolished the outward current, suggesting additivity of the Ba^{2+}- and Na^+-sensitive components of the opioid response.

We conclude that opioids turn off a resting Na^+-dependent conductance in LC neurons, in addition to opening K^+ channels. Since opiates inhibit adenylate cyclase activity in LC neurons, a possible candidate for the Na^+-dependent component of the opioid response is the resting cAMP-induced-Na^+-dependent inward current which drives pacemaker activity in these cells and reverses at ~ -30 mV.

417.13

CHRONIC MORPHINE INDUCES A PERSISTENT INCREASE IN RAT STRIATAL CALBINDIN D28K IMMUNOREACTIVITY VIA AN NMDA RECEPTOR-DEPENDENT MECHANISM. M.M. Garcia* and R.E. Harlan, Dept. of Anatomy, Tulane Univ. Medical School, New Orleans, LA 70112.

Calbindin D_{28k} (CD) is an intracellular calcium-binding protein which acts as a Ca^{++} buffer, thus protecting cells from the damaging effects of high intracellular Ca^{++}. As chronic morphine (M) has been reported to cause increased Ca^{++} levels in striatal synaptosomes, we studied its effect on CD immunoreactivity (ir) in rat brain, using immunocytochemistry. In the initial series of experiments, male rats were made M tolerant and dependent by sc implantation of 75 mg M pellets (1/day for 5 days); controls were implanted with vehicle pellets. Using a monoclonal antibody to CD, we found CDir in control brains in the matrix compartment of striatum but not in patches. In the brains of M-treated rats, there was a dramatic increase in CDir in matrix, with the appearance of intense CDir in patches. When M treatment was discontinued, the increased CDir in patches persisted through day 14 post-M. Because striatal patches receive glutamatergic (GLU) input from cortex, we studied the interactions between M and GLU in a second series of experiments using the NMDA antagonist, MK-801. Rats were injected twice daily for 5 days with saline(S)/S, S/M (10 mg/kg), MK (2 mg/kg)/S or MK/M. Levels and patterns of CDir in brains of S/Sand MK/S rats were as found in controls from the previous study, while the S/M CDir resembled the pattern seen in the M-treated brains. CDir in the MK/M brains, however, resembled control patterns, with CDir absent from patches. We suggest that chronic morphine increases glutamatergic transmission in striatum, increasing Ca^{++} and CD levels. These findings are consistent with reports that MK-801 inhibits tolerance to morphine, and provide a possible mechanism for this inhibition. (DA-05411 [MMG] and DA-06194 [REH])

417.14

MORPHINE REGULATES EXPRESSION OF JUN FAMILY MEMBERS IN RAT BRAIN. R.E. Harlan, O. Prakash* and M.M. Garcia. Department of Anatomy, Tulane Medical School (REH, MMG) and Division of Research, Ochsner Medical Foundation (OP), New Orleans, LA 70112.

Members of the Jun family of immediate-early genes dimerize with c-fos or fos-related antigens, to form a protein complex which binds to the AP-1 promoter sequence, activating or inhibiting expression of a variety of genes. We have used immunocytochemistry with antibodies to c-Jun (Oncogene Science AB-1 and AB-2) and to Jun-B and Jun-D (gift of R. Bravo) to study the cellular distribution of these proteins, and their regulation by morphine. In untreated rat brains, the four antibodies reveal four distinct distributions, suggesting that they may recognize four Jun family members. The distributions of cells recognized by the c-Jun AB-1, Jun-B and Jun-D antibodies are very similar to recent reports on distributions of cells expressing the c-Jun, Jun-B and Jun-D genes. Since the distribution revealed by the c-Jun AB-2 antibody is strikingly different, with immunostaining of neurons only in the nucleus accumbens, striatum, olfactory tubercle and central nucleus of the amygdala, this antibody may recognize a Jun-related antigen. In the striatum, the AB-2 antibody recognizes neurons in the matrix, leaving patches unstained. Chronic morphine treatment (one 75 mg morphine pellet, sc, daily for 5 days) greatly increased the number of neurons stained by the AB-2 antibody, especially in the patch compartment. Acute (3 hours after 10 mg/kg) or chronic treatment with morphine greatly increased the number of neurons stained with the Jun-B antibody in the nucleus accumbens, striatum and frontal cortex. These results extend previous work demonstrating morphine-regulated induction of immediate-early gene expression (c-fos) in the striatum and further suggest that the activated mu opiate receptor may be coupled to stimulatory as well as inhibitory cellular events. Supported by DA-06194, NS-24148 (REH) and DA-05411 (MMG).

417.15

HYPERTHERMIA INDUCED BY INTRAPREOPTIC MICRODIALYSIS OF A SELECTIVE μ OPIOID RECEPTOR AGONIST IN RAT. L. Xin*, E.B. Geller and M.W. Adler. Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140.

Previous studies from this and other laboratories have shown that the hyperthermic responses of the rat to opioids are mediated by μ receptors. To investigate the role of the preoptic area (POA) in the hyperthermia, the present study used the selective μ receptor agonist PL-017 (Try-Pro-N-MePhe-D-Pro-NH_2), the μ receptor antagonist CTAP (cyclic D-Phe-Cys-Tyr-Arg-Thr-Pen-Thr-NH_2), the κ receptor antagonist nor-binaltorphimine (nor-BNI) and naloxone (Nal, general opioid antagonist). The drugs were delivered directly into the POA of freely moving male S-D rats through a microdialysis probe, and rectal temperature (Tr) was measured at 21°C ambient and 50% relative humidity. All drugs were dissolved in the pyrogen-free saline (PFS) which had no effect on Tr alone. The efficiency of the probe during perfusion in vitro was 12% (1st h), 23% (2nd h) and 34% (3rd h). Three hours of dialysis of PL-017 (0.2, 1 and 2 μg/μl) at a speed of 1 μl/min induced a dose-related hyperthermia (ΔT± SEM: 0.74±0.21, 1.75±0.26, 1.94±0.35 °C, respectively). One hour of dialysis of PL-017 (1 μg/μl) produced a rise of 1.44 ±0.36 °C in Tr. PFS, Nal (1 μg/μl) and CTAP (1 μg/μl) were each microdialyzed into POA after 1 hour perfusion of PL-017. Nal and CTAP shortened the recovery time course from 180 min to 80 and 60 min, respectively, compared with the 160 min time course seen with the control PFS. In addition, one hour microdialysis of Nal or CTAP before PL-017 prevented the hyperthermia, but nor-BNI did not. The POA microdialysis route produced similar results to those seen after i.c.v. or s.c. administration of opioid, but at lower doses. These data not only support the hypothesis that μ receptors mediate hyperthermia in the rat, but indicate that the POA may be the primary site for opioid-induced temperature responses. (Supported by grant DA 00376 from NIDA)

418.1

ELECTROPHYSIOLOGIC EVIDENCE THAT VENTRAL PALLIDAL (VP) DOPAMINE MODULATES VP RESPONSES TO AMYGDALA STIMULATION. R.J. Maslowski-Cobuzzi* and T.C. Napier. Neurosci. Prog. and Dept. of Pharmacol., Loyola Univ. Chicago, Stritch Sch. of Med., Maywood, IL 60153.

The VP is a dopaminoceptive brain region that also receives amygdala (AMN) efferents. Receptor subtypes involved in dopamine (DA)-mediated VP responses and the effects of DA on AMN inputs to VP are unknown. Thus, the present study determined if VP responses to AMN stimulation could be modulated at the level of the VP by exogenous DA (microlontophoretic application), and/or endogenous DA (stimulation of midbrain dopaminergic regions; mDAr).

Single, spontaneously active VP neurons, recorded *in vivo* from male Sprague-Dawley rats anesthetized with chloral hydrate, were assessed for convergent responses to AMN and mDAr single pulse stimulation. Most VP neurons tested responded to both. Seventy percent of the short latency inhibitory VP responses to activation of the mDAr was attenuated by microiontophoretically applied SCH23390 (D1 DA antagonist), but only 45% were effected by the D2 DA antagonist, sulpiride. Similarly, the D1 DA agonist, SKF38393 suppressed VP activity more often than the D2 agonist, quinpirole (38% vs. 12.5%), and with a greater magnitude (61±7% vs. 30±5%).

AMN-evoked short and long latency inhibitory effects of VP neurons were attenuated by DA within the VP (64% and 80%, respectively), and by prior mDAr stimulation (10 pulse train; 85% and 78%). The results suggest a monosynaptic inhibitory influence on VP neurons by mDAr that often is mediated through D1 DA receptors. DA within the VP and mDAr activation produce a similar modulatory influence on AMN efferents to the VP. Thus, the VP may be an integrative site of limbic (AMN) and motor (mDAr) systems. Work supported by MH45180.

418.2

CORTICAL CHOLINE ACETYLTRANSFERASE (ChAT) ACTIVITY IS AFFECTED BY CHRONIC DOPAMINE ANTAGONISM. M.B. Muench*, I. Hanin, G. Battaglia, and T.C. Napier. Dept. Pharmacol. Loyola University Chicago, Maywood, IL 60153.

Pharmacologic and electrophysiologic evidence suggests that the dense population of cortically-projecting cholinergic cells in the ventral pallidum/substantia innominata (VP/SI) is dopaminoceptive. The present study demonstrates the sensitivity of rat basal forebrain cholinergic projection neurons to chronic dopamine (DA) receptor antagonism.

Rats received either SCH23390 (0.25 mg/kg s.c.), sulpiride (12.5 mg/kg s.c.), or vehicle (s.c.) for 21 days. On day 23, the rats were killed and their brains dissected and frozen. D1 and D2 DA receptors in rat basal forebrain tissue homogenates respectively were labeled with [3H]SCH23390 and [3H]spiperone. ChAT activity was measured in basal forebrain terminal regions.

ChAT activity significantly increased in frontal cortex (fCTX) and amygdala (AMYG) following chronic SCH23390 treatment as compared to vehicle controls. No changes occurred in hippocampus (HIPP) or anterior cingulate (AC). In contrast, after chronic sulpiride, ChAT activity significantly decreased in HIPP and significantly increased in AC. ChAT activity in fCTX and AMYG remained unaltered.

Radioligand binding data demonstrated selective upregulation of striatal D1, but not D2, receptors after chronic SCH23390. No changes in D1 sites were observed in septum, globus pallidus or VP/SI homogenates. No significant changes in [3H]spiperone-labeled D2 receptors occurred in any of these tissues following sulpiride; however, this might be a result of the low sulpiride dose used during this experiment.

These results suggest that basal forebrain cholinergic neurons are sensitive to DA and that they are modulated differently by D1 and D2 receptors. (Work supported by MH45180 to T.C.N.)

418.3

MOTORIC ANALYSIS OF DOPAMINE RECEPTOR SUBTYPE ACTIVATION WITHIN THE VENTRAL PALLIDUM AND DORSAL GLOBUS PALLIDUS. T.C.Napier* and F.Rehman. Department of Pharmacology, Loyola University Chicago, Stritch School of Medicine, Maywood, Il., 60153.

Dopamine (DA) projections ascending to forebrain striatal regions are known to mediate a complex repertoire of motor-related behaviors. Recent work demonstrates that pallidal regions also are dopaminoceptive, responding to both D1 and D2 DA receptor activation. Thus, the effect of this input on DA-mediated motor functions, and whether the contribution of the dorsal globus pallidus (dGP) differs from that of the infracommissural ventral pallidum (VP), were examined.

Under pentobarbital anesthesia, male Sprague Dawley rats were implanted bilaterally with guide cannulae to allow ic microinjection of treatments into the dGP and VP. Experimentation began 1 week after surgery, and various motor effects were quantified for 1 hr following ic treatments. Intrapallidal DA (.001 to 100 μg/0.5μl ic) produced a dose-related increase in locomotion and rearing/wall climbing. Quinpirole (QUIN, D2 agonist) elevated these behaviors only when injected into the dGP. In the VP, such effects were mimicked by SKF82958 (SKF, a full D1 agonist). SKF also produced robust "mouthing movements" that remained intact in rats with acute depletions of endogenous DA (in contrast to the other behaviors). Thus, dGP and VP regions contribute a distinct profile to dopaminergic modulation of motoric function. Since the magnitude of the locomotor and rearing/wall climbing measures were less than that observed in these animals with 1mg/kg ip amphetamine, pallidal structures serve as constituents of an engaged system for these behaviors. Whereas, mouthing movements resulting from VP D1 receptor activation, without the involvement of endogenous DA (and thus, D2 receptors) induced a response that was greater than any other treatment assessed, suggesting that VP D1 receptors play a critical role in this behavior. Supported by MH45180.

418.4

DO INHIBITORY G-PROTEIN COUPLED RECEPTORS ACTIVATE AN ATP-SENSITIVE POTASSIUM CHANNEL? G.A. Hicks, A.W. Twigg and G. Henderson. (SPON: Brain Research Association) Department of Pharmacology, University of Bristol, Bristol, BS8 1TD, U.K.

The D_2-dopamine receptor is a member of the inhibitory G-protein coupled superfamily of receptors which includes the μ-opioid receptor and the α_2-adrenoceptor. Roeper et al (1990, Pflugers Archiv 416: 473-475) have suggested that activation of D_2 receptors resulted in the opening of ATP-K channels. Using whole cell recording from substantia nigra zona compacta (SNc) neurones in a brain slice we have been unable to demonstrate that the membrane hyperpolarisation produced by dopamine is reversed by the ATP-K channel antagonist glibenclamide (Hicks and Henderson, 1992, Neurosci. Letts. in press). To exclude the possibility that we had washed out an intracellular component necessary for the inhibitory action of glibenclamide we have performed cell attached and conventional extracellular recordings of the firing rates of SNc neurones. Dopamine (30-100μM) inhibited neuronal firing but this effect was not antagonised by glibenclamide (10μM). The electrically evoked neurogenic contractions of the isolated guinea-pig ileum (GPI) and rat vas deferens (RVD) were inhibited by the ATP-K channel activator, lemakalim (0.03-3μM), in a concentration-dependent manner. Glibenclamide reversed the action of lemakalim. The site of action of lemakalim appears to be on the presynaptic nerves because directly evoked contractions of the smooth muscle were unaffected. The inhibition of neurogenic contractions produced by the μ-opioid receptor agonist morphine (10μM; GPI) and the α_2-adrenoceptor agonist clonidine (3nM; RVD) were not reversed by glibenclamide (1-6μM) but were antagonised by naloxone (1μM) and yohimbine (100nM) respectively. We have thus been unable to demonstrate the inhibitory G-protein coupled receptors activate an ATP-K channel on either neuronal somata or terminals.

418.5

EXCITATION OF RAT CAUDATE NEURONS BY INDIRECT AND DIRECT ACTING DOPAMINE AGONISTS. D.K.Hyslop,* W.E.Hoffmann and M.F.Piercey, The Upjohn Co., Kalamazoo, MI 49001.

The effects of dopaminergic (DA) drugs were evaluated for effects on neuronal firing rates of DA neurons in the substantia nigra pars compacta (SNPC) and the caudate nucleus (Cd), the major projection area for SNPC DA neurons, in chloral hydrate anesthetized rats. Using dye-filled glass microelectrodes, DA neurons in substantia nigra pars compacta (SNPC, Bunney et al., JPET 155:560, 1973) and caudate neurons were identified by classical electrophysiological/neuroanatomical criteria. Cells were located in central caudate and were selected for spontaneous activity and large positive-negative wave forms (Type II, Life Sci 25:419, 1979). Intravenous injections of the indirect DA agonist, amphetamine (AMPH, 1-3 mg/kg), excited spontaneously active Cd neurons and inhibited SNPC neurons by a HAL-sensitive mechanism. Similarly, quinpirole (QUIN, 0.3-3 mg/kg), a D2 agonist, and APO (3 mg/kg), a D1/D2 agonist, excited spontaneously active Cd neurons by HAL-sensitive mechanisms. However, much lower doses of APO and QUIN were effective in inhibiting SNPC neurons. SKF38393, a D1 agonist, did not alter Cd firing rates or responses to QUIN. The data is consistent with the view that, while AMPH inhibits SNPC neurons by striatonigral feedback pathways, QUIN and APO inhibit SNPC neurons by direct autoreceptor activation.

418.6

ELECTROPHYSIOLOGICAL STUDIES OF (-)-STEPHOLIDINE ON SNC AND VTA DA NEURONS.

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(-)-Stepholidine (SPD) is a novel DA receptor antagonist, but it possesses agonistic action on rotational behavior in the 6-OHDA-lesioned rats. It is presumed that D_1 receptors in the SNR are involved in this agonistic action. The present work attempts to elucidate whether SPD exhibits the antagonistic or agonistic action on extracellular firing recorded from SN and VTA DA neurons in the anesthesized, the reserpinized, and the 6-OHDA-lesioned rats.

It has been shown that SPD had antagonistic effect only to D_2 receptors in the SNC and VTA of anesthetized rats. However SPD was an antagonist both to D_1 and D_2 receptors in the reserpinized rats. Interestingly, SPD had the characteristic of depolarization inactivation in VTA, not in SNC, of the anesthetized rats. This action is the characteristic of atypical neuroleptic. Thus SPD would exist the potential possibility of atypical neuroleptic. Furthermore, when firing was recorded from the SNR in the 6-OHDA-lesioned rats, SPD showed the D_1 agonistic action. This result would be compatible with the rotational behavior.

418.7

DIFFERENTIAL INTERACTION OF DOPAMINERGIC D-1 AND D-2 RECEPTORS WITH GLUTAMATERGIC AND CHOLINERGIC TRANSMISSION IN THE 6-HYDROXYDOPAMINE MODEL OF PARKINSON.

M.Morelli *, S.Fenu, A.Cozzolino, A.Pinna, and G.Di Chiara. Dpt. of Toxicology, University of Cagliari, Cagliari, Italy.

The interaction of dopaminergic D-1 and D-2 agonists with antagonists of N-methyl-D-aspartate (NMDA) or muscarinic receptors, was examined after a unilateral 6-hydroxydopamine lesion of the dopaminergic nigro-striatal system. Blockade of NMDA receptors by MK-801 increased the contralateral rotational behavior induced by the D-1 agonist SKF 38393 and inhibited the rotation induced by the D-2 agonist LY 171555. Blockade of muscarinic receptors by scopolamine, like MK-801, potentiated D-1 mediated contralateral rotation but did not influence D-2 mediated rotation. D-1 dependent activation of the proto-oncogene c-fos in the lesioned caudate-putamen (CPu) was increased after both treatments. 2DG utilization studies evidenced an increased metabolic activity in the entopeduncular nucleus and substantia nigra reticulata after these treatments, indicating that the behavior observed is associated with specific biochemical modifications in the CPu, reflected by functional modifications in striatal afferent areas. The results suggest that administration of NMDA or muscarinic receptor antagonists might improve the therapeutic efficacy of D-1 agonists in the treatment of Parkinson's disease.

418.8

ALTERATIONS IN G PROTEIN LEVELS IN 6-OH DOPAMINE LESIONED RATS. E.R. Marcotte, S.K. Gupta, R.K. Mishra*, R. Sullivan, and H. Szechtman. Depts. of Psychiatry and Biomedical Sciences, McMaster University, Hamilton, Ont., Canada, L8N 3Z5.

6-OH dopamine (6-OHDA) is known to selectively destroy nigrostriatal pathways in the brain. In this study, we have investigated the effects of 6-OHDA lesions on G protein levels in the rat striatum. Male Sprague Dawley rats were given unilateral lesions of 6-OHDA and striata were removed from both hemispheres 1, 4, 8, or 16 days post-lesion. Quantitative immunoblotting of western blots, using specific antisera, was used to assess relative levels of $G_S\alpha$, $G_i\alpha$, and $G_o\alpha$ subunits. Both $G_S\alpha$ and $G_i\alpha$ levels were found to be depressed in striata ipsilateral to the lesion relative to non-lesioned striata, and this effect was most pronounced by day 16. Although both $G_S\alpha$ and $G_i\alpha$ levels showed similar patterns of alteration, $G_S\alpha$ levels consistently showed the greatest difference for each day post-lesion, suggesting that alterations in $G_i\alpha$ levels may follow changes in $G_S\alpha$ activity. $G_o\alpha$ levels showed no significant alterations in lesioned relative to unlesioned striata at any day post-lesion. Given that the molecular basis of many human neurodegenerative diseases are not at present well established, we have begun attempts to relate these results to healthy individuals and to the alterations in human neurodegenerative conditions. This work was supported by grants from the Parkinson's Foundation of Canada and NIH.

418.9

QUINPIROLE, BUT NOT SKF-38393 INHIBITS STRIATAL NEURONS IN FREELY-MOVING RATS. K.C. HOOPER*, I. M. WHITE, M.E. TAYLOR AND G.V. REBEC. Prog. Neural Science, Dept. Psychology, Indiana University, Bloomington, IN 47405.

Indirect dopamine agonists, such as amphetamine, typically increase motor-related, but suppress nonmotor-related neurons in rat striatum (Haracz et al., Brain Res. 489:365, 1989). In an initial attempt to assess the mechanisms by which dopamine regulates striatal activity, we monitored the effects of quinpirole (LY-171555), a dopamine agonist that has a high affinity for D2 and, especially, D3 receptors, and SKF-38393, a D1 agonist, on single-unit activity in the striatum of awake, behaving, male rats.

Like amphetamine, quinpirole (1.0-5.0 mg/kg sc) alone, or administered 30 minutes after the D1 agonist SKF-38393, inhibited nonmotor-related neurons in the striatum. Unlike amphetamine, quinpirole also inhibited motor-related neurons located in the lateral and central striatum. In medial striatum, however, neurons were particularly sensitive to the animal's state of arousal. After quinpirole, these cells showed complex changes in firing rate that may, in part, reflect behavioral activation induced by the drug. In contrast, SKF-38393 (5mg/kg sc) alone had no effect on the firing rate of striatal neurons. Nor did its effects differ from control when administered 30 minutes after quinpirole. Collectively, these results suggest an important role for D2 and/or D3 receptors in regulation of striatal activity.

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418.10

SOME STUDIES ON DA D1 & D2 ANTAGONIST-INDUCED CATALEPSY IN RATS. D.M. Jackson[1], J.A. Watson*[2], A. Bengtsson[1] and M Lindgren[1] Astra Arcus AB[1] and Astra Pain Control AB[2], Södertälje 151 54, Sweden.

Catalepsy (C) is produced in rats by either DA depletion or by blocking DA D1 & D2 receptors with antagonists. While C is a useful & predictive model, the measure itself is sensitive to environmental manipulations. In this rat study we investigated the method of testing on SCH23390 (SCH) & raclopride (R)-induced C. C was determined on a steel grid at an angle of 60 +/- 2° & the time each rat spent before moving 1 of its 4 paws determined. 3 experiments are described. 1. 2 methods were used to measure C after antagonist injection. First, the C time for each rat was determined 0.5, 1, 2, 4, 8 & 24 h after injection. Secondly, the C time was measured only once on each rat either 0.5, 1, 2, 4, 8 or 24 h after injection. With the first technique, both SCH & R produced dose dependent C that peaked 2 to 8 h after R & 0.5 to 1 h after SCH, peak time depending on dose. With the second technique, SCH induced C that was similar to that seen after repeated testing. In contrast, R produced weak C when each rat was tested once. 2. Rats were injected with R (60 µmoles/kg). Half were injected 10 min later with saline & the remaining remained untreated. All rats were then tested repeatedly beginning 0.5 h after saline injection for 24 h. The time course curve for C was altered by the saline injection. 3. Rats were given R (60 µmoles/kg) & half handled each 5 min, & half not handled, before determining C 0.5 h through 24 h after injection. Less C was evident in the handled group. It is clear that D1 & D2 induced C depends upon markedly different mechanisms, as proposed earlier. Some form of conditioning, which is extremely sensitive to environmental influences, seems to play a major role in D2,- but not D1, -induced C.

418.11

REM SLEEP DEPRIVATION AND DOPAMINE RECEPTOR BINDING IN RAT STRIATUM. A. Hamdi, J.Brock* K. Ross, S Payne, and C. Prasad. Pennington Biomed.Res.Ctr., Baton Rouge, LA, 70808; Dept. Medicine, LSUMC, New Orleans, LA, 70112.

Rats deprived of sleep express facilitation of dopamine-mediated behaviors, but the mechanisms involved are largely unknown. Male rats were divided into 4 groups (N=6 each). Group REMd was deprived of REM sleep for 96 hours using a water-tank procedure; group L/S resided on larger (control) pedestals for 72 hrs, then on small pedestals for 24 hrs; group TC resided on large pedestals for 96 hrs; and group CC remained in the home cage. The striata were analyzed for dopamine D1 and D2 receptor density and affinity using [^{3}H]SCH23390 and [^{3}H]YM-09151-2 as ligands, respectively. Group REMd showed increases in Bmax for D1 and D2, and increased Kd for D2, compared to group TC. Group L/S had increases in Bmax and Kd for D1 and D2, compared to group TC. Group TC showed decreased Bmax for D1 and D2 and decreased Kd for D2, compared to group CC. Thus, stress caused downregulation of both receptor subtypes, whereas REMd had an opposite effect. These data suggest that differences in D1 and D2 receptor binding may be used to distinguish the effects of stress from the specific effects of REMd.
(Supported by Dept. of Army)

418.12

SYNERGISTIC AND ANTAGONIST INTERACTIONS OF DOPAMINE D1 AND D2 AGONISTS: A 2-DEOXYGLUCOSE AUTORADIOGRAPHY STUDY. C.A.Ray, M.Stults, W.E.Hoffmann* and M.F.Piercey, The Upjohn Co., Kalamazoo, MI 49001 and Kalamazoo College, Kalamazoo, MI 49007.

D1 and D2 receptor subtypes are distributed unevenly and independently within the brain. D1 and D2 agonists act synergistically to stimulate behavior (Braun and Chase, Eur. J. Pharmacol. 131:301) and neuronal firing (Carlson et al., Brain Res. 400:205, but see Hyslop et al., this meeting). We have used the 2-deoxyglucose (2DG) autoradiography procedure (Sokoloff et al., J. Neurochem. 28:897) to evaluate the effects of the D1 agonist, SKF38393 (SKF, 20 mg/kg i.v.) and the D2 agonist, quinpirole (QUIN, 1 mg/kg i.v.) in lightly restrained unanesthetized rats. Both drugs stimulated SNPC and SNPR. SKF, but not QUIN, stimulated caudate, VTA, n. basalis, sup col., d horn, and olf., pyriform, and temp. cortex. QUIN, but not SKF, stimulated globus and inhibited AV thalamus. QUIN and SKF synergistically excited SNPC, globus, subthal., cent. amygdala, d and v horn. QUIN inhibited SKF stimulation in sup col., olf. and temp cortex. There were no interactions at other sites. It is concluded that D1, D2 interactions are not uniformly synergistic but vary greatly amongst neuroanatomical structures.

418.13

COMPARATIVE STUDIES OF F-18 LABELED BENZAMIDES AND [F-18]N-METHYLSPIPERONE . RH Mach,[1] JG Greenberg,[1] PA Nowak,[1] PH Evora,[1] RL Ehrenkaufer,[2] RR Luedtke,[1] CD Unsworth,[1] K Ivins,[1] S Childers,[2] PB Molinoff,[1] and M Reivich.[1*] [1]Univ. of Pennsylvania and [2]Bowman Gray School of Medicine.

A series of imaging studies using PET were carried out on a baboon comparing the uptake and retention of 2,3-dimethoxy-N-(p-fluorobenzyl)piperidin-4-yl benzamide (MBP) and 2,3-dimethoxy-N-(9-(p-fluorobenzyl)-9-azabicyclo[3.3.1] nonan-3ß-yl benzamide (MABN) with that of N-methylspiperone (NMSP). All three compounds displayed a high accumulation in the basal ganglia (BG) with NMSP exhibiting the slowest rate of washout from the cerebellum (Cb). MBP reached a maximum BG uptake at 40 min post-injection. Washout of MBP after maximal accumulation occurred with a half-life of 140 min. MABN and NMSP did not washout from the BG and a linear increase in the BG:Cb ratio was seen over the three hour data acquisition period. The rapid washout of MABN from the Cb resulted in a BG:Cb ratio twice that of NMSP. A series of in vitro binding studies were conducted in order to determine the selectivity of each analog for D2 vs 5-HT2 and α2 receptors. The radioligands and tissue sources used were: D2, [^{125}I]NCQ-298, rat striatum; 5-HT2, [^{125}I]I-LSD, P11 cells; α2, [^{3}H]Rauwolscine, rat cortex. The results of the in vitro studies indicate MBP and MABN possess a higher 5-HT2:D2 ratio than NMSP. All three analogs possess a low affinity for α2 receptors. The results of these studies suggest that MBP and MABN may be superior to NMSP for PET studies of D2 receptors.

Table (Ki nM)

compound	D2	5-HT2	α2	5-HT2/D2
NMSP	0.118	0.55	3000	4.66
MBP	0.38	1840	3000	4842
MABN	0.028	84	1300	3000

418.14

A NOVEL AND RAPID METHOD FOR ENRICHMENT OF LACTOTROPH CELLS (LC) FROM DISPERSED RAT ANTERIOR PITUITARY (AP). K. Bohmaker*, T. Puza, J. Diamond, J.Y. Lew, M. Schütte[¶], D. Okrongly[¶], M. Goldstein and E. Meller. Dept. of Psychiatry, NYU Medical Center, New York, NY 10016, [¶]Dept. of Biology, CCNY, New York, NY 10031 and [¶]Applied Immune Sciences, Inc., Menlo Park, CA 94025.

The rat AP contains at least 6 different cell types, complicating electrophysiological and pharmacological studies of signal transduction mechanisms regulating the release of hormones. Enriched LC preparations, obtained by density gradient sedimentation or centrifugation, have been used to study dopamine (DA)-regulated second messengers and membrane ion channels involved in prolactin (PRL) release. These methods require specialized equipment and are labor-intensive. A simple and rapid method for LC enrichment is described which yields results comparable to those obtained with other techniques.

An affinity-purified polyclonal Ab to the N-terminal region of the D_2 DA receptor (50 μg/ml in PBS) was covalently attached to MicroCELLector surface-activated 24-well polystyrene plates (Applied Immune Sciences, Menlo Park, CA) by incubation for 1 hr at RT. After washing (PBS) and blockade of nonspecific binding sites with BSA, appx. $1x10^6$ dispersed rat AP cells were added/well and incubated for 1 hr at RT. Non-adherent cells were removed and the wells were washed. After overnight incubation (37°C) and further washing, captured cells were transferred to poly-L-lysine-coated microscope slides or regular culture plates. Captured cells were fairly uniform in size; several days incubation revealed the absence of fibroblasts which normally proliferate extensively. Immunostaining with a rat PRL antiserum by the ABC method showed that about 80% of the cells stained positively, compared to about 20% in non-captured cells, i.e., about 4-fold enrichment. Comparison of functional response in enriched and standard cell preparations will be reported.

418.15

REVERSED RELATIVE EFFICACIES OF QUINPIROLE (QUIN) AND (+)3-PPP AT DOPAMINE (DA) D_2 RECEPTORS (D_2R) IN STRIATUM AND ANTERIOR PITUITARY (AP). E. Meller*, T. Puza, J. Diamond and K. Bohmaker. Dept. Psychiatry, NYU Medical Center, New York, NY 10016.

Previous studies have shown that D_2R on striatal DA nerve terminals and AP lactotrophs mediating, respectively, inhibition of transmitter synthesis and prolactin (PRL) release, both display a similar large receptor reserve (RR) for full agonists. Both effects involve receptor coupling to pertussis toxin (PTX)-sensitive G proteins(s); consequently, the efficiency of receptor/coupling (i.e., RR), is likely to be related to the ability of agonists to induce ternary complex formation. Treatments which reduce the amount of receptor or G protein are therefore expected to reduce the RR.

Treatment of AP cell cultures with 17ß-estradiol (EST; 10 nM, 3 days), recently shown to reduce PTX-sensitive G protein levels in AP, shifted the dose-response curve (DRC) for N-propylnorapomorphine (NPA) to the right 14-fold; receptor inactivation studies with phenoxybenzamine (PBZ; 1 μM, 60 min) showed that EST treatment abolished the RR for NPA. Surprisingly, EST had nearly identical effects on the DRCs for QUIN and (+)3-PPP (4.5-fold shift), although differential effects were expected since (+)3-PPP had been found to be a much weaker agonist than QUIN in striatum. Treatment of cultures with PTX (10 ng/ml, 24 hr) produced shifts in the DRCs for NPA, QUIN and (+)3-PPP which did not correlate with their previously determined relative efficacies; the PTX DRCs were also significantly shallower ($P<.001$). Receptor inactivation expts. revealed that half-maximal response in AP for QUIN and (+)3-PPP required 25.6 and 9.6% receptor occupancy, but 6.2 and 30%, respectively, in striatum. "Promiscuous" coupling of D_2R to different G proteins in the two tissues may underlie the reversal of relative efficacy, as recently predicted (Kenakin and Morgan, Mol. Pharmacol. 1989). Supported by NS 23618.

418.16

LOCALIZATION AND BINDING ANALYSIS OF [^{3}H]BTCP: A DOPAMINE UPTAKE SITE ANTAGONIST. M.E. Hunt*, F. Filloux, N. Narang, C. Johnson, and J.K. Wamsley. Neuropsychiatric Research Institute, Fargo, ND 58103 and Western Institute of Neuropsychiatry, Salt Lake City, UT 84108.

The phencyclidine analog, N-[1-(2-benzo(b)-thiophenyl cyclohexyl] piperidine, has recently been made available in tritiated form ([^{3}H]BTCP) from Dupont NEN. Specific binding of [^{3}H]BTCP in striatal tissue was maximized at a pH ranging from 6.8 to 7.0 and a NaCl concentration between 180 and 220 nM. Nonlinear regression analyses of association and dissociation experiments yielded a k_{+1} .0008min^{-1}nM^{-1} and a k_{-1} .023min^{-1}. The calculated k_d is equal to 30.22nM which is in agreement with the k_d derived from nonlinear regression analysis of the saturation isotherm for a single site model. However, two site modeling of the saturation isotherms proved to be significantly better than a one site model and revealed high and low affinity binding sites with k_d's of 1.3nM and 79.7nM respectively. Autoradiographic localization showed high binding in the caudate putamen, nucleus accumbens, and olfactory tubercle.

418.17

DOPAMINE SYNTHESIS, STORAGE, RELEASE AND RECEPTOR CONTENT IN A MESENCEPHALIC CELL LINE. S.P. Han*, K.L. O'Malley, H. Choi,[+] A. Heller[+] and R.D. Todd[#] Depts of Anatomy and Neurobiology and [#]Psychiatry and Genetics, Washington Univ. Sch. of Med., St. Louis, MO 63110 and [+]Dept. of Pharmacological and Physiological Sciences, The Univ. of Chicago, Chicago, IL 60637

An immortalized mouse mesencephalic dopamine (DA) containing cell line (MN9D) has been established by somatic cell fusion techniques (Choi et al., Brain Res., 552:67-76, 1991). This cell line contains DA, NE, and DOPA as well as DA metabolites. These cells show neurite formation, generation of action potentials and sensitivity to MPP^+. MN9D cells express tyrosine hydroxylase mRNA and immunoreactive protein. In the present study we have further characterized the dopaminergic properties of the MN9D cell line. Enzymatic assays show the presence of L-aromatic amino acid decarboxylase activity which can be inhibited by NSD1015, a specific inhibitor of the decarboxylase enzyme. Dense core vesicles are clearly visible under electron microscopy. DA can be released by potassium-induced depolarization in a concentration dependent manner. Differentiation with 1 mM sodium butyrate results in increased cellular DA levels. No D_1-like or D_2-like binding sites were detected in receptor binding assays using [^{3}H] SCH23390 or [^{3}H] spiperone. Furthermore, reverse transcription-polymerase chain reaction assays detect no D_1-like or D_2-like receptor mRNA. Given these findings, the MN9D cell line appears to be a suitable model system for examination of CNS DA neurobiology and a convenient system for studying autoreceptor function by transfection of specific receptor types. Supported by NS29343, MH45019 and MH28942.

419.1

ELECTROPHYSIOLOGICAL ACTIVITY MODULATES THE LEVEL OF ENDOGENOUS ADENOSINE IN THE HIPPOCAMPAL SLICE. J.B. Mitchell* and T.V. Dunwiddie. University of Colorado Health Sciences Ctr. and VA Medical Ctr., Denver, CO 80262

Adenosine is a potent inhibitory neuromodulator within the CNS. An increase in adenosine levels accompanies hypoxia, ischemia and seizures, but it is not known if adenosine has a role in normal electrophysiological function. We have found evidence for the activity-dependent release of adenosine from in vitro hippocampal slices maintained under physiological conditions. Bipolar stimulating electrodes were positioned in the Schaffer collateral-commissural fiber layers of areas CA1 and CA3, and test fEPSPs were recorded from a single recording electrode positioned in the stratum radiatum of CA1. A train of conditioning pulses was applied to one stimulating electrode, and then a response evoked by applying a test pulse to the second stimulator. A train of 6 conditioning pulses (100 Hz) beginning 250 ms prior to the test pulse decreased the amplitude of the test fEPSP to 78.6 $\pm$ 1.9 percent of control. Antidromic firing of the CA1 pyramidal cells, evoked by an electrode positioned in the alveus, decreased the amplitude of the test fEPSP to 84.1 $\pm$ 2.6 percent of control. The decrease in fEPSP amplitude induced by either conditioning stimulus could be prevented by superfusion with an adenosine antagonist. When 2 conditioning pulses were used, the decrease in fEPSP amplitude lasted 500 ms and the maximum decrease occured at 250 ms. Superfusion with the adenosine uptake inhibitor, dipyridamole, (50 μM) lengthened the duration of the decrease. These results suggest that endogenous adenosine levels can be increased in response to activation, and that this adenosine can then decrease excitatory neurotransmission in the hippocampus.

Supported by NS29173, VA Medical Research Services, MRC of Canada.

419.2

NMDA- AND NON-NMDA-EVOKED ADENOSINE RELEASE: DISTINCT PURINERGIC SOURCES AND MECHANISMS OF RELEASE. C.G. Craig* and T.D. White, Dept. of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7.

Activation of excitatory amino acid receptors releases the inhibitory neuromodulator adenosine from superfused rat cortical slices. Here we investigated the source of adenosine and its mechanism of release. Inhibition of the nucleoside transporter with dipyridamole greatly enhanced adenosine release evoked by glutamate, NMDA, kainate and AMPA. Inhibition of *ecto*-5'-nucleotidase with α,β-methylene ADP and GMP had no effect on either kainate- or AMPA-evoked adenosine release but decreased glutamate- and NMDA-evoked adenosine release by 23% and 68%, respectively. NMDA-evoked adenosine release, but not kainate- or AMPA-evoked release, was Ca^{2+}-dependent. These results indicate that activation of non-NMDA receptors releases adenosine *per se* in a Ca^{2+}-independent manner. In contrast, NMDA receptor activation evokes a Ca^{2+}-dependent release of a nucleotide which is subsequently converted extracellularly to adenosine. Although neither NMDA- nor non-NMDA-evoked adenosine release occurs via the nucleoside transporter, this transporter does appear to be a major route for removal of adenosine from the extracellular space.

(Supported by the MRC of Canada)

419.3

BLOCK OF NMDA-EVOKED ADENOSINE RELEASE BY IBMX IS NOT DUE TO INHIBITION OF PHOSPHODIESTERASE I OR IV. T.D. White* and C.G. Craig. Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7.

Activation of NMDA receptors in rat cortical slices evokes a Ca^{2+}-dependent release of a nucleotide which is then degraded extracellularly to adenosine. To test whether the released nucleotide might be cAMP, we examined the effects of PDE inhibitors on adenosine release. IBMX, a non-selective PDE inhibitor, blocked NMDA-evoked adenosine release but this was not accompanied by enhanced cAMP recovery in the medium. However, it seemed possible that cAMP may be degraded intracellularly to 5'AMP which is then released and converted to adenosine by *ecto*-5'-nucleotidase. PDE I, II and IV have been isolated in the cortex. Inhibitors of Ca^{2+}/calmodulin-dependent PDE I (8-methoxymethyl-IBMX, W-7 and calmidazolium) and cAMP-specific PDE IV (rolipram) did not mimic the effects of IBMX on NMDA-evoked adenosine release. It appears that IBMX decreases the NMDA-evoked release of purines from cortex either by inhibiting PDE II or by acting at a site other than PDE. (Supported by the MRC of Canada)

419.4

HETEROGENEITY OF ADENOSINE TRANSPORTERS AS BASIS FOR CNS-SPECIFIC ADENOSINE TRANSPORT INHIBITORS? J.Deckert*, A.Hennemann, B.Bereznai, W.Gsell, M.Götz J.Fritze, H.Beckmann and P.Riederer. Dept. of Psychiatry, Univ. Würzburg, 87 Würzburg, FRG.

Adenosine transport inhibitors have been suggested for the treatment of neuropsychiatric disorders. Heterogeneity of adenosine transporters has been described with differential sensitivity to dipyridamole (DPR) and nitrobenzylthioinosine (NBI) in the brain of some mammals.

Preparation of crude menbranes from human parietal cortex and (^{3}H)DPR as well as (^{3}H)NBI radioreceptor assays were performed as described.

(^{3}H)DPR and (^{3}H)NBI marked approximately the same number of binding sites with a Bmax of about 1000 fmol/mg protein and Kd`s in the low nanomolar range. They displaced each other completely from their respective binding sites with Ki`s equivalent to their respective Kd`s.

In conclusion, the data obtained so far in human brain makes the development of CNS-specific adenosine transport inhibitors on the basis of DPR- and NBI- sensitive transport unlikely.

419.5

CHARACTERIZATION OF ADENOSINE UPTAKE SITES IN CULTURES OF CHICK EMBRYO RETINAL CELLS. R. Paes-de-Carvalho* and R. Visser. Dep. Neurobiologia, Univ. Fed. Fluminense, Niterói, RJ 24000, Brazil.

The presence of a specific uptake system for adenosine that can be inhibited by nitrobenzylthioinosine (NBI) or dipyridamole (DPR) was previously demonstrated in cultures of chick embryo retinal cells. Here we show that (^{3}H)NBI binds with high affinity to adenosine uptake sites on intact retinal cells. Cultures obtained by dissociation of 8-day-old chick embryo retinas and incubated in BME with 5% fetal calf serum for 6 days at 37°C were washed and incubated in Hanks' with 2-5nM (^{3}H)NBI in the absence or presence of unlabeled NBI (10µM) to determine nonspecific binding. The binding was blocked approximately 70% in the presence of unlabeled NBI or DPR and attained equilibrium after 10 min at 37°C. The addition of NBI or DPR after equilibrium completely displaces specific binding, indicating the binding to surface sites rather than (^{3}H)NBI uptake into cells. The data indicate that adenosine uptake sites labeled with (^{3}H)NBI can be directly studied in living intact retinal cells and that primary cultures of chick embryo retinal cells are excellent models for the study of these sites and their regulation by external factors.

419.6

[^{3}H]ADENOSINE TRANSPORT IN POSTMORTEM HUMAN BRAIN. J.G. Gu*, G. Kala, and J.D. Geiger. Dept. Pharmacology, Univ. of Manitoba, Faculty of Medicine, 770 Bannatyne Avenue, Winnipeg, Manitoba, R3E 0W3

The kinetics of [^{3}H]adenosine transport was characterized in cerebral cortical synaptoneurosomes prepared from postmortem human brain. For this assay, it was determined that the adenosine transport inhibitors dypridamole and dilazep were instantaneously and completely effective in blocking adenosine transport. For 5 sec incubations, two kinetically distinguishable processes were identified; the Kt and Vmax values for high affinity adenosine transport were 89 uM and 0.98 nmol/min/mg protein and for low affinity adenosine transport were 4.5 mM and 15.2 nmol/min/mg protein. With incubation of 1 uM [^{3}H]adenosine, [^{3}H]adenosine accumulation was 0.3 uM at 5 sec and 1.0 uM at 600 s; hence adenosine transport was not a concentrative process. For 5, 15, 30, 60 and 600 sec incubations, 14, 23, 34, 43 and 80% of transported [^{3}H]adenosine was metabolized mainly to its phosphorylated derivatives AMP, ADP, and ATP. The concentration (uM) of total accumulated radiolabeled purines at these times was 0.3, 0.5, 1.0, 1.3 and 5.6, respectively; hence the accumulation of radiolabeled purines was concentrative only when extensive metabolism was present. In the presence of 10 uM EHNA, an adenosine deaminase inhibitor, and 10 uM iodotubercidin, an adenosine kinase inhibitor, metabolism was 14, 14, 16, 14, and 38% of the transported [^{3}H]adenosine and total radiolabeled purine accumulation was 0.3, 0.5, 0.5, 0.7, and 1.8 uM; hence concentrative accumulation of radiolabeled purines can be inhibited by the inhibition of adenosine metabolism. Thus, adenosine transport in human brain synaptoneurosomes is via a facilitated diffusion system that exhibits low affinity for adenosine.

419.7

INHIBITORY ADENOSINE A_1 RECEPTORS EXIST ON MYENTERIC AH/DOGIEL TYPE II NEURONS OF THE SMALL INTESTINE. F.L. Christofi, H.J. Cooke* and J.D. Wood. Dept. of Physiology, Ohio State Univ., Columbus, OH 43210.

Intracellular microelectrodes were used to characterize the adenosine receptors of 102 myenteric neurons. Biocytin was injected from microelectrodes to characterize the morphology of responsive neurons. Superfusion of adenosine, the A_2 agonists 5'-N-ethylcarboxamidoadenosine and CGS 21680 or the A_1 selective agonists N^6- cyclopentyladenosine and 2- chloro-N^6-cyclopentyladenosine (CCPA) evoked an inhibitory response in 93 of 102 AH/type 2 neurons (91.3%). The response consisted of membrane hyperpolarization, associated with a decrease in input resistance, increase in the hyperpolarizing after-potentials, inhibition of spike discharge and a decrease in cell input resistance. The potency profile for inhibition of cell input resistance was CCPA > > CGS 21680 > ADO (N=40); the EC_{50} for CCPA (5.15+2.17 nM) was 1000 fold lower than that of CGS 21680 (5.60 +2.54 μM). This effect was reversible with the A_1 selective antagonist 8-cyclopentyl-1,3-dimethylxanthine (0.10-1.00 μM). Of 30 responsive neurons, 27 neurons (90%) had Dogiel Type II and 3 neurons (10%) had Filamentous morphologies. It is concluded that inhibitory A_1 adenosine receptors exist mainly on myenteric AH/Dogiel Type II neurons.**(Supported by NIH R29 DK44179 to FLC and R01 DK 37238 to JDW).**

419.8

ADENOSINE AND 5-HT INHIBIT SUBSTANCE P RELEASE FROM ENTERIC NERVES THROUGH DISTINCT G PROTEINS. R.M. Broad* and M.A. Cook. Dept of Pharm. & Tox., Univ. of Western Ontario, London, CANADA N6A 5C1.

Evidence suggesting that both adenosine and 5-HT, released from enteric nerves, can function to inhibit the release of excitatory neurotransmitters is well documented. It is possible that both of these compounds cause hyperpolarization of neurons and that transduction may involve G protein coupling. Examination of the *pertussis* toxin-sensitivity of the inhibitory actions of these neuroactive substances was undertaken. A perifused preparation of enzymatically dispersed guinea pig myenteric ganglia was used to examine the inhibitory actions of 5-HT and N^6-cyclopentyl-adenosine (CPA, an A_1-selective adenosine analog) on the $\uparrow[K^+]_o$-evoked release of Substance P-like immunoreactivity (SP-LI). The release of SP-LI was inhibited, in a simple graded manner, by CPA. Incrementing concentrations of 5-HT also inhibited SP-LI release yielding a biphasic concentration-response relationship. The selective A_1 antagonist DPCPX (1μM) abolished the inhibition due to CPA while 5-HT-mediated inhibition was abolished by the 5-HT_{1A}-selective antagonist NAN-190 (10μM). In the presence of DPCPX alone, evoked SP-LI release was enhanced supporting the presence endogenous adenosine. The inhibition mediated by both A_1 and 5-HT_{1A} agonists was insensitive to tetrodotoxin (1μM) implicating a prejunctional locus for both receptors on tachykininergic nerve endings. Pretreatment of ganglia with *pertussis* toxin had no effect on CPA-mediated inhibition while that to 5-HT was abolished. Treatments designed to eliminate the possible influence of adenosine receptor occupancy by endogenous adenosine on binding of PTX to the G proteins did not alter these results. The findings demonstrate directly the presence of functional adenosine A_1 and 5-HT_{1A} receptors on enteric nerve endings, coupled negatively to tachykinin release, and that they are coupled through distinct mechanisms, putatively distinct G proteins, (G_i or G_o). Supported by the Medical Research Council of Canada.

419.9

MODULATION OF PITUITARY TRANSCRIPTION FACTOR EXPRESSION BY ADENOSINE A_1 RECEPTOR LIGANDS. M.T.C. Jong, F.B. Tuazon, E.J. Simon*, H.H. Samuels and M.R. Sherman. Depts. of Med. and Pharmacol., New York Univ. Sch. of Med., New York, NY 10016 and Depts. of Biol. Sci. and Chem., Rutgers Univ., Newark, NJ 07102.

Pit-1 (GHF-1) is a pituitary-specific transcription factor that regulates the synthesis of growth hormone (GH) and prolactin. Hormones and other agents regulate Pit-1 synthesis by various mechanisms, including effects on adenylate cyclase (AC). Since AC activity can be decreased or increased by analogs of adenosine (Ado) *via* A_1 or A_2 receptors, respectively, we studied the effects of these ligands on mRNA synthesis under the control of the Pit-1 promotor. GH-producing rat pituitary tumor cells (GH_4C_1) were transiently transfected with a chimeric reporter construct containing part of the 5'-flanking sequence of the Pit-1 gene (-389/+11) linked to the coding sequence for chloramphenicol acetyl transferase (CAT). After 24h, various concentrations of forskolin (Forsk), a direct activator of AC, *R-*N^6-phenylisopropyladenosine (PIA, an A_1 agonist), 8-cyclopentyltheophylline (CPT, an A_1 antagonist) and/or Ado deaminase (ADA) were added. Cells were harvested 24h later and lysates were assayed for CAT activity. ADA altered both the magnitude and dose-dependence of Forsk-stimulated CAT activity, suggesting a role for secreted Ado. In the presence of 2 U/ml ADA, the Forsk concentration giving half-maximal increase in CAT activity was increased 2- to 3-fold, and CAT activity was stimulated 25-fold by 0.5 µM Forsk. PIA (0.1 or 1 µM) consistently *inhibited* the expression of this construct to 40-60% of the basal value in the presence of 0.25 µM Forsk and 2 U/ml ADA. The effect of 0.1 µM PIA was completely blocked by 1 µM CPT. In the absence of ADA, responses to PIA were variable, *e.g.* 10 nM PIA *stimulated* construct expression 2-fold in some experiments. Our results indicate that adenosine should be added to the growing list of neurotransmitters and hormones which regulate pituitary hormone production at the transcriptional level. *(Supported by NIH grant DK16636 and the Cohen Fund)*

419.10

ADENOSINE A1 RECEPTOR/G-PROTEIN COUPLING DURING EARLY DEVELOPMENT IN THE RAT. R. Guillet*. Depts. Pediatrics and Psychology, University of Rochester, Rochester NY 14642.

Caffeine, an adenosine receptor antagonist, is commonly administered to human neonates with apnea of prematurity. In an animal model of such exposure, it has been shown that neonatal exposure to caffeine (1) alters the ontogeny of adenosine A1 receptors in certain brain regions, (2) alters developmental sensitivity to adenosine receptor ligands, and (3) alters control of locomotor activity in the developing rat. All of the observed effects have age and/or brain region specificity. Morgan et al. have demonstrated in guinea pigs both a region- and age-specific efficacy of displacement of 3H-CHA (an adenosine receptor agonist) by a guanine nucleotide analogue. This may reflect a region- and age-dependent degree of adenosine A1 receptor/G-protein coupling. To investigate this phenomenon in the developing rat as well as the effect of early caffeine exposure on such development, rats were exposed to 15-20 mg/kg caffeine (p.o.) over postnatal days 2-6. Membranes were prepared from cortex, cerebellum and hippocampus obtained from control (nonhandled and vehicle-exposed) and caffeine-exposed rats on postnatal days 10, 14, 18, 21, and 28. Membranes were incubated with 0.5nM 3H-CHA in the absence and presence of 100µM GTP for 2 hours at room temperature. Specific binding±GTP and % decrease in binding in the presence of GTP were calculated. As demonstrated previously, there was an increase in specific binding as a function of neonatal caffeine exposure in cortex ($p<.02$), but not in cerebellum. There was a trend toward a greater decrease in cortical specific binding in the presence of GTP in neonatally-caffeine exposed rats compared with controls. There was no difference in % decrease in specific binding in the presence of GTP as a function of age in any region examined. These results suggest a regionally-specific alteration in coupling between the adenosine A1 receptor and its associated G-protein as a function of neonatal caffeine exposure, but not as a function of age during the second and third weeks of life in the rat.

419.11

MECHANISMS OF ADENOSINE A_1 RECEPTOR-MEDIATED EFFECTS ON CELLULAR PROPERTIES IN RAT VAGAL MOTONEURONS. J.D. Marks*, D.F. Donnelly, and G.G. Haddad. Dept. of Pediatrics, Section of Resp. Med., Yale U. Sch. of Medicine, New Haven, CT 06510.

We have previously shown that specific adenosine A_1 receptor activation in the rat medullary slice preparation decreases vagal motoneuron excitability and increases input resistance (R_N). This decrease in excitability is due in part to an adenosine-mediated increase in afterhyperpolarization (AHP) amplitude. In order to understand better the mechanisms underlying A_1 receptor effects, we recorded intracellularly from vagal motoneurons using a rat brainstem slice preparation before and during subfusion with either adenosine (10 μM-1 mM) or a specific A_1 receptor agonist, cyclopentyladenosine (CPA, 10 μM). Synaptic blockade with either TTX or a perfusate containing high Mg^{++} (10 mM), low Ca^{++} (0.5 mM) blocked the CPA-induced increase in R_N. Blockade of Ca^{++} entry with $CoCl_2$ (2 mM) abolished the AHP increase. In addition, selective K^+ channel blockade with either 4-aminopyridine (4-AP, 1 mM) or apamin (0.5-2.5 nM) failed to block the increase in AHP amplitude. Blockade of native adenosine with 8-cyclopentyltheophylline (0.8 mM) decreased both R_N and AHP amplitude, and increased excitability. Interestingly, no effect was seen with exogenous adenosine itself, unless adenosine uptake had been blocked with dipyridamole 25 μM. We conclude that 1) the adenosine-induced R_N increase, and possibly the AHP increase as well, is due to A_1 receptor actions on presynaptic mechanisms, 2) the ion channel mediating the increase in AHP amplitude is sensitive to cobalt but not 4-AP or apamin, and 3) the adenosine uptake system in the vagal motor nucleus effectively buffers extracellular adenosine.

419.12

MDL 102,234: A SELECTIVE ADENOSINE A_1 RECEPTOR ANTAGONIST REFLECTING A NEW BINDING MODE TO THE RECEPTOR. M. Dudley, M. Racke, A.M. Ogden, N. Peet, R. Secrest, R. McDermott. Marion Merrell Dow Research Institute, Cincinnati, OH 45215.

The accepted binding mode for a xanthine within the A_1 receptor superimposes N^1,N^3,N^7 and N^9 of the xanthine with the 4 identical nitrogens of the adenine base. This mode does not overlap the xanthine C-8 nor the adenosine C^6-N positions, the positions which impart potency and selectivity to xanthine-based antagonists and substituted adenosine agonists. With superimposition of N^1,N^3 and N^9 of adenosine and N^9, N^3 and N^1 of the xanthine, the C^6-N and C-8 positions are in close proximity. MDL 102,234 (MDL) [(R)-1,3-dipropyl-8-(1-phenylpropyl)xanthine] is a C-8 substituted xanthine with the same stereochemistry as the C^6-N phenylisopropyl substituent of the A_1-selective agonist R-PIA. MDL 102,234 has K_i values at the adenosine A_1 and A_2 receptors of 3 and 900 nM, respectively. The compound is a very weak phosphodiesterase inhibitor with IC_{50} values against PDE Type III and PDE Type IV of 514 µM and 28.3 µM, respectively. This is 10,000-fold weaker than its potency at the A_1 receptor. In guinea pig atria, the compound antagonizes A_1 agonist-induced negative chronotropic and inotropic responses. MDL at a concentration of 10 µM has no effect on heart rate or developed tension in the tissue. In conclusion, MDL 102,234 is a selective A_1 antagonist and supports the new binding mode of xanthines to the A_1 receptor.

419.13

IN VIVO ACTIVATION OF A1 ADENOSINE RECEPTOR BY CCPA ENHANCES ^{35}S-TBPS BINDING IN THE MOUSE BRAIN. A.Concas*, E. Maciocco, L. Dazzi, G. Santoro, M.P. Mascia, M. Trampus[1], E. Ongini[1] and G. Biggio Dept. of Experimental Biology, Chair of Pharmacology, University of Cagliari and [1]Schering-Plough, Research Laboratories, Comazzo (MI) - Italy

The effect of 2-chloro-N_6-cyclopentyladenosine (CCPA), the most selective A_1 adenosine receptor agonist, was studied on ^{35}S-TBPS binding measured "ex vivo" in the mouse brain. In fact, the increase and decrease of ^{35}S-TBPS binding in the rat and mouse brain homogenate reflect a reduction and an enhancement in the function of the $GABA_A$ receptor-coupled chloride channel, respectively. CCPA (0.5 - 10 mg/kg i.p.), like negative modulators of GABAergic transmission, elicited in 30-60 min a dose-dependent increase of ^{35}S-TBPS binding in the mouse cerebral cortex, hippocampus, striatum, but in the cerebellum. The effect of CCPA lasted for more than 4 hrs. Saturation studies revealed that the effect of CCPA on ^{35}S-TBPS binding was entirely due to an increase in the apparent affinity for TBPS recognition sites with no changes in the Bmax. The effect of CCPA on ^{35}S-TBPS binding was abolished by the concomitant administration of the specific A_1 receptor antagonist DPCPX (3 mg/kg i.p.). Moreover, abecarnil (0.5 mg/kg, i.p.), an anxiolytic and anticonvulsant benzodiazepine receptor ligand, completely abolished the increase of ^{35}S-TBPS binding induced by CCPA. In spite of its capability to reduce the function of GABA-coupled chloride channel, CCPA (1-3 mg/kg) completely antagonized the convulsant activity of pentylentetrazol and isoniazid, two GABA function inhibitors, while failed to antagonize kainic acid and strychnine-induced seizures.

419.14

EFFECTS OF ALCOHOL ON ADENOSINE A_1 AND A_2 RECEPTOR-MEDIATED CHANGES IN ADENYLATE CYCLASE ACTIVITY IN BRAIN MEMBRANES FROM LONG-SLEEP MICE. M.R. Sherman*, F.B. Tuazon, E.N. Shafik and J.J. McArdle. Dept. of Biol. Sci., Rutgers Univ., Newark, NJ 07102; Dept. of Pharmacol. and Toxicol., Univ. of Med. and Dent., Newark, NJ 07103.

Prolonged exposure to alcohol (alc) is known to diminish adenosine receptor-mediated activation of adenylate cyclase (AC) in a cultured neural cell line and in human lymphocytes *(PNAS 83:2105, 1986; PNAS 84:1413, 1987)*. However, definitive evidence for the involvement of a particular receptor subtype has been difficult to obtain. We have addressed this question by assaying AC activity in membranes from various brain regions of mice selectively bred for alc sensitivity (long-sleep mice). Mice were kept on liquid diets ± 3.8% alc for 10d. AC activity (α^{32}P-ATP → ^{32}P-cAMP) in membranes from hippocampus (hip), cortex and striatum was measured in the presence of adenosine deaminase and various concentrations of forskolin (Forsk, a direct activator of AC), *R-N*^{6}-phenylisopropyladenosine (PIA, an A_1 agonist), 8-cyclopentyltheophylline (CPT, an A_1 antagonist) and/or 2-(carboxyethylphenylethylamino)adenosine-5'-carboxamide (CGS21680, an A_2 agonist). We detected no effect of alc on the A_1 receptor-mediated inhibition of AC by PIA in either hip or cortex. In striatal membranes, 1 μM CGS21680 stimulated AC to 140 % of basal activity in the absence of Forsk and the presence of 0.1 or 1 μM CPT (which presumably blocks CGS21680 binding to A_1 receptors), but no effect of alc was detected. Only in freshly prepared cortical membranes was there clear evidence for an effect of alc on A_2 receptors. When cortical membranes from control mice were assayed with 20 nM Forsk and 1 μM CPT, AC activity was increased 2-fold by 1 μM CGS21680 and to a lesser extent by 10 or 100 μM. In membranes from alc-treated mice, no stimulation of AC activity was detected at any tested concentrations of CGS ± CPT. These results demonstrate that 10 days of alc ingestion by alc-sensitive mice abolishes the responsiveness of AC in their cortical membranes to a ligand selective for A_2 but not A_1 adenosine receptors.

419.15

EXPRESSION OF ADENOSINE A1 AND A2 RECEPTOR SUBTYPES IN PRIMARY CULTURED NEURONS FROM FETAL RAT FOREBRAIN. J.L. Daval*, F. Nicolas, J. Oillet and V. Koziel. INSERM U.272, 30 rue Lionnois, 54013 NANCY, FRANCE.

The expression of both adenosine A1 and A2 receptors, which are physiologically coupled to adenylate cyclase via G proteins, was investigated by radioligand binding methods in primary cultured neurons isolated from fetal rat forebrain and grown in serum-free medium for 8 days. A1 receptors were labeled by incubating intact neurons in 50 mM Tris-HCl buffer (pH 7.4) for 120 min at 25°C with 2 IU/ml adenosine deaminase and increasing concentrations of (3H)2-chloro-N6-cyclopentyladenosine (CCPA), using cyclohexyladenosine for the determination of non-specific binding. A2 binding sites were analyzed by incubating the cells for 90 min at 25°C with deaminase and (3H)CGS 21680. Non-specific binding was assessed with NECA. Additionally, the presence of adenylate cyclase was tested by analyzing (3H)forskolin specific binding. Each radioligand bound specifically and with high affinity to a single population of sites. Scatchard plots yielded Kd-values of 2.9 nM for (3H)CCPA, 1.7 nM for (3H)CGS 21680 and 33 nM for (3H)forskolin. Maximum numbers of sites were 160±14, 10±3 and 254±23 fmol/mg protein for CCPA, CGS 21680 and forskolin, respectively. The addition of 1 μM G(pp)NHp, a GTP analogue, increased significantly KD-values for both CCPA and CGS 21680, suggesting that receptors are, at least partly, linked to G proteins.

419.16

DEPRESSION OF LOCOMOTOR ACTIVITY BY ACTIVATION OF A_2 RECEPTORS IN THE NUCLEUS ACCUMBENS IN MICE, K. Martens[1], M. Parizon[1], H.J. Normile[1,2] and R.A. Barraco*[1]. Depts. of [1]Physiology and [2]Psychiatry, Wayne State Univ. Sch. of Med., Detroit MI 48207

Adenosine agonists are potent locomotor depressants in rodents- an effect which has been shown to be centrally mediated. In the present study, we examined the locomotor effects of A_1 and A_2 agonists when infused into the nucleus accumbens, a ventral striatal structure which appears to functionally link limbic and motor systems. The selective A_1 agonist N^6-cyclopentyladenosine (CPA), and the selective A_2 agonists 5'-N-ethylcarboxamidoadenosine (NECA) and 2-[4-(2-carboxyethyl)phenethylamino]-5'-N-ethylcarboxamido adenosine (CGS) were injected (0.0002, 0.002, 0.02, 0.2, and 2.0 nmoles per mouse) bilaterally (0.5 ul/side) into the nucleus accumbens through permanent indwelling cannulae in mice. Each of the A_2 agonists, NECA and CGS, significantly suppressed locomotor activity in a dose-dependent fashion, whereas the A_1 agonist, CPA, had no effect on locomotor activity. These findings suggest that selective activation of A_2 receptors in the nucleus accumbens may suppress locomotor activity.

419.17

ADENOSINE-2 RECEPTOR SEQUENCES ON HUMAN CHROMOSOME 22. RA Peterfreund*, M MacCollin, M MacDonald, RH Lekanne Deprez, EC Zwarthoff, J Gusella and JS Fink. Depts. of Anesthesia and Neurology, Mass. Gen. Hosp., Boston, MA 02114, USA and Dept. Pathology, Erasmus U., Rotterdam, The Netherlands

Adenosine-2 receptors (A2Rs) are abundantly expressed in the striatum, suggesting an important role in motor function and extrapyramidal diseases. Complementary DNAs (cDNAs) for the A2R have been isolated from dog (BBRC 173:1169) and rat (Mol Br Res, *in press*). These cDNAs exhibit substantial nucleic acid and amino acid homology in the transmembrane (TM) domains but diverge in their carboxy termini. To further understand the functional significance of sequence divergence at the carboxy terminus of the A2R, we have begun to characterize the human A2R gene.

A panel of somatic cell hybrids containing various human chromosomes on a rodent background was screened with a 540bp fragment of coding sequence from the rat A2R. Hybridization was detected with the chromosome 22 containing cell line. Localization on chromosome 22 proximal to a t(4:22) breakpoint at 22q11 was then established by hybridization with a regional somatic cell hybrid panel. A human chromosome 22 cosmid library was screened with the same probe and two clones were isolated. A restriction fragment from one of the cosmid clones which selectively hybridized to the rat probe was subcloned and sequenced. The predicted amino acid sequence of this fragment was highly homologous to the dog and rat A2Rs (70% overall identity and greater than 90% identity in the transmembrane domains). We conclude that A2R-like sequences homologous to the rat and dog A2R reside on human chromosome 22.

419.18

MOLECULAR CLONING AND CHARACTERIZATION OF A NOVEL ADENOSINE RECEPTOR: THE A3 ADENOSINE RECEPTOR. Q.-Y. Zhou, C. Li, M. E. Olah, J. Williams*, G. Stiles, and O. Civelli. Vollum Institute, Oregon Health Sciences University, Portland, OR 97201 and Department of Medicine, Duke University, Durham, NC 27710.

We have previously reported the selective amplification of several rat striatal cDNA sequences that encode novel G-protein coupled receptors. One of these sequences (R226) exhibited high sequence identities (58%) with the two previously cloned adenosine receptors RDC7 (A1) and RDC8 (A2). A full-length cDNA clone for R226 has been isolated from a rat brain cDNA library. The cDNA clone encodes a protein of 320 amino acids that can be organized into seven transmembrane stretches. When stably expressed in CHO cells, R226 could bind the nonselective adenosine agonist [^{3}H]NECA and A1-selective agonist [^{125}I]APNEA but not A1-selective antagonists [^{3}H]DPCPX and [^{3}H]XAC or the A2-selective agonist ligands [^{3}H]CGS21680 and [^{125}I]PAPA-APEC. Extensive characterization with [^{125}I]APNEA showed that R226 binds [^{125}I]APNEA with Kd of17 nM, which is ten fold lower than typical A1AR, and the specific [^{125}I]APNEA binding could be inhibited by adenosine ligands with a potency order of R-PIA = NECA > S-PIA > adenosine > ATP = ADP but not by antagonists XAC, IBMX and DPCPX. In R226 stably transfected CHO cells, adenosine agonists R-PIA, NECA, and CGS21680 inhibited by 40-50% the forskolin-stimulated cAMP accumulation through a pertussis toxin-sensitive G protein with EC50 of 18±5.6 nM, 23±3.5 nM, and 144±34 nM, respectively. Based on these observations we conclude that R226 encodes an adenosine receptor with non-A1 and non-A2 specificity and thus name it the A3 adenosine receptor. mRNA analyses revealed that the highest expression of R226 was in the testis and low level mRNAs were also found in the lung, kidneys, heart, and some parts of the CNS such as cortex, striatum and olfactory bulb. The high expression level of the A3 receptor in the testis suggests its possible role in reproduction.

420.1

NUCLEOTIDE RECEPTORS IN PHEOCHROMOCYTOMA (PC12) CELLS.
L. de Souza[1], S. Raha[1], A. Lange[2]* and J.K. Reed[3]. Depts of Biochemistry[1], Zoology[2] and Chemistry[3], University of Toronto, Erindale College, Mississauga, Ont. L5L 1C6

The effect of extracellular ATP was studied in PC12 cells, a neurosecretory line that co-releases ATP with catecholamines upon stimulation. We have examined the effects of ATP by monitoring both cytosolic free Ca^{2+} concentrations $[Ca^{2+}]_i$ and inositol phosphate (IP) levels. Micromolar concentrations of ATP evoked a transient increase in $[Ca^{2+}]_i$ as measured with the Ca^{2+}-sensitive dye fura-2. The increase was eliminated by EGTA. UTP and ITP also evoked an increase while GTP and CTP had little effect. A variety of ATP cogeners were screened to identify the purinergic receptor subtype involved. The rank order potency of these analogues is not consistent with either P_{2X} or P_{2Y} purinergic receptors. The effect of ATP on IP levels was determined using ^{3}H-inositol. IP levels increased in response to ATP and other nucleotides such as UTP and ITP, but not GTP. The rank order potency for agonist-induced stimulation of IP production followed closely the rank order potency for the calcium response. We propose that PC12 cells express a P_{2N} nucleotide receptor that is distinct from classical purinergic receptors.

This research was supported by NSERC.

420.2

SECOND MESSENGER PATHWAY FOR ATP-EVOKED INCREASES IN INTRACELLULAR Ca^{2+} IN GLIA CULTURED FROM THE DORSAL SPINAL CORD.
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ATP increases intracellular Ca^{2+} in glial cells from the spinal cord and other regions of the CNS by activating P_{2y}-purinergic receptors. Signal transduction mechanisms of this effect of ATP were investigated by measuring $[Ca^{2+}]_i$ in individual cells and delivering agents intracellularly by whole-cell patch clamp electrodes. Glia were studied in primary dissociated cultures prepared from the dorsal spinal cord of E17-E19 rats and grown for 7-14 days. $[Ca^{2+}]_i$ was measured using the fluorescent dye, Fura-2. Cells were loaded with the dye by incubating the cultures with the membrane permeant form, Fura-2 AM (2 μM). The standard intracellular solution used for whole-cell recording contained (in mM): 140 KCl, 1 $MgCl_2$, 10 HEPES, 0.1 EGTA, 0.05 K_5-Fura-2; pH 7.25. ATP (1-5 μM) was applied by pressure (5-20 s pulses) from a pipette located near the cell. ATP was tested before breakthrough into whole-cell mode and caused a rapid, transient increase in $[Ca^{2+}]_i$ in cells studied. Increases in $[Ca^{2+}]_i$ were evoked by ATP for up 30 min after breakthrough when the standard intracellular solution was used. In contrast, when heparin (1 mg/ml), an IP_3-receptor antagonist, was included in the intracellular solution, ATP was found to have no effect on $[Ca^{2+}]_i$ within 5 min following breakthrough. Including the G-protein activator GTPγS (50-500 μM) produced spontaneous increases in $[Ca^{2+}]_i$ and occluded the effect of ATP. GDPβS (100 μM), a G-protein inhibitor, did not affect resting $[Ca^{2+}]_i$ but attenuated the effect of ATP. Also, pretreatment with pertussis toxin (1 μg/ml, 24 hr) had no effect on the responses to ATP. These results indicate that ATP evokes release of Ca^{2+} from IP_3-sensitive stores via P_{2y}-receptors coupled to phospholipase C by a pertussis toxin-insensitive G protein. (Supported by the MRC of Canada and the Nicole Fealdman Memorial Fund.)

420.3

ACTIONS OF ATP ON SYNAPTIC TRANSMISSION IN THE MYENTERIC PLEXUS OF THE GUINEA-PIG ILEUM.
K.Morita*, T.Kamiji and Y.Katayama. Department of Autonomic Physiology, Tokyo Medical and Dental Univ., Tokyo, 101 Japan.

Intracellular recordings were made from myenteric neurons *in vitro* to study the actions of ATP applied by superfusion (10 nM-100 µM). ATP showed pre- and postsynaptic actions in a concentration-dependent manner. ATP produced a membrane depolarization associated with an increase in input membrane resistance in S cells and a hyperpolarization accompanied by a fall in input resistance in AH cells; both responses were reversed in polarity near the K^+ equilibrium potential and were markedly reduced or abolished in Ca^{2+} free/high Mg^{2+} solution. These suggest that they may be due to inactivation and activation of Ca-sensitive K conductance, respectively. Furthermore, ATP inhibited both fast and slow EPSPs in S cells and slow EPSPs in AH cells. The inhibitory effects of ATP on the synaptic potentials were observed, when the ATP-induced depolarization and hyperpolarization were nullified with manual clamp method. It should be added that ATP at lower concentration ($\leq$100 nM) did not affect ACh-nicotinic depolarizations but at higher concentration ($\geq$1 µM) augmented the ACh-responses. These results suggest that ATP may inhibit presynaptic release of ACh. Thus, it is concluded that ATP may act pre- or postsynaptically or both and may regulate synaptic transmission in the myenteric plexus. This was supported in part by a Grant-in-Aid from the Ministry of Education, Japan.

420.4

Fura-2 Imaging of Calcium mobilization by two different P_{2}-Purinoceptors on Single Rat Parotid Acinar Cells.
L. Tenneti* and B.R. Talamo, Neurosciences Labs, Tufts Med. Sch., Boston,MA.

Receptor-mediated cytosolic Ca^{2+} (Ca_i) regulation is readily studied in the rat parotid acinar cell model, where at least five different neurotransmitter receptors participate in Ca_i elevation. Previously, we observed that extracellular ATP stimulated Ca^{2+} influx without IP_3 formation in cell suspensions, and that the concentration-response curve was biphasic. Fluorescence microscope ratio-imaging methods were used to examine Ca_i responses in perfused, single acinar cells loaded with Fura-2. All cells (93%) which respond to muscarinic and Substance P activation through IP_3-stimulated Ca^{2+} release also respond to ATP. Analysis of the concentration-response curves showed that two responses to ATP can be resolved in most cells. The high affinity response, detectable at 0.1 μM ATP, saturates at 1- 3 μM and rapidly desensitizes in 50% of the cells, fully recovering within about 6 min after washout. At much higher concentrations of ATP (300-600μM), a large, more slowly developing Ca_i rise occurs, which can be blocked by Mg^{2+} as expected for an ATP^{4-}-specific response. The latter response does not desensitize, and is difficult to fully reverse on washout, possibly because a large influx of extracellular Ca^{2+} is handled differently from an equally high Ca_i level produced through muscarinic receptor activation, which is readily and rapidly reversed. Examination of these two Ca^{2+}-mobilizing purinergic receptors as well as PLC-activating receptors reveals the participation of different mechanisms of Ca_i homeostasis in recovery of resting Ca_i levels in individual cells.

420.5

EFFECT OF ATP ON VOLTAGE-SENSITIVE CALCIUM INFLUX AND PROTEIN PHOSPHORYLATION IN SYNAPTOSOMES ISOLATED FROM RAT CEREBRAL CORTEX.
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Adenosine triphosphate (ATP) is co-released from nerve terminals together with neurotransmitters such as acetylcholine. Aside from its action on purinergic receptors, other functional aspects of extracellular ATP are not yet known. Since extracellular ATP has been implicated in the disruption of intracellular Ca^{2+} homeostasis, we examined the effect of ATP on the Ca^{2+} transport system. Results indicated that ATP at 1-2 mM enhanced the voltage-dependent calcium influx (VDCI). The enhancement of VDCI activity was specific for ATP and activity was blocked by ADP and AMP. Furthermore, the slowly hydrolyzable ATP, ATPγ-S, inhibited synaptosomal VDCI significantly, indicating that ATP hydrolysis was involved in ATP-induced enhancement of VDCI. Under similar conditions a 43 kD protein was phosphorylated by an ecto-kinase. The level of phosphorylation of this protein was dependent on exogenous calcium and was dephosphorylated upon depolarization. It is possible that the phosphorylation of the 43 kD protein is functionally linked to the ATP-induced VDCI activity.

420.6

GUANOSINE AND GTP ENHANCE NGF-STIMULATED NEURITE OUTGROWTH IN PC12 CELLS.
John W. Gysbers and Michel P. Rathbone*. Departments of Biomedical Sciences and Medicine, McMaster University Health Sciences Center, Hamilton, Ontario, Canada L8N 3Z5.

Extracellular guanosine or GTP (30-300µM), but not adenosine or ATP (0.3-300 µM) enhanced the neuritogenic effect of NGF in rat pheochromocytoma (PC12) cells. None of these purines induced significant neurite outgrowth in the absence of NGF. The effects of guanosine and GTP were synergistic with that of NGF in increasing the proportion of cells bearing long neurites. Adenosine - receptor agonists such as N^6-cyclopentyladeonsine (CPA), 5'-(N-cyclopropyl)-carboxamidoadenosine (CPCA) or 5'-N- ethylcarboxamideadenosine (NECA), also increased NGF-stimulated neuritogenesis, but to a lesser extent than did guanosine with NGF. High concentrations (1µM) of PACPX (1,3-dipropyl-8-(2-amino-4-chloro)xanthine, a preferential adenosine A_1 receptor antagonist, blocked the synergistic effect of guanosine and NGF. However, adenosine A_2 receptor antagonists DPMX (1,3-dipropyl-7-methylxanthine) or CGS15943 did not inhibit the effects of guanosine plus NGF. Interestingly, guanosine also acted synergistically with NECA (an adenosine A_1/A_2 receptor agonist) to induce significant neurite outgrowth in the absence of NGF, although neither of these compounds alone stimulated significant neurite outgrowth in PC12 cells. These data indicate that guanosine may not act via the purinergic P_1 (A_1 and A_2) receptors to enhance neuritogenesis. Moreover, neither ADPβS and ATP (purinergic P_2 receptor agonists) enhanced neurite outgrowth, indicating that the neuritogenic effects of GTP may not be mediated through classical P_2 purinergic receptors. Support: Hospital for Sick Children Foundation, Toronto. J.W.G. is a Neuro-Oncology Foundation Research Fellow.

421.1

BOVINE CHROMAFFIN GRANULE MEMBRANES UNDERGO CALCIUM-REGULATED EXOCYTOSIS IN XENOPUS LAEVIS OOCYTES [1]D.L. Scheuner, [2]C.D. Logsdon, and [1]R. W. Holz*, [1]Dept. of Pharmacol., [2]Dept. of Physiology, Univ. of Mich., Ann Arbor, MI 48109-0626.

We have devised a new method that permits the investigation of exogenous secretory vesicle function using bovine chromaffin granules and frog oocytes. Highly purified chromaffin granule membranes were injected into Xenopus laevis oocytes. Exocytosis was detected by the appearance of dopamine-β-hydroxylase (DBH) of the chromaffin granule membrane in the oocyte plasma membrane. The appearance of DBH on the oocyte surface was strongly Ca^{2+}-dependent and was stimulated by co-injection of the chromaffin granule membranes with $InsP_3$ or Ca^{2+}/EGTA buffer (18 μM free Ca^{2+}) or by incubation of the oocytes in medium containing the Ca^{2+} ionophore ionomycin. Similar experiments were performed with a subcellular fraction from cultured chromaffin cells enriched with [^{3}H]norepinephrine-containing chromaffin granules. The Ca^{2+}-dependent appearance of DBH on the oocyte surface correlated with [^{3}H]norepinephrine secretion. Therefore, it is likely that intact chromaffin granules and chromaffin granule membranes undergo exocytosis in the oocyte. This system may allow the study of secretory vesicle membrane constituents required for exocytosis.

421.2

GABA PRODUCTION AND RELEASE FROM CELLS PROGRAMMED TO EXPRESS GAD_{65} AND GAD_{67}. A. Sandrasagra[1], C.H.J. Ruppert[1], R.C. Weatherwax[1*], B. Anton[2], C.J. Evans[2], E.S. Schweitzer[3], and A.J. Tobin[1,4,5], Departments of [1]Biology, [2]Psychiatry and Biobehavioral Sciences, [3]Anatomy and Cell Biology, [4]Molecular Biology Institute, and [5]Brain Research Institute, University of California, Los Angeles CA 90024.

GABA is an important signalling molecule in both neuronal and non-neuronal systems, but the mechanisms of GABA production, GABA compartmentalization and GABA release are still obscure. To address these questions directly, we are using a retroviral GAD-cDNA expression system to obtain *in vitro* cell culture models of GABA producing cells that are of neuronal, neuro-endocrine, and pancreatic origin. We have obtained stable clones of PC12, and B65 neuroblastoma cells that express either or both GADs (GAD_{65} and GAD_{67}). These cells produce GAD mRNA(s), enzymatically active GAD, and GABA. In addition we are currently characterizing G418 resistant AtT-20 and RINm5F clones for GAD expression. Experiments that detail the calcium-dependent and calcium-independent release of GABA from the engineered cells are in progress. (Supported by NIH Grants NS23084-07 to E.S.S., and NS22256 to A.J.T.)

421.3

SKF 89976A INCREASES EXTRACELLULAR GABA IN RAT DORSOLATERAL STRIATUM IN VIVO. K.L.Drew* and U.Ungerstedt. Dept. Pharmacology, Karolinska Institute, Stockholm, Sweden.

The effects of SKF 89976A, a non-substrate inhibitor of GABA uptake, on extracellular levels of GABA in rat dorsolateral striatum were studied as a prelude to further studies of the role of the GABA transporter in voltage dependent GABA release. Procedures were as described in Drew and Ungerstedt (1991). SKF 89976A (0.02, 0.2 and 2.0 uM) was dissolved in modified Ringer's solution (1.2 mM $CaCl_2$, 0.85 mM $MgCl_2$, 2.7 mM KCl and 148 mM NaCl) and delivered through the probe. The drug dose dependently increased extracellular levels of GABA. SKF 89976A failed however, to significantly decrease veratridine-evoked release of GABA. Bupropion (6.0 uM), an inhibitor of dopamine uptake was also shown to increase extracellular levels of dopamine in rat dorsolateral striatum and to reduce veratridine-evoked release of dopamine. Further studies are required to determine if bupropion interfers with carrier mediated release of dopamine or if an increase in extracellular dopamine and consequent reduction in the frequency of cell firing explains the bupropion-induced inhibition of veratridine-evoked dopamine release.

421.4

EFFECT OF DRUG-INDUCED CHANGES IN THE DISPOSITION AND METABOLISM OF NEUROTRANSMITTER ON THE QUANTITATION OF ^{3}H-SEROTONIN (5HT) RELEASE. P.J. Monroe*, D.L. Smith, B.K. Kradel, D.J. Smith, and P. Blier, Dept. of Anesthesiology, West Virginia University Health Sciences Center, Morgantown, WV 26506 and Department of Psychiatry, McGill University, Montreal, Canada

While evaluating the effect of 2-methylserotonin (2-Me-5HT) on the release of ^{3}H-5HT from rat spinal cord tissue, it was determined that the drug altered the intrasynaptosomal disposition of the neurotransmitter. Superfusion of the tissue with 2-Me-5HT (0.3 - 10 μM) produced a concentration-dependent increase in ^{3}H-5HIAA efflux which occurred without a concomitant elevation in the efflux of ^{3}H-5HT. This effect was blocked by the co-superfusion of an uptake inhibitor, suggesting the drug disrupts intraneuronal storage of neurotransmitter following active uptake. The effect of 2-Me-5HT on ^{3}H-5HIAA efflux was slow in onset. Fifteen minutes was required before changes in ^{3}H-5HIAA efflux could be detected. However, this length of drug exposure is frequently achieved in the course of routine neurotransmitter release assays. Therefore, if total ^{3}H-efflux is quantitated (rather than separated neurotransmitter and metabolite), it is possible that a drug induced increase in ^{3}H-metabolite efflux could be mistakenly associated with an enhancement of depolarization-evoked ^{3}H-neurotransmitter efflux. For example, when calculated using total ^{3}H-efflux, 10 μM 2-Me-5HT caused an apparent 2.2 fold enhancement above the control (no 2-Me-5HT) response to a 15 mM K+ stimulus. On the other hand, when superfusate fractions were analyzed for ^{3}H-5HT and ^{3}H-5HIAA content, K^+-evoked increases in ^{3}H-5HT efflux were comparable in drug treated and control conditions. However, drug-induced increases in ^{3}H-5HIAA efflux were of the same magnitude as those seen from tissues which were not exposed to elevated K^+ concentrations. Thus, these results indicate that the separation of transmitter from metabolites is necessary to ensure that drug-induced changes in the disposition of neurotransmitter do not adversely affect the assessment of radiolabelled neurotransmitter release evoked via depolarization induced mechanisms.

421.5

RELEASE OF GLUTAMATE AND ASPARTATE FROM SYNAPTOSOMES OF HIPPOCAMPAL AREA CA1. M. Zhou, C.P. Duncan and J.V. Nadler*, Depts. Neurobiology and Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

When stimulated with elevated K^+, slices of hippocampal area CA1 release endogenous glutamate and aspartate in a 5:1 ratio. Release of these amino acids from CA1 slices originates predominantly from the Schaffer collateral-commissural projection and is Ca^{2+}-dependent. Axon terminals of this pathway thus appear to release both glutamate and aspartate. However, studies of neocortical synaptosomes have revealed little evidence of K^+-evoked aspartate release, in contrast to the substantial release evoked from neocortical slices. It has therefore been proposed that aspartate release in slice preparations reflects exchange of intracellular aspartate with released glutamate. This idea was tested by comparing glutamate and aspartate release from CA1 slices and synaptosomes.

Transverse slices of the rat hippocampal formation were prepared, area CA1 (excluding stratum lacunosum-moleculare) was isolated and synaptosomes were prepared by the method of phase partition. Synaptosomes were layered onto moistened filter paper disks and superfused in the same manner as CA1 slices. When superfused for 1 min with 25 μM K^+ or for 2 min with 300 μM 4-aminopyridine, the synaptosomes released both glutamate and aspartate in a Ca^{2+}-dependent manner. The glutamate/aspartate ratio was about 5:1. This result demonstrates that excitatory synaptic terminals can release aspartate, even under conditions where membrane transport of released amino acid is expected to be minimal. (Supported by NIH grant NS 16064.)

421.6

KINETIC ANALYSIS OF Na^+-DEPENDENT PROLINE AND GLUTAMATE UPTAKE IN RAT HIPPOCAMPAL FORMATION DURING POSTNATAL DEVELOPMENT. S. M. Cohen* and J. V. Nadler. Depts. Pharmacology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

Some of the excitatory glutamate pathways in the rat hippocampal formation, including the Schaffer collateral-commissural fibers and the lateral perforant path, take up proline by a high affinity Na^+-dependent process. The role of proline in glutamate transmission is unknown. This study compared the uptakes of proline and glutamate by crude synaptosomal preparations of the hippocampal formation during ontogenesis. Uptake of both amino acids was determined from the same tissue samples.

At all ages studied from 9 d onward, glutamate was taken up by a single high affinity, Na^+-dependent process ($K_T \approx 2$ μM). In contrast, proline was taken up by both high affinity ($K_T \approx 5$ μM) and low affinity ($K_T \approx$ 100-1000 μM) Na^+-dependent processes. High affinity uptake of proline increased 4-fold and high affinity uptake of glutamate increased 2.5-fold between 9 and 15 d after birth. At 15 d postnatal, glutamate uptake had reached adult values and it remained relatively constant afterward. In contrast, proline uptake remained elevated above adult values during the 15-21 d period. These results demonstrate that high affinity uptakes of proline and glutamate, which are co-expressed by many of the same pathways, develop during the same postnatal period. The developmental overshoot in proline uptake suggests that this process may play a more significant role in synaptic physiology during adolescence in the rat than during adulthood. (Supported by NIH grant NS 16064.)

421.7

EFFECTS OF ACUTE AND CHRONIC INSULIN TREATMENT ON NOREPINEPHRINE (NE) UPTAKE AND NE TRANSPORTERS IN RAT BRAIN AND PC-12 CELLS. D. Figlewicz Lattemann*, P. Szot, P.A. Israel, and C. Payne. Depts. of Psych. and Med., U. Washington, Seattle, WA 98195 and VA Medical Center, Seattle, WA 98108.

Previous work from this laboratory has led to the suggestion that some insulin (INS) receptors in the CNS may be located presynaptically on noradrenergic neurons. To test whether insulin may serve as a neuromodulator for NE neurons, we measured 3H-NE uptake into hypothalamic slices, and into the rat pheochromocytoma PC-12 cells. Acute INS treatment significantly inhibited 2-min NE uptake into hypothalamic slices and PC-12 cells over a concentration range of 0.1--10 nM, in good agreement with the Kd of the insulin receptor (-31±15% and -29±4% inhibition, respectively, with 10 nM insulin). Chronic INS treatment (96 hr) of partially serum-deprived PC-12 cells also resulted in significant inhibition of 3H-NE uptake (-45±9% inhibition with 10 nM insulin). Chronic third ventricular INS infusion *in vivo* resulted in a significant decrease of NE transporter mRNA levels vs. vehicle-treated controls (grain area=3598±716 vs 9782±592). Together, these results suggest that INS may play a physiologic role in the acute and chronic regulation of synaptic NE concentrations via modulation of its re-uptake within certain populations of NE neurons in the CNS.

421.8

R75231 IS AN IRREVERSIBLE INHIBITOR OF [^{3}H]NITROBENZYLTHIOINOSINE BINDING TO THE NUCLEOSIDE TRANSPORTER OF RABBIT CNS CORTICAL SYNAPTOSOMES. K.W.Jones*, and J.R.Hammond. Dept. of Pharmacology and Toxicology, University of Western Ontario, London, Ontario, Canada, N6A 5B8.

The nucleoside adenosine has a wide range of physiological effects including modulation of CNS neuronal activity. Adenosine effects are terminated primarily by uptake, via the nucleoside transporter, from the extracellular milieu into the cell. Inhibitors of the nucleoside transporter are able to increase extracellular adenosine levels, and thereby potentiate the effects of endogenously released adenosine.

R75231, a mioflazine derivative, has been reported to be unique as a extremely long-lasting, tightly-binding inhibitor of nucleoside transport (Van Belle and Janssen, Nucleosides and Nucleotides, **10**:975, 1991). We have examined the nature of R75231 interaction with the nucleoside transporter in rabbit CNS cortical synaptosomes, using [^{3}H]nitrobenzylthioinosine ([^{3}H]NBMPR) as a specific probe. Synaptosomes were incubated in the absence and presence of 4nM R75231 or 4nM nitrobenzylthioguanosine (NBTGR) for 45 min, after which, an aliquot of each was removed for analysis of [^{3}H]NBMPR (0.4nM) binding. The remaining synaptosomes were washed (10 min centrifugation at 40 000*g*, resuspension in ≈50 vol., 30 min incubation at room temperature) and the assay repeated. The inhibition of [^{3}H]NBMPR binding by NBTGR (≈70%) was eliminated after 2 washes, whereas the inhibition of [^{3}H]NBMPR binding by R75231 after 5 washes (≈85%) was not significantly different from that observed before washing, nor did inclusion of either 10mM adenosine or 10μM nitrobenzylthioguanosine in the wash procedure induce the dissociation of R75231. Saturation analysis of [^{3}H]NBMPR binding to synaptosomes showed that R75231 caused a decrease in B_{max} and an increase in K_D (ie. mixed inhibition). These results are consistent with a model in which R75231 binds irreversibly to membrane components associated with the nucleoside transporter.

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421.9

EFFECT OF CHOLINE ON BASAL ACETYLCHOLINE RELEASE *IN VIVO*. D.L.Marshall, M.D.Greaney[1], A.Erfurth and R.J.Wurtman. Dept. of Brain & Cogn. Sci., M.I.T, Cambridge MA 02139, [1]ESA, Bedford, MA 01730.

Acetylcholine (ACh) formation in brain neurons depends on choline (Ch) levels, because of the high K_M of choline acetyltransferase for this substrate. Using microdialysis, Farber *et al.* (1992) observed a slight increase in basal ACh release in anesthetised rats after intraperitoneal (i/p) Ch, using a high dialysate neostigmine concentration (10^{-5}M) to allow detection of the small amount of ACh present. This may have influenced ACh synthesis by depriving the neuron of a Ch source, thus making it more sensitive to exogenous Ch, or by causing excessive activation of presynaptic M_1 receptors, thus reducing ACh synthesis. To reduce such artefacts we examined ACh release using *low* neostigmine concentrations and a sensitive new ESA HPLC system for ACh. The effects of i/p Ch (120mg/kg) or saline on striatal ACh release were determined in anesthetised rats, using high (10^{-5}M) and low ($5x10^{-8}$M) neostigmine concentrations. With 10^{-5}M neostigmine, basal ACh release was unaffected one hour after Ch (n=6) or saline (n=4). However, with $5x10^{-8}$M neostigmine, ACh levels were significantly increased by 50±22% (mean±S.E.M.; n=6; $p<0.05$) one hour after Ch, but not changed (-6±11%; n=4) by saline. These initial data show that the ability of Ch to enhance basal ACh release is greater using low neostigmine concentrations.

Farber, S., Kischka, U. and Wurtman, R.J. (1992). In preparation.

421.10

MICRODIALYSIS WITH HYPERTONIC NaCl CAUSES CENTRAL RELEASE OF AMINO ACIDS AND DOPAMINE, T.Horn, L.Bauce, R.Landgraf and Q.J.Pittman*; Section of Biosciences, Univ.of Leipzig, Germany & Neuroscience Research Group, Univ. of Calgary, Canada.

Recent studies have shown that the neuropeptides arginine vasopressin (AVP) and oxytocin (OXT) are released within the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus in response to microdialysis of these nuclei with hypertonic perfusion media. These results suggest an inherent osmosensitivity of SON and PVN neurons. In order to investigate whether the observed release of these peptides is a unique phenomenon, several brain regions were examined for the release of amino acids or dopamine in response to hyperosmotic stimulation. Urethane-anaesthetized male Sprague-Dawley rats were perfused with five ion solution using chronically implanted U-shaped microdialysis probes (3 mm total length, 6 kDa cut off). Samples were collected at 30 min intervals and analyzed by HPLC.

In all perfusion areas, including the SON, PVN, dorsal hippocampus (DH) and striatum, concentrations of aspartate, glutamate, serine, glutamine, glycine, taurine and GABA were significantly increased during perfusion with hypertonic NaCl. This release was found to be dose dependent in the DH and striatum when tested with ion solution containing 0.25, 0.5 or 1M NaCl. In addition dopamine levels in striatal perfusates was significantly increased during hyperosmotic stimulation (1M NaCl). These results indicate that hyperosmotic stimulation with high NaCl affects the release of several neurotransmitters and is not specific for AVP and OXT. Furthermore, release of amino acids and dopamine may influence local release of neuropeptides and play a role in the previously described "rebound" release of AVP.

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421.11

AMPHIPHILIC WEAK BASES INCREASE EXTRACELLULAR DOPAMINE IN THE NUCLEUS ACCUMBENS OF FREELY MOVING RATS IN A NON-EXOCYTIC DOSE-DEPENDENT MANNER. E. Pothos*[1], D. Sulzer[2], S. Rayport[2] and B.G. Hoebel[1]. [1]Dept. of Psychology, Princeton Univ., Princeton, NJ 08544 and [2]Dept. of Psychiatry, Columbia Univ., Dept. Neuropathology, NYS Psychiatric Inst., New York, NY 10032.

As amphiphilic weakbases, amphetamine (AMPH) and phencyclidine (PCP) may release dopamine (DA) by reducing synaptic vesicle proton gradients, thereby discharging vesicular DA into the cytosol and, via the DA transporter (Sulzer et al., this meeting), into the extracellular space. Local infusion of either AMPH or PCP increases extracellular DA in the nucleus accumbens (NAC) of freely moving rats (Br. Res. Bull. 19:623, 1987; Life Sci. 42:1713, 1988) and abolishes pH gradients in cultured DA neurons (Neuron 5:797, 1990). To show that weak base action mediates psychostimulant-like synaptic DA elevation in vivo, we infused ammonium chloride (NH_4Cl), benzylamine ($C_6H_5CH_2NH_2$) and methylamine (CH_3NH_2) into the NAC of freely moving rats by reverse dailysis over 30 minutes. All compounds increased extracellular DA in a dose-dependent manner (n=6 for 25, 50, 75 and 100mM; $p<.05$). DOPAC and HVA levels increased significantly during ammonium or methylamine infusions ($p<.05$); in contrast, DOPAC output decreased significantly ($p<.05$), but HVA output did not change during benzylamine infusions. The addition of 15uM TTX in physiological Ringer's or 1mM verapamil in CA^{2+}-free Ringer's reduced DA and metabolite basal levels but failed to attenuate the weak base-induced DA increase or the increase in metabolites. These results suggest that (a) amphiphilic weak bases not known as drugs of abuse mimic the effects of AMPH or PCP in vivo, (b) their mechanism of action involves non-exocytic DA release, and (c) benzylamine like AMPH, but not like ammonium or methylamine, seems to act as a DA transporter substrate and/or monoamine oxidase inhibitor.

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421.12

ELECTROGENIC TRANSPORT OF GABA BY HORIZONTAL CELLS OF THE CATFISH RETINA. J. Cammack and E. A. Schwartz*, Dept. Pharmacological & Physiological Science, University of Chicago, Chicago, IL 60637.

GABA transport has two roles in synaptic transmission. GABA is removed from the extracellular space when a cell is hyperpolarized, and released into the extracellular space when a cell is depolarized[1]. Membrane voltage plays an essential role in regulating influx and efflux. We have investigated the electrophsyiology of GABA transport in solitary horizontal cells whose membrane voltage was controlled with a whole-cell voltage clamp. Two GABA-activated currents were identified. A GABA-activated Cl^- curent could be blocked with 0.5 mM picrotoxin. Current produced by GABA transport was voltage dependent, and was blocked by the GABA uptake inhibitors nipecotic acid and NNC-711. Transport currents produced by influx and efflux were observed. The dependence of each component on monovalent cations and anions was determined. The electrical properties of GABA transport can be compared to the properties of glutamate transport previously characterized in retinal Müller cells[2].

1. Schwartz, E. A. (1987). *Science* **238**, 350-355.
2. Schwartz, E. A. & Tachibana, M.(1990). *J.Physiol.* **426**, 43-80.

422.1

DELAYED INCREASE OF EXTRACELLULAR ARGININE (ARG), THE NITRIC OXIDE (NO) PRECURSOR, FOLLOWING ELECTRICAL WHITE MATTER STIMULATION IN RAT CEREBELLAR SLICES. C. Hansel[1], A. Batchelor[2], M. Cuenod*[1] J. Garthwaite[2], T. Knöpfel[1] and K.Q. Do[1]. [1]Brain Res. Inst., Univ. of Zürich, Switzerland ; [2]Dept. of Physiology, Univ. of Liverpool, U.K.

Amino acid levels were measured in perfusates from L-shaped rat cerebellar slices installed in a Krebs-filled three-compartment system following electrical white matter stimulation. The lateral compartments housed white matter and a cortical section containing parallel fibres respectively, whereas the central compartment housed cortical structures at the point of bending. This arrangement allows electrical stimulation while the perfusion medium passing the central chamber can be collected for amino acid analysis with HPLC. Following a 2-minutes stimulation period of either 2 Hz (n=6) or 5 Hz (n=5) Arg levels were significantly rised above levels already present in the Krebs-solution (6.62±1.75 pmol/min respectively 7.31±2.66 pmol/ min). Arg is the precursor of NO, a neuronal messenger in the brain, which is synthesized by NO synthase. Since Arg and the NO synthase are located in different cell types it can be suggested that Arg passes through the extracellular space in order to replenish the precursor pool for NO synthesis.

422.2

IN VIVO MICRODIALYSIS OF NITRIC OXIDE-DEPENDENT cGMP EFFLUX IN THE RAT CEREBELLAR CORTEX. D. Luo*, E. Leung and S.R. Vincent. Kinsmen Laboratory of Neurological Sciences, Department of Psychiatry, The University of British Columbia, Vancouver, B.C. Canada.

Nitric oxide activates soluble guanylyl cyclase causing a transient increase in cGMP levels in target cells. We have found using intracerebral microdialysis that nitric oxide also mediates an increase in the extracellular levels of cGMP. Rats were implanted with a transverse microdialysis probe in the cerebellar cortex, which was perfused two days later with an artificial CSF at 5 µl/min. Samples were collected and assayed for cGMP by radioimmunoassay. Perfusion at ultra-slow flow rates gave an approximation for the basal extracellular cGMP concentration of 4 nM. Inhibition of cerebellar phosphodiesterase activity with IBMX in the dialysate doubled the levels of cGMP. Pharmacological activation of the climbing fiber input to the cerebellum, with systemic harmaline, produced a rapid, 12-fold increase in the extracellular levels of cGMP. This was dose-dependently attenuated by prior administration of various inhibitors of nitric oxide synthase, either systemically, or via the microdialysis probe. Blockade of sodium channels with TTX, or removal of calcium from the microdialysate also blocked the harmaline-induced increase in cGMP. Inhibition of soluble guanylyl cyclase, with methylene blue or LY83583 in the perfusion solution, also inhibited the cGMP increase. These treatments also decreased the basal levels of cGMP. These results indicate that activation of the climbing fiber synapses in the cerebellar cortex stimulates the production of NO which in turn causes a large increase in the extracellular levels of cGMP. The efflux of cGMP may serve to decrease intracellular cGMP levels. It may also indicate a role for cGMP as an intercellular messenger.

(Supported by the Medical Research Council of Canada)

422.3

NITRIC OXIDE SYNTHASE-CONTAINING CELLS IN THE CEREBRAL CORTEX OF RATS R.J. Weinberg*[1], I.G. Valtschanoff[1], V.N. Kharazia[1], M. Nakane[2], H.H.H.W. Schmidt[3] and A. Rustioni[1] [1]Dept. Cell Biology & Anatomy, UNC, Chapel Hill, NC 27599; [2]Dept. Vascular Research, Abbott Labs, Abbott Park, IL 60064; and [3]Dept. Pharmacology, Northwestern U., Chicago, IL 60611.

We have employed histochemistry for NADPH diaphorase and immunohistochemistry using antibodies prepared against neuronal nitric oxide synthase (NOS-1) to reveal neurons and fibers in the rat cortex likely to synthesize nitric oxide. Nembutal-anesthetized animals were perfused with mixed aldehydes; Vibratome sections 20-100 µm thick were stained for NOS. The distribution of NOS-stained cells was plotted; detailed drawings showing their morphology were made with camera lucida. Particular attention was focused on sensorimotor cortex.

NOS-positive neurons comprised about 0.5% of neurons in SmI. They were densest in layers 2, 3 and 6 and in the subcortical white matter. Stained neurons were morphologically heterogeneous, but did not include typical pyramidal neurons. Most had aspiny dendrites and axons branching close to the soma. Stained axons had predominantly radial orientation. Boutons were visible throughout the cortex, densest in layers 4 and 6. The morphological impression that NOS-positive neurons are local circuit neurons was supported by double-label experiments in which injections of CTB-gold or WGA-HRP were placed in contralateral cortex, thalamus, caudoputamen or spinal cord. In all cases, retrogradely labeled neurons and NOS-positive ones belonged to separate populations. In tangential sections through the barrel field NOS-positive neurons and dendrites were confined to the septa between barrels. Preembedding and postembedding immunocytochemistry in combination with NADPH diaphorase histochemistry demonstrated that a large fraction of NOS-positive somata also contained GABA.

422.4

NITRIC OXIDE GENERATION RELEASES [^{3}H]-NOREPINEPHRINE FROM HIPPOCAMPAL SLICES. K.M. Johnson* and G. Lonart, *Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77555-1031.*

Nitric oxide (NO), a diffusible second messenger, has been suggested to potentiate neurotransmitter release. Cross-chopped hippocampal slices (300 X 300 µm) were prepared from male Sprague-Dawley rats, washed and incubated with [^{3}H]-norepinephrine ([^{3}H]-NE) for 30 min in Krebs- bicarbonate buffer, containing 10 µM pargyline and saturated with O_2/CO_2 (95:5). Efflux of [^{3}H]-NE was determined and the data are expressed as fractional release. Hydroxylamine (3-300 µM), which is known to generate NO subsequent to cellular metabolism, induced a concentration dependent increase in the basal [^{3}H]-NE release. The EC_{50} value was about 30 µM and 100 µM hydroxylamine produced a maximal 10-fold increase in the basal [^{3}H]-NE release. 10 µM hydroxylamine increased basal [^{3}H]-NE release about 2-fold. Since NO stimulates the activity of guanylate cyclase, we tested the effect of methylene blue, a inhibitor of this enzyme. 3 µM methylene blue alone induced a steady 2-fold increase in the basal [^{3}H]-NE release and significantly potentiated the response to 10 µM hydroxylamine. 20 min pretreatment with 3 µM or 10 µM hydroxylamine did not significantly influence NMDA (300 µM) stimulated [^{3}H]-NE release, while higher concentrations had inhibitory effects. Ongoing experiments are being carried out to characterize the mechanism underlying the effects of hydroxylamine and methylene blue in this preparation. Supported by DA-02073.

422.5

AN NMDA/NITRIC OXIDE PATHWAY MEDIATES LIGHT RESPONSIVENESS BY THE SUPRACHIASMATIC NUCLEUS. S. Amir*, CSBN, Concordia University, Montreal, Quebec, Canada.

The messenger molecule nitric oxide (NO) is produced in brain on stimulation of N-methyl-D-aspartate (NMDA) receptors, and receptors for NMDA have been implicated in the transmission of light to the hypothalamic suprachiasmatic nucleus (SCN), site of a circadian pacemaker. We assessed the involvement of the NMDA/NO pathway in SCN responsiveness to light by blocking NMDA receptors or NO production in the SCN and evaluating the effect on heart rate during photic stimulation in dark-adapted urethane-anaesthetized rats. Photic stimulation (300 Lux, 3 min) caused a rapid increase in heart rate (Δ = 37.3±4.4 bpm, n=8). This effect was blocked by prior infusion of a competitive NMDA antagonist (CPP, 20 nmol) in the SCN (Δ = 12.9±1.6 bpm, n=8, p<0.01). Infusion 2 mm dorsal to the SCN had no effect, suggesting a role for NMDA receptors specific to the SCN. Infusing a competitive blocker of NO synthesis, N^G-nitro-L-arginine methyl ester (L-NAME; 40 nmol) in the SCN, but not 2 mm dorsal to the SCN, blocked the rise in heart rate in response to light (Δ = 16.1±1.8 bpm, n=16, p<0.01). This effect was selective and stereospecific: addition of L-arginine (80 nmol), which competes with L-NAME for the substrate site on the NO-generating enzyme, reversed the effect of L-NAME (Δ = 42.1±4.9 bpm, n=8), and infusion of an inactive isomer of L-NAME, D-NAME (40 nmol), had no effect (Δ = 38.8±5.4 bpm, n=12). The ability of light to stimulate heart rate and of infusion of a competitive NMDA antagonist or a competitive blocker of NO production in the SCN region to inhibit this effect suggests a functional link between activation of an NMDA/NO pathway and SCN light responsiveness.

422.6

EVIDENCE THAT NITRIC OXIDE (NO) MEDIATES THE CYCLIC GMP RESPONSE TO SYNAPTIC ACTIVITY IN THE RAT SUPERIOR CERVICAL GANGLION (SCG). Marion L. Hughes[1], Hong Sheng[2], Ferid Murad[2], and Clark A. Briggs[1]*. [1]Neuroscience Research and [2]Signal Transduction, Abbott Laboratories, Abbott Park , IL 60064.

Preganglionic stimulation increases cyclic GMP levels in the SCG. It is not known what neurotransmitter mediates this response. However, exogenous nitroprusside and azide, which liberate NO, also have been shown to stimulate cyclic GMP synthesis (Volle & Quenzer 1983). We investigated whether NO is the physiological mediator of the cyclic GMP response to preganglionic stimulation.

Paired SCG were isolated and superfused *in vitro* at ambient temperature (21-23°C) with oxygenated Locke's solution containing 0.3 mM 3-isobutyl-1-methyl-xanthine. Various drugs were applied for a period of 75-85 minutes before stimulation. In each pair, one SCG served as a non-stimulated control and the other received a preganglionic stimulation of 10 Hz for 30 sec. Cyclic GMP was determined by radioimmunoassay.

Upon stimulation, cyclic GMP levels increased 8-fold and this response was Ca^{2+} dependent. The NO synthase inhibitor, N^G-nitro-L-arginine (L-NNA), inhibited the cyclic GMP response in a concentration dependent manner which was partially reversed by the NO synthase substrate, L-arginine (1 mM). L-NNA (10 µM) blocked the response and this effect was stereoselective because D-NNA (10 µM) showed no inhibition. Additionally, the cyclic GMP response was inhibited 65% by 10 µM oxyhæmoglobin. Thus, it appears that NO or a similar substance mediates, in an extracellular fashion, the cyclic GMP response to synaptic activity in the rat SCG.

422.7

TRANSCRIPTION OF THE BRAIN NITRIC OXIDE SYNTHASE GENE IN NEURAL CELL CULTURES. D.Minc-Golomb & J.P.Schwartz*. Clinical Neuroscience Br., NINDS, NIH, Bethesda, MD 20892.

Nitric oxide synthase (NOS) generates the second messenger nitric oxide from arginine. The brain NOS has been cloned and found in highest density in the cerebellum and accessory olfactory bulb. We now present data on the expression of the NOS gene in cerebellar granule cells (CGCs), prepared from postnatal day 8 rat pups, after 10 days in culture. Using specific oligonucleotide primers to the brain NOS gene, reverse transcription of CGC RNA followed by polymerase chain reaction (RT-PCR) produced a 366 bp fragment, the expected size. Identity was confirmed by hybridization of a blot containing the electrophoresed product with a full-length cDNA probe for NOS, followed by high stringency washes (0.1XSSC-65°), which showed a positive signal for the same 366bp band. NOS activity was also detected in CGCs, by measuring conversion of arginine to citrulline, thus showing that CGCs express the brain NOS. Preliminary results suggest that type 1 astrocytes also express brain NOS mRNA and activity. These culture systems are being used to assess the modulation of NOS in cerebellar neural cells both in physiological and neurotoxic conditions.

422.8

CARBON MONOXIDE AS A PHYSIOLOGICAL MESSENGER INDICATED BY HEME OXYGENASE LOCALIZATIONS AND CYCLIC GMP REGULATION. David J. Hirsch*, A. Verma, C. Glatt, G. Ronnett and S.H. Snyder. Dept of Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD, 21205.

Abundant evidence indicates that nitric oxide(NO) is a major physiologic molecule, accounting for endothelial derived relaxing factor(EDRF) of blood vessels, the turmoricidal and bactericidal actions of macrophages, and serving as a putative neurotransmitter in the central and peripheral nervous system. The notion that a normally noxious gas such as NO might serve as a physiologic messenger raises the possibility that other gases might have similar functions. One such candidate might be carbon monoxide. Carbon monoxide(CO) is endogenously generated by the enzyme heme oxygenase. Heme oxygenase(HO), in concert with cytochrome P450 reductase, catalyzes the conversion of heme into biliverdin with the concomitant release of CO. We thought that this CO could function in an analogous manner to NO. The brain has been previously reported to have high levels of HO. We localized HO2, the constitutive form of this enzyme, by *in situ* hybridization. Highest densities of HO2 mRNA are evident in the olfactory neurons of the olfactory epithelium as well as the olfactory neuronal layer of the olfactory bulb along with the granule cells in the bulb. We also observed discreet localizations in the cerebellum, hippocampus, pontine nucleus, olfactory tubercle, and habenulae. To investigate the physiological role of CO we utilized a primary culture of olfactory neurons which expressed HO2 at high levels as revealed by the *in situ* localizations. These neurons have very high levels of cGMP which could be potently blocked by hemoglobin but were insensitive to inhibitors of nitric oxide synthase(NOS). When zinc protoporphyrin-9 (Zn PP-9) which is a potent inhibitor of HO was added to the cultures, cGMP levels plummeted These findings suggest that CO might be a messenger molecule similar to NO

422.9

G_{OLF} EXPRESSION IN THE BRAIN: MOLECULAR CLONING, *IN SITU* HYBRIDIZATION AND IMMUNOHISTOCHEMISTRY.

L.A. Williams*, C.D. Hodson, T.P. Snutch & S.R. Vincent. Dept. Psychiatry & Biotechnology Lab., Univ. of British Columbia, Vancouver, B.C. Canada.

Striatal neurons express high adenylyl cyclase activity and large amounts of a stimulatory G protein, but very low levels of Gsα mRNA. We have previously presented evidence suggesting that the novel stimulatory G protein Golf is expressed in the basal ganglia. In support of this hypothesis, we have now used synthetic oligonucleotides, derived from the published Golf cDNA sequence, to identify and characterize four independent cDNAs from a rat brain cDNA library. These code for a protein of 381 amino acids with a predicted molecular mass of 44.3 kD, whose sequence is identical to the Golf sequence originally derived from a rat olfactory epithelium library, except for one conservative amino acid substitution. Thus Golf is expressed in rat brain. To determine the cellular localization of Golf in the brain *in situ* hybridization and immunohistochemistry were used. Golf mRNA was expressed in neurons in the striatum, nucleus accumbens and olfactory tubercle. For the immunohistochemical localization of Golf, rabbits were immunized with a synthetic peptide corresponding to amino acids 73-87 of Golf, conjugated to KLH. This yielded an antibody that upon affinity-purification, specifically labelled a striatal protein of about 46 kD in Western blots. In immunohistochemical studies, this antibody selectively stained many neurons within the striatum, nucleus accumbens and olfactory tubercle. In addition, stained fibers were seen leaving the striatum, and forming terminal networks in the globus pallidus, entopeduncular nucleus and substantia nigra pars reticulata. In conclusion, striatal projection neurons express a novel stimulatory G protein, Golf, which may couple the D1 receptors in these neurons to adenylyl cyclase.

(Supported by The Parkinson Foundation of Canada)

422.10

CHARACTERIZATION OF THE HIGH-AFFINITY FORSKOLIN BINDING SITE IN THE RAT STRIATUM. S.R. Vincent* and B. Dhillon. Kinsmen Laboratory of Neurological Sciences, Department of Psychiatry, The University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

The diterpene forskolin is a potent activator of adenylyl cyclase. We have characterized the high-affinity binding site for [^{3}H]-forskolin in the rat striatum using quantitative autoradiography. Saturation analysis revealed a single high-affinity binding site with a Kd of 26 nM and a Bmax of 694 fmole/mg tissue. Lesion studies indicate that these binding sites are largely present on striatal neurons, including the striato-nigral, medium-spiny, projection neurons which contain D1 dopamine receptors. The abilities of various forskolin analogues to displace high-affinity [^{3}H]-forskolin binding correlated well with their effectiveness in activating adenylyl cyclase. The density and affinity of the striatal binding sites were decreased if Mg^{2+} was absent from the incubation medium. This is consistent with the hypothesis that binding of forskolin to adenylyl cyclase involves an interaction with a stimulatory G protein. However, pretreatment of rats with SCH-23390, which blocks D1 dopamine receptors, and should therefore largely reduce the ability of endogenous dopamine to activate stimulatory G proteins in striatal neurons, had no effect on forskolin binding. Similarly, pretreatment with the atypical neuroleptic clozapine, a potent inhibitor of dopamine-stimulated adenylyl cyclase, was without effect on high-affinity forskolin binding in the striatum. These data suggest that endogenous D1 receptor activation does not account for the very high levels of forskolin binding present in the striatum.

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422.11

P_{2Y} PURINOCEPTOR-MEDIATED PHOSPHOINOSITIDE TURNOVER, Ca^{2+} INFLUX AND HOMOLOGOUS DESENSITIZATION IN C_6-GLIOMA. W.W. Lin* and D.-M. Chuang[1]. Dept. of Pharmacology, National Taiwan Univ. Taipei, Taiwan and [1]Biological Psychiatry Branch, NIMH, MD 20892.

In response to ATP, C_6-glioma cells accumulated inositol phosphates and elevated $[Ca^{2+}]_i$ dose-dependently with an EC_{50} of 60 μM and 10 μM, respectively. For stimulation of phosphoinositide (PI) turnover, the rank order of potency of adenine nucleotides was ATP>ADP>ADPβS >>AMP, adenosine, α,β-MeATP, β,γ-MeATP. ATP-stimulated PI metabolism was found to be partially dependent on $[Na^+]_o$ and $[Ca^{2+}]_o$, but resistant to tetrodotoxin, amiloride, ouabain and inorganic Ca^{2+} blockers. In Ca^{2+}-free medium, ATP caused only a transient increase in $[Ca^{2+}]_i$ as opposed to a sustained $[Ca^{2+}]_i$ increase in normal medium. The ATP-induced elevation of $[Ca^{2+}]_i$ was resistant to Na^+ depletion, verapamil and nisoldipine, but was attenuated by La^{3+}. The ATP-induced PI hydrolysis showed homologous desensitization following agonist prestimulation and was unaffected by PKC inhibitors (staurosporine, H-7) and depletion of PKC activity. On the contrary, the inhibition caused by PMA or octylindolactam V was inhibited by staurosporine, H-7 and polymyxin B. Our results suggest that P_{2Y} purinoceptors are coupled to PI turnover and Ca^{2+} influx in C_6-glioma and its desensitization does not involve PKC activation.

422.12

FACTORS INFLUENCING THE EFFECT OF CALRETININ ON PHOSPHORYLATION OF A 39 kDa MITOCHONDRIAL PROTEIN FROM RAT BRAIN. **L. Winsky* and D.M. Jacobowitz. Lab. of Clin. Sci., NIMH, Bethesda, MD**

The calcium binding protein calretinin (CR, 100 to 1000 nM) produced a significant reduction in the phosphorylation of a 39 kDa band in mitochondrial membranes. This 39 kDa band was maximally phosphorylated within 15 sec while the inhibition by calretinin was most apparent after 2 min. Both the phosphorylation of the 39 kDa protein and inhibition by calretinin were pH dependent. The 39 kDa band was phosphorylated in the presence of 5 mM EDTA and a stimulation was observed in the presence of Mg^{2+} (4.5 or 10 mM) or 4.5 mM Mn^{2+}, Ni^{2+}, Ca^{2+} or Co^{2+}. The inhibitory effect of calretinin was enhanced in the presence of Mg^{2+}, Co^{2+} and $Ni^{2+}$and was attenuated by 5 mM EDTA. Zn^{2+} (4.5 mM) inhibited both the phosphorylation of the 39 kDa band and the inhibition of phosphorylation produced by calretinin. Calretinin also produced a slight inhibition in the phosphorylation of a 42 kDa band in the presence of 4.5 mM Ni^{2+}, or Co^{2+}. This band was tentatively identified as the alpha subunit of pyruvate dehydrogenase based on immunoblots and comigration with purified phosphoenzyme. These results suggest a possible functional role of calretinin in modifying mitochondrial enzyme activity through effects on protein phosphorylation.

422.13

MOBILIZATION OF INTRACELLULAR CALCIUM IN CULTURED GLIA CELLS FROM THE RAT NEURAL LOBE BY VASOPRESSIN CAN BE INHIBITED BY OPIOID PEPTIDES. C.J.C. Boersma*, F.W. van Leeuwen, W.G. O'Brien[1], G.J. Law[1], W.T. Mason[1] and J.R. Bicknell[1] Netherlands Institute for Brain Research, Amsterdam, the Netherlands. and [1]Department of Neuroendocrinology, AFRC, Babraham, Cambridge, U.K. (Spon: ENA)

Evidence is accumulating that the resident astrocytes (pituicytes) in the neural lobe of pituitary are involved in the regulation of the release of neurohormones (oxytocin and vasopressin) into the circulation. Pituicytes possess opioid binding sites and the modulatory actions of opioids on neurosecretion have been extensively studied. Using real time quantitative fluorescence microscopy and ratiometric imaging (Miracal, Applied Imaging, Santa Clara, USA) we measured changes in free intracellular calcium $(Ca^{2+})_i$ levels in FURA-2 loaded pituicytes in culture in response to administration of Dynorphin 1-17 (DYN 1-17) and/or AVP.

Hatton et al. (Brain Res., in press) have previously demonstrated that administration of 10 nM AVP results in a large transient mobilisation of Ca^{2+} which can be blocked by V_1-antagonists. We have now shown that preincubation with DYN 1-17 both delays and decreases the $(Ca^{2+})_i$ response to AVP, effects which can be blocked by Naloxone. DYN 1-17 by itself does not affect basal $(Ca^{2+})_i$ levels. The present results show that opioid receptors are present on pituicytes and are coupled to a second messenger pathway by which they can affect Ca^{2+} mobilising effects of other neuropeptides, such as AVP. Phosphoinositides (PI) are likely candidates for this pathway as AVP application activates PI turnover.

422.14

THE EFFECT OF GUANINE NUCLEOTIDES ON [^{3}H]-IDAZOXAN BINDING SITES IN HUMAN AND GUINEA-PIG CEREBRAL CORTEX. J.F.Hussain, V.G.Wilson and D.A.Kendall. SPON: Brain Research Association. Dept. Physiology and Pharmacology, University of Nottingham Medical School, Nottingham NG7 2UH, U.K.

Idazoxan is an imidazoline compound which binds to both α_2-adrenoceptor and non-adrenoceptor imidazoline binding sites (NAIBS) in a variety of tissues and species. We have examined the potential interaction of NAIBS with G-proteins, investigating whether GTP alters [^{3}H]-idazoxan binding to human and guinea-pig cortical membranes in the presence and absence of α_2-adrenoceptor blockade.

Membranes were incubated with 1-2 nM [^{3}H]-idazoxan in Tris HCl buffer (pH 7.4) for 60-90 mins at 25°C in a total volume of 0.5 ml. Non-specific binding was defined by 10 μM cirazoline; bound radioligand was recovered by rapid vacuum filtration and quantified by liquid scintillation spectrometry.

The selective α-antagonists rauwolscine and corynanthine were used to identify [^{3}H]-idazoxan binding to NAIBS. A concentration of 10 μM rauwolscine was used to block [^{3}H]-idazoxan binding to α_2-adrenoceptors. Of the total binding sites labelled by [^{3}H]-idazoxan, 40 % in the human cortex and 30% in the guinea-pig cortex were NAIBS. Saturation curves were then constructed using increasing concentrations of [^{3}H]-idazoxan in the presence and absence of 10 μM rauwolscine and 300 μM GTP. The B_{max} values in the presence of GTP were significantly less than those without GTP, regardless of α_2-adrenoceptor blockade.

These results suggest that NAIBS may activate an intracellular mechanism via interaction with GTP-binding proteins and that idazoxan may be acting as an agonist at these sites.

JF Hussain is a SERC CASE student in conjunction with Syntex (UK).

422.15

PURIFICATION AND CHARACTERIZATION OF BOVINE FOREBRAIN CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II M. Tanji*, R. Horwitz & J. C. Waymire, Department of Neurobiology & Anatomy, University of Texas Medical School., Houston, TX 77030.

Calcium/calmodulin-dependent protein kinase II (Ca^{2+}/CaM kinase), is an abundant brain protein proposed to mediate Ca^{2+}-regulated signal transduction in neuronal tissue. The enzyme purified from rat brain is reported to exists as isoenzymes (M_r 550,000 & 615,000) that vary in their relative ratio of three subunits, M_r 50,000, 58,000, and 60,000.

In this study we have purified Ca^{2+}/CaM kinase from bovine forebrain. The tissue was frozen in liquid N_2 within 5 min of the animals death. The purification revealed two isoenzymes with apparent Ca^{2+}/CaM kinase properties based on the phosphorylation of a known Ca^{2+}/CaM kinase substrate peptide (MHRQETVD). The two isoenzymes have similar purification characteristics, subunit composition, and physical properties. Native gradient-gel electrophoresis provided an estimated M_r of 560,000 for the major isoenzyme and 870,000 for the minor isoenzyme. The autophosphorylated subunits of the major kinase have apparent M_r of 72,000 and 59,000 while those of the minor kinase are M_r 72,000 and 53,000. Both bovine kinases bind calmodulin. The major kinase weakly cross-reacts with monoclonal anti-rat Ca^{2+}/CaM kinase IgG in a spot blot assay while the minor kinase dose not cross-react at all. Western blotting technique using polyclonal anti-rat kinase IgG shows that a M_r 72,000 subunit of bovine kinase cross-reacts with the antibody. These results suggested that although the two isoenzymes of bovine kinase are similar to rat Ca^{2+}/CaM kinase, their are several important differences between the rat and bovine enzymes. Supported by USPH NS11061.

422.16

MULTIPLE MECHANISMS OF SLOW SYNAPTIC EXCITATION IN MYENTERIC NEURONS. P. P. Bertrand*, J. J. Galligan. Dept. Pharmacol./Toxicol., Michigan State University, MI 48824.

Conventional single electrode voltage clamp recordings were made in vitro from individual myenteric neurons of guinea pig ileum; (holding potential -70 mV). Drugs were applied by superfusion or by pressure ejection from a micropipette; sEPSCs were elicited by focal stimulation of interganglionic nerve tracts (500 ms, 40 V, 20 Hz). Myenteric neurons fell into two categories, S-cells (receiving fast nicotinic input) or AH-cells (spike afterhyperpolarization > 1 s). Superfused forskolin (.01-3 μM) produced an inward current in S and AH cells (EC_{50}, 80 and 200 nM respectively). IBMX (25 μM) produced a similar current in AH, but not S cells (n=5). IBMX potentiated submaximal responses to forskolin (< 100 nM) but did not affect maximal doses of forskolin (1-3 μM) or substance P (SP) (0.1-0.3 μM). In 75% of cells (n=59), forskolin-induced currents were associated with a decreased conductance (g_M) (max. current: 230 pA/S, 283 pA/AH). SP or the NK-3 agonist senktide (.01-.3 μM) produced a transient (1-1.5 min.) inward current with an initial decrease in g_M, and a later increase in g_M (max. late current: 423 pA/S, 530 pA/AH). Nerve stimulation produced an inward current that reversed near E_K within the first 5 seconds, and a secondary current with an unclear g_M change (max. late current: 200 pA/S, 304 pA/AH). Reversal potentials were determined for forskolin (-111 mV), SP (early: -98 mV, late: -9 mV) and the sEPSC (early: -80 mV). Forskolin (1 μM) occluded the sEPSC by 50%/S and 57%/AH as did SP (100 nM)(98%/S, 85%/AH). Forskolin did not occlude the peak late current from pressure applied senktide (3 μM), but preferentially occluded the early g_M decrease. These data suggest that increases in cAMP induced by forskolin can only mimic part of the conductance change seen during the sEPSC. It is possible that one or more transmitters with multiple transduction pathways and ionic mechanisms mediate the sEPSC. (Supported grant DK 40210)

422.17

IDENTIFICATION OF SIGMA RECEPTORS AND THEIR POTENTIAL ROLE IN ARACHIDONIC ACID RELEASE IN RAT CEREBELLAR GRANULE CELLS. J.B. Starr and L.L. Werling*. Dept. Pharmacology, The George Washington University Medical Ctr., Washington, D.C. 20037.

The sigma receptor has been well characterized in terms of ligands that bind to it, but its functional significance has remained elusive. The goals of this study were to 1) identify its presence in neonatal rat cerebellar granule cells in culture and 2) to test its potential role in the arachidonic acid (AA) cascade in these cells.

Cerebella of eight-day-old neonatal rats were dissected and chopped into pieces. Cells were mechanically and enzymatically dissociated, subjected to differential centrifugation, resuspended and plated on poly-D-lysine coated dishes. Cultures were treated with 10 μM cytosine arabinofuranocide to prohibit growth of non-neuronal cells. Cells were allowed to develop for at least eight days in culture (DIC) before use in any assay.

Sigma receptors were identified by the specific binding of 2 nM [^{3}H]haloperidol in the presence of spiperone to prohibit labeling of dopamine receptors. The sensitivity of [^{3}H]haloperidol binding to a variety of competing ligands supported identification of the receptors labeled as sigma. A 10 μM concentration of the sigma-selective agonist (+)pentazocine inhibited approximately 40-50% of specific binding. The binding of [^{3}H]haloperidol and its competition by (+)pentazocine was stable over a period of 3 days (8-11 DIC). This interval was chosen for second messenger studies.

Cells in culture were incubated with 1 μCi/dish [^{3}H]arachidonic acid ([^{3}H]AA) overnight, then washed to remove residual, unaccumulated labeled eicosanoid. The release of [^{3}H]AA was stimulated by 50 μM NMDA, resulting in the release of approximately 0.5% of total accumulated [^{3}H]AA. About 30% of stimulated release could be inhibited by 10 μM (+)pentazocine.

Collectively, these findings provide the first evidence that sigma receptors reside on cerebellar granule cells, and that they may be involved in the regulation of the release of arachidonic acid in these cells. This is the first direct association of sigma receptors with this metabotropic second messenger system.

422.18

ARACHIDONIC ACID METABOLISM MAY INVOLVE VOLTAGE-SENSITIVE COMPONENTS IN ACUTELY DISSOCIATED EMBRYONIC CHICK SPINAL CORD CELLS. K.S. Madden*, A. Prasad, S.V. Smith, and G.D. Lange. Laboratory of Neurophysiology and Instrumentation and Computer Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892

Prostaglandin E_2 and most other types of eicosanoids are metabolites of arachidonic acid (AA) formed by multi-factorial enzymatic pathways with components distributed throughout cell membranes and the cytosol. The pathways can be blocked at particular branch points by exposing preparations to various inhibitors of AA metabolism. We studied the effects of several of these inhibitors on the steady-state membrane potential of individual chick cells using two different electrophysiological approaches. Flow cytometry coupled with a voltage-sensitive oxonol dye monitored the effects of bath applications on populations of cells in suspension. Conventional whole cell patch techniques for intracellular voltage recordings measured the effects of superfusing single plated cells that were not stained with the oxonol dye. We found that four molecularly different agents, indomethacin, ETYA (5,8,11,14-eicosatetraynoic acid), BW755C and NDGA (nordihydroguiaretic acid) decreased the intensity of oxonol signal and hyperpolarized cells at concentrations known to block oxygenase activity. Subsequent exposure to PGE_2 did not counteract these effects unless preparations were first depolarized by elevating extracellular potassium ion concentrations. Then, the PGE_2 increased the hyperpolarization elicited by the drugs. These findings support the possibility that PGE_2 acts through an intracellular mechanism which is normally protected by electrical and chemical gradients opposing its net influx. These gradients would also promote its net efflux after *de novo* synthesis.

422.19

INTRASTRIATAL FORSKOLIN INCREASES NEURONAL PREPROENKEPHALIN AND PREPRODYNORPHIN mRNA IN RATS. J.N. Simpson*, S.R. Childers†, and J.F. McGinty. Dept. Anat. and Cell Bio., School of Medicine., East Carolina Univ., Greenville, NC 27858-4354, †Dept. Physiol. and Pharm., Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, NC 27103.

This experiment utilized in situ hybridization histochemistry (ISHH) to examine the effects of intrastriatal forskolin on pro-opioid and c-fos gene expression in the rat striatum. Bilateral 21 G guide cannulae were implanted above the striata of rats. Alza 1003D mini-osmotic pumps (pumping rate of 1 µl/h) were loaded with 500 µM, 1 mM, or 10 mM forskolin dissolved in 70% dimethyl sulfoxide (DMSO) solution with 0.1% pontamine sky blue dye (PSB). PSB was included to examine the extent of drug diffusion. As controls, other pumps were loaded with only the 70% DMSO / 0.1% PSB solution. Tubing was used to attach the pumps to 26 G injection cannulae. The pumps with attached injection cannulae were placed in sterile saline at 37°C overnight to fill the tubing and injection cannulae with solution. Four to seven days after guide cannulae implantation, two pumps were implanted subcutaneously in each rat and the attached injection cannulae were inserted through the guide cannulae and cemented in place. The brains were removed after 24 h of continuous infusion. The right hemispheres were quickly frozen for ISHH and the striato-accumbens were immediately dissected out of the left hemispheres for adenylate cyclase assays. ISHH for preproenkephalin, preprodynorphin, and c-fos was performed using ^{35}S-dATP labeled oligos on 12 µm sections taken through the infusion area. Infusion of forskolin for 24 h caused some tissue damage around the infusion area. However, forskolin increased preproenkephalin and preprodynorphin mRNA in neurons around the damaged area in a dose-dependent manner as compared to controls. The area of increased mRNA correlated positively with the distribution of PSB. There did not appear to be any forksolin-stimulated induction of c-fos mRNA in these rats. Digital image analysis of this tissue is currently underway. Initial results of adenylate cyclase assays on 10 mM forskolin-infused striatal tissue indicates a 2 - 3 fold forskolin-induced increase in cyclase activity. These results indicate that continuous in vivo activation of the adenylate cyclase cascade induces expression of the pro-opioid peptide genes in striatal neurons. Supported by DA 05470 (JNS), DA 02904 (SRC), and DA 03982 (JFM).

422.20

REGULATION OF SUBSTANCE P RECEPTOR SYSTEM IN THE SPINAL CORD BY NALTREXONE. O.J. Igwe*, Division of Pharmacology, Schools of Pharmacy & Medicine, UMKC, Kansas City, MO 64108.

Substance P-immunoreactive (SP-ir) and opioid peptide-ir nerve terminals interact in the regulation of nociceptive pathway in the spinal cord as two opposing systems. Opiate antagonist, naltrexone (NALT), increases opioid peptides and upregulates opioid receptors in the CNS. Recent evidence strongly suggests that SP system is also regulated by opioid peptides. Here, NALT was used to examine the functional link between SP receptor system and the activation of opioid receptors in the spinal cord by chronic blockade. Under ether anesthesia, male Sprague-Dawley rats were implanted SubQ with Alzet® miniosmotic pumps, filled with either NALT HCl (70 mg/ml) or vehicle (control), for 7 days. Animals were sacrificed on day 8, spinal cord rapidly removed, and used to determine SP and inositol-1,4,5-trisphosphate (IP_3) tissue contents, and to examine the regulation of their specific receptors using in vitro binding assays. SP and IP_3 levels in the spinal cord were significantly increased by 53% and 34%, respectively, over controls. Using [^{125}I] Bolton Hunter SP and [^{3}H]IP_3 as ligands, the affinities (K_d) for SP and IP_3 were unaffected by chronic NALT treatment. However, the density (B_{max}) of IP_3 receptors, increased by 120% over control, while the B_{max} for SP receptors remained unchanged. The data suggest that IP_3 production, coupled to functional SP receptor activation, and IP_3 receptors, which mediate IP_3-induced alterations in intracellular Ca^{2+} flux, are increased in the spinal cord by chronic blockade of opioid receptors. Supported by UMKC-FRG and USPHS grant AR 41606.

422.21

ESTROGEN DESENSITIZES HYPOTHALAMIC ß-ADRENOCEPTORS WITHOUT ALTERING RECEPTOR DENSITY OR ANTAGONIST BINDING AFFINITY. S. Ungar* and A.M. Etgen. Depts. Psychiat. & Neurosci., Albert Einstein Coll. Med., Bronx, NY 10461

We have shown previously that, in membranes prepared from female rat hypothalamus and preoptic area, estrogen desensitizes ß-adrenergic receptor stimulation of adenylate cyclase without modifying G protein stimulation of the enzyme. The current experiments examined the possibility that estrogen modulates receptor function through changes in either receptor density or binding affinity. We examined receptor binding affinity and density using the high specific activity radiolabeled antagonist [^{125}I]Iodocyanopindolol (^{125}ICYP) in membranes prepared from combined hypothalamus-preoptic area of ovariectomized (OVX) and OVX, estrogen-primed rats. OVX rats received oil vehicle or 2 µg of estradiol benzoate 48 and 24 h prior to sacrifice. Scatchard analysis of saturation binding experiments revealed a single class of binding sites with Kd in the range of 30-50 pM and Bmax in the range of 30-40 fmol/mg protein. Estrogen had no effect on either the Kd or Bmax of ^{125}ICYP binding in hypothalamic-preoptic area membranes. Saturation experiments carried out in cortical membranes also showed no measurable effect of estrogen on ß antagonist binding affinity or receptor density. These data suggest that estrogen-dependent uncoupling of ß-adrenergic receptor from adenylate cyclase is not contingent on hormonal modulation of receptor density or antagonist binding affinity. Agonist affinity studies are currently underway to determine if estrogen regulates the coupling of ß-adrenergic receptors to adenylate cyclase by modulation of agonist binding.

422.22

EFFECTS OF THE AMINOSTEROID U-73122 ON NEUROTENSIN OR MUSCARINIC RECEPTOR MEDIATED CGMP FORMATION AND PPI HYDROLYSIS IN N1E-115 CELLS. M. Yamada, Mi. Yamada and E. Richelson.* Mayo Foundation, Jacksonville, FL 32224.

The aminosteroid, U-73122 (1-{6-{[17ß-3-methoxyestra-1,3,5(10)-trien-17-yl]amino}hexyl}-1H-pyrrole-2,5-dione) reportedly inhibits muscarinic receptor sequestration and polyphosphoinositide (PPI) hydrolysis in SK-N-SH human neuroblastoma cells. Like the muscarinic receptor, the neurotensin receptor couples to cyclic guanosine 3',5'-monophosphate (cGMP) synthesis and PPI hydrolysis. For this study, we examined the effects of U-73122 on neurotensin or muscarinic receptor mediated cGMP formation and PPI hydrolysis in murine neuroblastoma clone N1E-115. Both cGMP formation and PPI hydrolysis stimulated with 10 nM neurotensin or 1 mM carbamylcholine (a muscarinic receptor agonist) were inhibited dose dependently after incubation with U-73122 (dissolved in DMSO, 1% final) at 37°C for 15 min. Maximum inhibition occurred with 10 µM U-73122. We showed before that 10 µM U-73122 inhibits neurotensin receptor down-regulation in N1E-115 cells. Molecular mechanisms underlying these phenomena are unknown. However, our results suggest that U-73122 inhibits the down-regulation, cGMP formation, and PPI hydrolysis in many other receptors that couple with these responses (Supported by Mayo Foundation and USPHS grant MH27692).

422.23

DEPOLARIZATION CHANGES THE EXPRESSION OF NEUROPEPTIDES AND CATECHOLAMINES IN THE CHICK SYMPATHOADRENAL SYSTEM. G. Maynard-Salgado* and J. E. García-Arrarás. Biology Department, University of Puerto Rico, Río Piedras, PR 00931.

The sympathoadrenal system is characterized by the expression of catecholamines (CA) and several neuropeptides. Electrical activity, elicited by the presynaptic fibers, influence the survival, maintenance and phenotypic expression of the sympathoadrenal cells. We are using adrenal gland and sympathetic neuronal cultures from 11 day chick embryos to study the effect of depolarization on CA and neuropeptides, enkephalin (ENK), neuropeptide Y (NPY) and somatostatin (SS) expression. Histochemical studies demonstrate that, as occurs in vivo, CA, ENK-, NPY- and SS-like immunoreactivity are expressed by sympathoadrenal cell cultures. Radioimmunoassays for ENK and NPY show that their expression is enhanced in both cell cultures upon depolarization by 30 mM K^+ or veratridine (10 µM). SS levels, on the contrary, are lowered or do not change. These effects can be mimicked by the Ca^{+2} channel agonist (±) Bay K 8644 (1 µM) but not by the adenylate cyclase activator forskolin (10 µM), suggesting the involvement of Ca^{+2} but not cAMP on the depolarization effect. CA levels are also affected by high K^+ depolarization; exposure to 30 mM K^+ cause a lowering in epinephrine and norepinephrine levels in adrenal cells, as quantified using high performance liquid chromatography . However, no changes are observed in norepinephrine levels in sympathetic neuron cultures. Our results show that electrical activity modulates the expression of neuropeptides and CA in the avian sympathoadrenal system in vitro and suggest that a similar effect might occur in vivo.

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422.24

REGULATION OF CALCITONIN GENE-RELATED PEPTIDE mRNA IN PRIMARY CULTURES OF DORSAL ROOT GANGLIA NEURONS. M.D. Christensen,* D.J. DiPette, K.N. Westlund and S.C. Supowit. Depts. of Int. Med., Marine Biomed. Inst., Human Biol. Chem. & Genetics, Univ. of Texas Med. Br., Galveston, TX 77550

Calcitonin gene-related peptide (CGRP) is produced by the alternative processing of the calcitonin/CGRP gene primary transcript. CGRP is a potent vasodilatory neuropeptide and has been implicated in regulation of cardiovascular function. Neuroendocrine and behavioral effects of centrally administered CGRP also suggest that this neuropeptide may play an important role in CNS function, however, very little is known about the factors and mechanisms that modulate neuronal CGRP synthesis and release. We have therefore begun to use primary cultures of dorsal root ganglia (DRG) neurons to identify the factors that modulate CGRP mRNA levels. DRG were chosen because this is the site of neuronal cell bodies known to produce abundant CGRP levels and to send axons peripherally to periadventitial tissue and centrally to spinal cord. It has been previously documented that isolated DRG neurons produce CGRP and that levels of both CGRP mRNA and protein are positively regulated by NGF. Using Northern blot analysis and immunocytochemical staining we have confirmed that DRG neurons contain immunoreactive CGRP and CGRP mRNA. Preliminary results indicate that treatment (20h) of isolated neurons with either forskolin (10uM) or phorbol 12-myristate 13-acetate (PMA; 20µM) in absence of NGF also increase CGRP mRNA levels. These results suggest that regulatory agents which act through protein kinase A and protein kinase C signal transduction pathways may modulate neuronal CGRP expression.

422.25

IDENTIFICATION AND CHARACTERIZATION OF A NOVEL GTP-BINDING PROTEIN IN BOVINE HIPPOCAMPAL MEMBRANES. J. Zhu, M.L. Toews, R.G. MacDonald and T.D. Hexum.*, Depts. of Pharmacology and Biochemistry, Univ. Nebraska Med. Ctr., Omaha, NE 68198-6260

A novel GTP-binding protein with an apparent molecular weight of 55 kDa was identified in purified membranes from bovine hippocampus. [α-^{32}P]GTP was covalently linked to GTP-binding proteins by incubating membranes in buffer for 3 min at 32° followed by exposure to UV light for 5 min at 3°. The reaction was stopped by removal of the light source and dilution in cold buffer. The 55 kDa protein was the most prominently labeled species under these conditions. Labeling could be moderately promoted by neuropeptide Y (NPY) but greatly enhanced by the C-terminal fragments, NPY(18-36) and NPY(20-36), in a concentration-dependent manner. The labeling was sensitive to inhibition by GTP-γ-S and GDP but not ATP. [α-^{32}P]ATP labeled a 53 kDa protein which did not increase after the addition of any of the peptides and was sensitive to inhibition by ATP but not GTP-γ-S. Labeling of membrane proteins by both nucleotides was strictly dependent on UV irradiation. Photoaffinity-labeling of the 55 kDa protein was facilitated, in a dose-dependent fashion, by dithiothreitol but was inhibited by pretreatment with N-ethylmaleimide. Western-blot analysis showed that an antibody against a consensus sequence of G-protein alpha subunits recognized a protein band co-migrating with the band that was photoaffinity-labeled. Thus, a novel GTP-binding protein exists in bovine hippocampus which may mediate an NPY effect in brain. (Supported by NS26479 and the Amer. Heart Assoc., Neb. Affil.)

422.26

G PROTEIN INVOLVEMENT IN COCAINE SENSITIZATION. C.D. Striplin* and P.W. Kalivas. Department of VCAPP, Washington State University, Pullman, WA. 99164-6520.

Behavioral sensitization is associated with a significant increase in dopamine neurotransmission in the nucleus accumbens (NA); while the neurochemical initiation of behavioral sensitization is thought to occur in the ventral tegmental area (VTA). A role for G proteins has been hypothesized since daily cocaine (30 mg/kg X 10 days) has been shown to decrease the levels of Gi and Go proteins. Also five daily injections of 15 or 30 mg/kg cocaine was shown to decrease the level of in vitro pertussis toxin ribosylation 1-6 hours after the last injection and a significant correlation has been found between the level of behavioral sensitization observed at 15 mg/kg and pertussis toxin ribosylation in the VTA . To determine if the G protein changes were transient or long-lasting, rats were sensitized to cocaine for 5 days and then given a final challenge of cocaine 14 days later. Rats were killed at 1hour after the final injection and G proteins were analyzed in the VTA, NA, substantia nigra, striatum and prefrontal cortex by Western blot analysis. The levels of Go, Gs, and Gi2 did not show any significant change in any of the brain areas tested. However, a significant decrease in Gi1 levels was observed in the NA in chronic cocaine treated animals given an acute cocaine challenge (85.5%) and chronic cocaine treated animals given an acute saline challenge (89.2%). These data indicate that G proteins in the VTA may play a role in the initiation of sensitization while long-term sensitization is associated with G protein changes in the NA.

422.27

CANNABINOID RECEPTOR-COUPLED G-PROTEIN ACTIVITIES IN CEREBELLAR MEMBRANES. M.A. Pacheco* and S.R. Childers. Dept. Neuroscience, University of Florida Sch. of Med., Gainesville, FL 32610. Dept. Phys./Pharm., Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, NC 27157.

Cannabinoid (Cn) receptors inhibit adenylyl cyclase (AC) and stimulate low K_m GTPase in cerebellar membranes. The AC inhibition is markedly reduced in cerebellar but not striatal membranes from Staggerer and Weaver mutant mice deficient in cerebellar granule cells, thus suggesting that these receptors are localized to granule cells. Studies of Na^+ effects on Cn receptor-coupled G-protein activities showed that Na^+ decreased specific binding of the Cn agonist ^{3}H-WIN 55212 in both cerebellar and striatal membranes, while Cn inhibition of AC in cerebellar membranes was maximal in the absence of Na^+. However in striatal membranes, AC inhibition was minimal in the absence of Na^+ and maximal at 50 mM Na^+. Cn agonists stimulated low K_m GTPase by 75-90% with potencies parallel to Cn-inhibted AC. Cn and $GABA_B$ stimulated GTPase was additive while corresponding agonist inhibition of AC was not additive. Na^+ dependence of Cn-stimulated GTPase in cerebellar and striatal membranes paralleled AC inhibition in these membranes: Na^+ decreased Cn-stimulated GTPase in cerebellar membranes, but in striatal membranes Cn-stimulated GTPase was maximal at 50 mM Na^+ with less than half that activity at without Na^+. These data suggest that in cerebellum Na^+ is not required for Cn-inhibited AC or Cn-stimulated GTPase activity.

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422.28

THE FUNCTION AND QUANTITY OF GTP BINDING PROTEINS IN POST-MORTEM BRAIN OF ALCOHOLISM. H. Ozawa*[1], Y. Katamura[1], E. Hashimoto[1], S. Hatta[2], T. Saito[1], L. Frolich[3], N. Takahata[1] , P. Riederer[3]. Dept. of [1]Neuropsychiatry and [2]Pharmacology, Sapporo Medical College, Sapporo 060, Japan, [3]Dept. of Psychiatry, Wurzburg University, D-8700 Wurzburg , Germany.

Our and other lab suggested that ethanol may have direct effects on GTP (G) binding proteins, particularly stimulatory G protein (Gsα). The chronic exposures of ethanol in animal or peripheral tissues from alcoholism provides a reduction of Gs-mediated adenylate cyclase and the amount of Gsα . Taken together with these evidences ,alcoholism may be associated with the abnormality of postreceptor signaling system. The present study examines the function and quantity of G proteins in membrane preparation from several regions (frontal, parietal ,temporal, occipital cortex) in postmortem brain obtained from alcoholism (N=5) and aged and postmortem delay time matched control (N=5). G proteins (Gsα ,Giα ,Goα ,Gqα , beta subunit) were identified by Western blotting with polyclonal antibody (RM/1, AS/7, GC/2,QL,SW/1,respectively). The quantitative immunoblotting showed that immunoreactivity of high molecular weight Gsα in temporal cortex was significantly decreased (30% , $p<0.05$) in alcoholism compared with control. Additionally functional photoaffinity GTP [azidoanilido GTP(AAGTP)] labeling to Gsα was decreased in frontal and parietal cortex in alcoholism even though no alteration of the immunoreactivity. These results indicate that disturbances of G protein mediated signal transduction may be involved in the pathophysiology of alcoholism.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION: STRESS—DEVELOPMENTAL ASPECTS

423.1

Reversible Depletions of 5-Hydroxytryptamine in the Neo-Natal Rat Exaggerates Stress Responses in Adulthood. J.W.Smythe[1]*, C.M.McCormick[1], S.Bhatnagar[1], J.R.Seckl[2] and M.J.Meaney[1], Dept. Psychiatry, McGill Univ. Montreal, Canada, and [2]Dept. Medicine, Univ. Edinburgh, Scotland.

5-Hydroxytryptamine (5-HT) systems subserve both neurotransmitter and neurotrophic functions in the rat. Recent data showed that 5-HT can regulate the density of glucocorticoid receptors (GCr) in fetal hippocampal cell cultures. Elevated GCr levels are associated with enhanced negative-feedback control of the hypothalamic-pituitary-adrenal axis (HPA). We examined the effects of perinatal 5-HT depletions on stress responses of the adult rat in order to assess the trophic action of 5-HT on HPA function.

Neonatal rats were injected with PCPA or vehicle (VEH) on alternate days, up to day 8. Plasma corticosterone (B) was measured in response to 20 min restraint stress in adulthood.

Although basal levels of B were the same for both groups, PCPA-treated rats hypersecreted B upon termination of stress and at 20 + 60 min post-stress. By 120 min post-stress both groups had returned to basal levels.

These results indicate that 5-HT can regulate HPA function, presumably by some trophic action. We are currently investigating the specific brain locations and nature of this regulation.

423.2

CHANGES IN PLASMA ACTH, CORTICOSTERONE, CBG, AND HIPPOCAMPAL GLUCOCORTICOID RECEPTOR OCCUPANCY IN RAT PUPS FOLLOWING MATERNAL SEPARATION AND/OR ETHER STRESS. Shakti Sharma*, V. Viau, S. LaRocque and M.J. Meaney, Douglas Hos. Res. Ctr., Depts of Psychiatry, and Neurology and Neurosurgery, McGill Univ, Montreal, Canada H4H 1R3

Plasma ACTH and corticosterone (B) responses to stress are often reduced in the neonatal rat. However, plasma cortiosteroid-binding globulin (CBG) levels of the neonate are substantially decreased, which might enhance the biological significance of existing glucocorticoid levels. We examined this question by estimating hippocampal glucocorticoid receptor (GR) occupancy in Day 6, Day 15, and adult animals under basal and stressful conditions. The results showed that: 1) Plasma ACTH levels were elevated in Day 6 animals in response to acute exposure to ether, maternal separation, and maternal separation + ether, however, ACTH responses were substantially lower than in Day 15 or adult animals. 2) Plasma total B levels followed a similar pattern; most noteworthy was the potent glucocorticoid response in Day 15 animals to the combination of maternal separation + ether. 3) Plasma CBG levels in Day 6 animals were extremely low (<3% adult values); by Day 15 CBG levels were about 25% of adult levels. Interestingly, maternal separation was associated with a substantial decrease in plasma CBG levels. 4) Hippocampal GR occupancy was similar at all ages under both basal and stress conditions. The only noteable exception occurred during maternal separation in Day 15 animals, where hippocampal GR occupancy was higher than that observed at any time in either Day 6 or adult animals. This finding is likely related to the decrease in plasma CBG that occurs following separation of Day 15 pups from the dam. Thus, despite the differences in plasma B levels, GR occupancy was generally comparable across all ages either under basal conditions, or following stress. These receptor data underscore the importance of developmental changes in plasma CBG levels.

423.3

DEVELOPMENTAL MANIPULATIONS DIFFERENTIALLY AFFECT HPA AXIS OF MALE AND FEMALE RATS. C.M. McCormick*, J.W. Smythe, S. Sharma, & M.J. Meaney, McGill University, Dept. of Psychiatry, Douglas Hospital Research Centre, Montreal, CANADA H4H 1R3.

Hypothalamic-pituitary-adrenal (HPA) function was investigated in adult animals in relation to both prenatal stress (PS: 20 min restraint of mothers on days 15-19 post-conception) and neonatal handling (H: separation from mothers for 15 min daily during first 10 days of life). Blood samples were drawn from catheters prior, during, and following 20 min restraint. PS females showed elevated levels of ACTH and corticosterone (B) during and following stress compared to NS females. No marked differences in B, ACTH, or corticosteroid binding globulin (CBG) were observed between PS and NS males. Despite similar basal levels of B, PS females showed elevated levels of CBG compared to NS females, which may account for their increased HPA response to stress. PS was associated with increased glucocorticoid receptor (GR) levels in the frontal cortex (FC) in females, decreased levels in FC of males, and increased levels in septum (SPT) of males. PS was not associated with GR levels in hippocampus (HPC), hypothalamus (HYPO), and amygdala (AMG). In contrast, neonatal handling (H) was associated with a reduced HPA response to stress in both males and females. H animals showed increased levels of GR in HPC (both sexes) and FC (males only), and decreased GR levels in SPT (males only) compared to non-H animals. H was not associated with GR levels in HYPO or AMG. Across experimental groups, when sex differences were observed, they were in the direction of higher GR levels in females than in males.

The results reflect sex differences in the effects of early environmental events on (1) HPA response to stress, and (2) GR levels in brain regions implicated in HPA regulation, and (3) plasma levels of CBG.

423.4

CENTRAL GLUCOCORTICOID RECEPTORS AND IMMUNE FUNCTION IN CHRONICALLY COLD STRESSED HANDLED AND NON-HANDLED RATS. S. Bhatnagar*, N. Shanks & M.J. Meaney, Douglas Hospital Research Center, Depts. of Psychiatry and Neurology & Neurosurgery, McGill University, Monteal, Quebec, Canada H4H 1R3.

Neonatally handled (H) animals secrete less corticosterone (B) and adrenocorticotropin (ACTH) in response to acute stress than their non-handled (NH) counterparts. Additionally, H animals have increased concentrations of Type II glucocorticoid receptors in the hippocampus and frontal cortex, structures known to be involved in feedback inhibition of HPA activation. We have previously shown that when chronically cold stressed H and NH animals (H CHR and NH CHR, respectively; 4h at 4 ° C a day for 21 days) are exposed to a novel stressor (20 min of restraint), NH CHR exhibit delayed recovery such that by 120 min following termination of the strss, plasma B does not return to baseline. We examined Type II glucocorticoid receptor concentrations in hippocampus, frontal cortex, hypothalamus and pituitary in these animals. Chronic stress does not alter Type II concentrations in any of these areas such that hippocampal glucocorticoid receptor density in both H CTL and H CHR is greater than in NH groups. The effects of chronic cold stress on immune function was examined in H CTL and NH CTL, H CTL and NH CTL animals exposed to one 4 h period of acute cold stress (4 ° C) 92 h after inoculation with sheep red blood cells, and in H CHR and NH CHR re-exposed to cold stress (4 ° C) 92 h after inoculation. We found that H CTL and NH CTL animals did not differ in their plaque forming cell (PFC) response or serum anitbody titers. A single exposure to cold strss reduced the PFC response equally in both H CTL and NH CTL. However, when H CHR and NH CHR were re-exposed to cold stress, immune responsiveness was reduced even further in NH CHR compared to NH CTL, whereas H CHR did not differ from H CTL or NH CTL exposed to one period of cold stress. These data suggest that early environmental experience may modulate adaptation to chronic stress and influence predisposition to stress-related pathologies.

423.5

PLASMA CORTICOSTERONE IS SIGNIFICANTLY INCREASED BY COLD STRESS IN THREE DAY-OLD NEONATAL RATS. S.-J. Yi*, L. Schultz, T.Z. Baram, Div. of Neurology, Childrens Hospital Los Angeles, Los Angeles, CA 90027

The hypothalamic-pituitary-adrenal axis underlies the hormonal stress response. The responsiveness of the neonatal hypothalamic-pituitary-adrenal axis to stress has been thought to be impaired ("stress hyporesponsive period"). We studied the response of neonatal rats to cold stress by measuring plasma corticosterone levels.

Rats aged 3 to 17 days were placed in compartmentalized plastic cages in a cold room (4^0C) for 20 to 60 min. Trunk blood was collected 5 min, 20 min, 1 hour, 4 hours and 28 hours after the onset of cold stress for plasma corticosterone measurement by RIA. Littermate control rats were kept under "stress free" conditions.

Animals exposed to cold stress showed a marked increase in plasma corticosterone starting on the third postnatal day, compared to non-stressed controls. We are currently studying the response of plasma ACTH and CRH-mRNA abundance in the paraventricular nucleus to cold stress during the first postnatal week.

423.6

POSTNATAL HANDLING ALTERS GLUCOCORTICOID FEEDBACK REGULATION OF HYPOTHALAMIC-PITUITARY-ADRENAL FUNCTION. D.S.L. MacIntosh*, V. Viau, S. Sharma, P.M. Plotsky and M.J. Meaney. Douglas Hosp. Res. Ctr., McGill Univ, Montreal H4H 1R3 and Peptide Biology, Salk Institute, La Jolla CA 92138.

Postnatal handling of rat pups is known to permanently alter hypothalamic-pituitary-adrenal (HPA) responses to a wide variety of stressors and negtaive feedback sensitivity to circulating glucocorticoids. In the present studies, we found that H animals show lower levels of plasma ACTH in response to both restraint and ether stress. However, H and NH did not differ in glucocorticoid fast feedback: Administration of 30 µg/kg corticosterone (B) immediately prior to restraint stress significantly dampened the subsequent increase in plasma ACTH levels to the same extent in H and NH animals. H and NH animals adrenalectomized (ADX) 5 days prior to testing did not differ in plasma ACTH responses to restraint stress, demonstrating that the differences between the groups is dependent upon the presence of circulating B. However, the handling effect was apparent in ADX animals provided with a low level of B replacement (50% B pellets producing plasma B levels of ~5 µg/dl). H/ADX + B rats showed lower levels of plasma ACTH both during and following restraint stress than did H/ADX animals. In contrast, there were no differences in plasma ACTH levels between NH/ADX and NH/ADX + B animals. Thus, a low level of B replacement was able to restore the difference between H and NH animals in plasma ACTH responses to stress. This finding shows that the differences between H and NH animals in HPA responses to stress can occur independent of stress-induced elevations in plasma B levels. Finally, we found that resting-state levels of corticotropin-releasing factor (CRF) and arginine vasopressin (AVP) in median eminence were significantly higher in NH animals compared with H animals. Taken together, these findings suggest that H and NH animals differ in delayed negative-feedback, that this difference occurs in response to low levels of B and is reflected in differential rates of CRF and AVP synthesis.

423.7

EARLY, POSTNATAL EXPERIENCE ALTERS HYPOTHALAMIC CORTICOTROPIN-RELEASING FACTOR (CRF) mRNA, MEDIAN EMINENCE CRF CONTENT AND STRESS-INDUCED RELEASE IN ADULT RATS. Michael J. Meaney* and Paul M. Plotsky. Douglas Hospital Res. Ctr., McGill Univ., Montreal H4H 1R3, Canada and Peptide Biology Lab., The Salk Inst., La Jolla, CA 92037.

The early studies of Levine, Denenberg and others have shown that early handling permantently alters hypothalamic-pituitary-adrenal responses to stress. Nevertheless, despite the long history of the neonatal handling paradigm and its importance for the study of the environmental regulation of development, we know rather little about the mechanisms that underly differences in HPA activity between handled (H) and nonhandled (NH) animals, and less about the effects of other forms of alterations in early rearing environment on the development of the HPA axis. In these studies, rat pups 2-14 days of age were exposed daily to handling (15 min of separation from mother and home cage), maternal separation (MS; 180 min of separation), or were left entirely undisturbed (nonhandled; NH). As adults, MS rats showed increased hypothalamic corticotropin-releasing factor (CRF) mRNA levels compared with NH rats, while CRF mRNA levels in H rats were significantly lower than either MS or NH animals. Hypothalamic CRF content under basal conditions followed exactly the same pattern. A 20-min period of restraint stress produced significant CRF depletion in all groups, although the percentage of depletion was significantly lower in H animals compared with either MS or NH animals. Restraint stress produced significantly higher increases in plasma corticosterone in MS and NH animals than in H animals. These data reflect the importance of early environmental factors in regulating the development of the hypothalamic CRF system and the responsiveness of the hypothalamic-pituitary-adrenal axis to stress.

424.1

PLASTICITY OF ß-ADRENOCEPTORS ON SPLENOCYTES FROM YOUNG AND OLD F344 RATS FOLLOWING ACUTE CHEMICAL SYMPATHECTOMY. S.M. Breneman*, D.L. Bellinger, K.S. Madden, A. Tong, J. Housel, S.Y. Felten, and D.L. Felten. Department of Neurobiology & Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

ß-adrenoceptor (ßAR) density on splenocytes was examined in 3- and 21-month-old (mo) male F344 rats between 1 and 56 days (D) after chemical sympathectomy (SympX) with 6-hydroxydopamine. In splenocytes from 3-mo SympX rats, ß-adrenoceptor density was not significantly different from nontreated or vehicle-treated controls at D1-3 post-treatment, but progressively increased through D10, and then declined from D10 through D56. ßAR density at D56 still was slightly elevated compared with both control groups. The initial rise in ßAR density suggests an upregulation of receptors in response to NA denervation of the spleen. The subsequent progressive decline closely parallels the time course of reinnervation of NA nerve fibers into the spleen. ßAR density on splenocytes from nontreated and vehicle-treated 21-mo rats was significantly higher compared with that seen in 3-mo rats (approximately 780-900 and 475-525, respectively). Vehicle-treated 3- and 21-mo animals possessed a slightly higher density of ßAR on splenocytes than did nontreated age-matched controls. In splenocytes from 21-mo SympX rats, a progressive decline in ßAR density was observed between D1 and D3, followed by a progressive rise in ßAR density through D56. At D56 the density of ßAR on splenocytes from denervated 21-mo rats was not significantly different from vehicle-treated controls. These findings indicate that regulation of ßAR density on splenocytes following acute denervation is impaired with age; upregulation of ßAR in response to denervation was not observed, only a slow progressive increase in the receptor density that closely parallels the reinnervation of the spleen in 21-mo rats. The initial decline in ßAR following SympX in 21-mo rats may result from a decrease in the ability to metabolize norepinephrine released from damaged sympathetic nerves. Supported by 1 R29 MH47783, R37 MH42076, and Markey Foundation Center. Award.

424.2

PRESENCE AND AVAILABILITY OF VIP IN PRIMARY AND SECONDARY LYMPHOID ORGANS. D.L. Bellinger*, D.J. Earnest, M. Gallagher, and D.L. Felten. Department of Neurobiology & Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

A large body of pharmacological and immunological data indicate that vasoactive intestinal peptide (VIP) acts as a neurotransmitter with cells of the immune system as targets. VIP receptors have been found on human and murine T and B lymphocytes. In functional studies, VIP inhibits mitogen-induced proliferation of lymphocytes from murine Peyer's patches and spleen, inhibits natural killer cell activity, alters antibody production, and promotes vasodilatation of vascular beds during local inflammatory responses. VIP receptors on T lymphocytes mediate their migration into gut-associated lymphoid tissue (GALT) and mesenteric lymph nodes (MLNs). While a few immunocytochemical (ICC) studies have reported VIP-positive (+) nerves in thymus, LNs and GALT, the presence and availability of VIP in lymphoid organs has not been examined thoroughly. We examined VIP innervation of primary and secondary lymphoid organs using ICC for localization of VIP and radioimmunoassay (RIA) for neurochemical measurement of VIP. A sparse density of VIP+ fibers was present in the thymus, residing exclusively in the capsule and intralobular septa, often in close proximity to mast cells. VIP+ nerves were most abundant in MLNs. These fibers coursed along the vasculature in internodal regions of the cortex, and to a lesser extent into the surrounding parenchyma and along medullary cords. No innervation was observed in the popliteal LNs (PLNs) or in the spleen. RIA for neurochemial measurement of VIP demonstrated high levels on MLNs (1.58 picograms/milligram tissue wet weight), and very low VIP content in thymus. VIP was not detectable in bone marrow, PLNs or spleen. Collectively these findings support the presence and abundant VIP innervation of MLNs and sparse VIP innervation of thymus. Supported by 1 R29 MH47783, R37 MH42076, and Markey Foundation Center. Award.

424.3

PLASTICITY OF NORADRENERGIC NERVES IN SPLEENS FROM AGED F344 RATS FOLLOWING CHEMICAL SYMPATHECTOMY. K.S. Madden*, D.L. Bellinger, A. Tong, J. Housel, N.L. Costello, C. Richardson, S.Y. Felten, and D.L. Felten. Department of Neurobiology & Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

The time course and pattern of noradrenergic (NA) nerve ingrowth into the spleen following acute chemical sympathectomy with 6-hydroxydopamine (6-OHDA) was examined in 3- and 21-month (mo)-old male F344 rats using glyoxylic acid fluorescence histochemistry and neurochemical measurement of norepinephrine (NE). In spleens from 3-mo-old rats, NA nerve ingrowth occurred along the splenic artery and entered the hilus (1-5 days, D1-5), extended into the hilar region (D5-10), and then proceeded into regions distal from the hilus (D21-56), suggesting orderly ingrowth from the hilus to distal regions. NA nerves reinnervated the same compartments seen in vehicle controls, but nerve fiber density in these compartments differed based on distance from the hilus and time after denervation. By D56 post-lesion, regions distal from the hilus had fewer NA nerves than vehicle controls, suggesting that reinnervation does not restore nerve density identical to that of vehicle controls. In contrast, splenic NE concentration at D56 post-lesion returned to vehicle control values, suggesting that functional restoration of NA nerves in the spleen may involve metabolic and receptor compensation for the lack of complete fiber regrowth into regions of white pulp distal from the hilus. In spleens from 21-mo-old rats NA nerves still were capable of ingrowth into the spleen following denervation; however, the initial ingrowth was delayed until D15 after the last dose, ingrowth occurred over a slower time course, and the density of NA nerves returning to the spleen was reduced at D56 compared with all other young and old treatment groups. Splenic NE levels at D56 post-lesion also were significantly lower than in 21-mo-old vehicle controls, suggesting that plasticity of NA innervation is compromised with age. Supported by 1 R29 MH47783, R37 MH42076, and Markey Foundation Center. Award.

424.4

MORPHINE-INDUCED ALTERATIONS OF IMMUNE STATUS: EVIDENCE FOR ß-ADRENERGIC RECEPTOR INVOLVEMENT. Karamarie Fecho,* Donald T. Lysle & Linda A. Dykstra. Department of Psychology & Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27599.

There is evidence suggesting that morphine's immunomodulatory effects are mediated through the central nervous system. For example, the systemic administration of morphine, but not of N-methylmorphine, has been found to produce a naltrexone-reversible suppression of splenic NK cell activity. However, little is known about how the activation of central opiate receptors by morphine translates into peripheral immune alterations. The purpose of the present experiments was to investigate the involvement of the ß-adrenergic system as one possible mediator of morphine's effects.

Prior to a subcutaneous (s.c.) injection of either 15 mg/kg morphine or saline, male Lewis rats (N=180) were administered either the nonselective ß-adrenergic receptor antagonist nadolol, the selective $ß_1$-adrenergic receptor antagonist atenolol or the selective $ß_2$-adrenergic receptor antagonist ICI-118,551 in doses of 0, 0.125, 0.5, 2.0 or 8.0 mg/kg, s.c. All three antagonists dose-dependently attenuated the suppressive effects of morphine on the proliferative responses of splenic lymphocytes to Con-A, PHA, LPS and Iono/PMA. In contrast, none of the antagonists exhibited any effect on the morphine-induced suppression of the proliferative responses of blood lymphocytes to Con-A or PHA; likewise, there was no antagonism of the suppression of splenic NK cell activity produced by morphine. Although the immunosuppressive effects of morphine appear to involve multiple mechanisms, these data clearly implicate the ß-adrenergic system.

424.5

PAVLOVIAN CONDITIONING OF MORPHINE-INDUCED IMMUNE ALTERATIONS: EVIDENCE FOR OPIOID RECEPTOR INVOLVEMENT Mary E. Coussons*, Linda A. Dykstra, and Donald T. Lysle, Departments of Psychology and Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3270

Our prior work has shown that morphine's immunomodulatory effects can become conditioned to environmental stimuli that predict drug administration. These alterations include conditioned changes in natural killer cell activity, interleukin-2 production, and mitogen-stimulated lymphocyte proliferation. The present studies were aimed at determining the involvement of opioid receptor activity in the establishment and expression of morphine-induced conditioned immune alterations. During the training phase, Lewis rats received two conditioning sessions during which a subcutaneous injection of 15 mg/kg morphine sulfate was paired with exposure to a distinctive environment. On the test day, animals were reexposed to the distinctive alone prior to sacrifice. Saline or naltrexone (0.3, 1.0, 3.0, or 10.0 mg/kg) was administered either prior to training or to test. Administration of naltrexone prior to training resulted in attenuation of the conditioned immune alterations, whereas naltrexone administration prior to testing had no effect. Taken together, these studies show that opioid receptor activity is involved in the establishment, but not the expression, of conditioned morphine-induced immune alterations. (Supported by PHS grants DA02749, DA07244 and MH46284.)

424.6

MODULATION OF ANTIBODY PRODUCTION AND ANTIGEN-INDUCED PROLIFERATION BY A CONDITIONED AVERSIVE STIMULUS. Elizabeth H. Bennett, Lynn Perez, Maureen E. Bronson* and Donald T. Lysle, Department of Psychology & Curriculum in Neurobiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599.

Our research program is designed to characterize the immunomodulatory effect of a conditioned aversive stimulus (CS) in rats. The present study examined the effect of presentation of the CS on the proliferative response of lymphocytes to the T-dependent antigen, keyhole limpet hemocyanin (KLH) and the production of specific antibody to KLH. The results showed that presentations of the CS on days 0, 2, and 4 following immunization had no effect on the proliferative response to KLH in either spleen or lymph node lymphocytes. Furthermore, presentation of the CS on those days had no significant effect on antibody production. In contrast, presentations of CS on days 10, 12, and 14 following immunization induced a significant suppression of the KLH-induced proliferative response of lymph node lymphocytes, but not splenic lymphocytes, and a reduction in serum antibody levels to KLH. These findings indicate that a conditioned aversive stimulus can modulate the immune response to specific antigen, and that the effect is dependent upon the temporal relationship between antigen administration and presentation of the CS. (Supported by MH46284.)

424.7

CHARACTERIZATION OF OPIOID RECEPTOR INVOLVEMENT IN PAVLOVIAN CONDITIONED IMMUNE ALTERATIONS. Lynn Perez and Donald T. Lysle*, Department of Psychology & Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27599.

Previous work from our laboratory has shown that naltrexone can antagonize alterations in immune status induced by presentation of a conditioned aversive stimulus (CS). The present studies were designed to further this investigation by determining the specific opioid receptor subtype involved in conditioned immune alterations. ß-funaltrexamine (ß-FNA), a highly selective μ opioid-receptor antagonist, was administered (0, 5.0, and 25.0 mg/kg, s.c.) 24 hours prior to exposure to the CS. Alterations of immune status were determined by in-vitro assessment of natural killer cell (NK) activity, lymphocyte proliferation induced by Con-A, PHA, LPS, and ionomycin/PMA, and production of interleukin-2 (IL-2). ß-FNA antagonized the CS-induced suppression of NK activity, but did not attenuate the suppression of lymphocyte proliferation or IL-2 production. In a subsequent study, naltrindole, a selective δ opioid-receptor antagonist, was administered (0, 0.1, 1.0, 10.0, and 30.0 mg/kg, s.c.) prior to exposure to the CS. The results showed that naltrindole had no effect on the CS-induced alterations of immune status. To further characterize opioid receptor involvement in conditioned immune alterations, additional studies will investigate the role of kappa opioid receptors using nor-binaltorphimine, a k-selective antagonist. Collectively, the results of these studies provide an extensive analysis of the involvement of opioid receptor subtypes in conditioned immune alterations. (Supported by MH46284.)

424.8

CATECHOLAMINE MODULATION OF LYMPHOCYTE MIGRATION AND HOMING TO LYMPHOID TISSUES. S. L. Carlson*, Dept. of Anatomy & Neurobiology, Univ. of Kentucky Medical Center, Lexington, KY 40536-0084

Catecholamines from the sympathetic nervous system have been shown to modulate many *in vivo* assays of immune function. In part, this may result from modulation of the migration patterns of lymphocytes into lymphoid tissues, producing different ratios of T-cell subsets or B-cells and subsequent alterations in immune responses. To test this hypothesis, peripheral lymph node lymphocytes from male C3H/HeN mice were obtained and labelled with fluorescent supravital dyes such as CFSE and the CellTracker dyes (Molecular Probes). Some of the cells were treated with isoproterenol (Iso, 1μM) for 15 min to stimulate the ß-adrenergic receptor, whereas control cells were not exposed to Iso. The cells (10-20 x 10^6) were infused into the tail vein of recipient mice, and allowed to circulate for various lengths of time. Tissues were either frozen for histological analysis or prepared for cell sorter (FACS) analysis of the number of fluorescent cells that migrated into each tissue. After 30 minutes migration, the labelled cells were distributed predominantly in the red pulp and marginal zones of the spleen, with some cells beginning to migrate into the white pulp. The distribution of Iso-treated and control cells appeared to be essentially the same. In peripheral and mesenteric lymph nodes, labelled cells were found throughout, again with the Iso-treated and control cells having a similar distribution. An increase in the number of Iso-treated cells that migrated to the peripheral lymph nodes has been noted, but additional experiments are needed to determine if a significant change will be found. In addition, other time points are being examined, as is the effect of NE and EPI stimulation on lymphocyte migration. (Supported by R29 MH48644-01)

424.9

TYPE I AND II ADRENAL STEROID RECEPTOR AGONISTS HAVE SELECTIVE EFFECTS ON PERIPHERAL BLOOD IMMUNE CELLS IN THE RAT. A.H.Miller*, R.L.Spencer, J.Hasset, C.H.Kim, A.Husain, B.S. McEwen, M.Stein. Mt Sinai Med Ctr, 1 Gustave L. Levy Pl., NY, NY 10029; Rockefeller Univ,1230 York Ave., NY, NY 10021.

Administration of adrenal steroids is classically associated with increases in neutrophils and decreases in lymphocytes and monocytes in the peripheral blood. Although immune cells possess two types of adrenal steroid receptors, type I (mineralocorticoid) and type II (glucocorticoid), it is unknown which receptor subtype is involved in these various adrenal steroid effects. To examine this issue, male rats were treated with selective receptor agonists for 7 days via osmotic minipumps, and the number and percentage of immune cells in the peripheral blood was determined. For each receptor agonist (aldosterone for the type I receptor, RU28362 for the type II receptor), five groups of rats (300g) were studied (n=5 per group); sham adrenalectomy (ADX), 7-day ADX, 7-day ADX+1ug/hr, 7-day ADX+4ug/hr and 7-day ADX+10ug/hr. Whereas ADX alone had no effect on the total white blood cell (WBC) count, both aldosterone and RU28362 resulted in a significantly decreased WBC count. While aldosterone decreased the number of neutrophils, lymphocytes, and monocytes; RU28362 decreased lymphocytes and monocytes but was associated with a significant increase in neutrophils. As for lymphocyte subsets, RU28362 had the greatest inhibitory effect on T helper cells and B cells, while aldosterone was associated primarily with decreases in T cells and NK cells. These results suggest that the two adrenal steroid receptor agonists have different effects on peripheral blood immune cells in the rat. Since selective activation of adrenal steroid receptor subtypes can occur under physiologic conditions, these differences allow for complex and varied effects of adrenal steroids on immune cells under basal hormone conditions and following stress. (Supported by MH00680, MH47674)

424.10

EFFECTS OF ENDOTOXIN ON THE INDUCTION OF C-FOS PROTEIN IN THE BRAIN, PLASMA LEVELS OF CORTICOSTERONE, AND NOREPINEPHRINE AND VIP LEVELS IN THE SPLEEN OF THE RAT. C. Y Vriend*, W. Wan, L. Janz, J. M. Sorvillo^, A.H. Greenberg and D.M. Nance. Departments of Pathology, Psychology, and Institute of Cell Biology, University of Manitoba, Winnipeg, MB., R3E 0W3, Canada and ^Oncogene Sci., Inc., Uniondale, NY, 11553.

Lipopolysaccharide (LPS), an endotoxin associated with gram-negative bacteria, is a potent activator of the immune system. We have tested the effects of ICV infusions of LPS (10 ng) or Ringer's solution on the induction of the proto-oncogene protein c-fos in the brain as well as plasma levels of corticosterone and splenic concentrations of norepinephrine (NE) and VIP. The c-fos was visualized by ICC procedures with a polyclonal antibody to c-fos (1/200, Oncogene Sci.) and the PAP technique. At 3 hr post-ICV infusion of LPS, we observed numerous labeled neurons focused in the paraventricular nucleus (PVN) of the hypothalamus and the A2 region of the brain stem. Corticosterone levels as well as splenic NE and VIP levels were all elevated 3 hr post-ICV LPS, relative to Ringer's solution. In additional studies, we examined the time course for the induction of c-fos labeled neurons in the brain following IP injections of LPS as well as establish a dose response curve for c-fos labeling in the PVN vs IP dose of LPS. Labeled cells first appeared in the PVN 1 hr following IP injection (100 µg), increased further at 2 and 3 hr post-injection and then returned to control levels at later intervals. The dose response curve for IP LPS vs the number of c-fos labeled neurons in the PVN showed a few labeled cells detectable at a dose of 4.0 µg, but the number and staining intensity of labeled neurons increased up to a dose of 100 µg, with no further increase in the number of labeled neurons at higher doses. In contrast to ICV injections, we observed additional labeled cells in the supraoptic nucleus (SON), arcuate nucleus and the A1 regions of the brain stem at IP doses of 40 µg and higher. These results indicate selective and differential effect of central and peripheral LPS on the induction of c-fos protein in hypothalamic and brain stem nuclei. The concurrent changes in corticosterone level and splenic neurotransmitter levels produced by ICV LPS suggest that the endocrine and autonomic nervous systems are primary targets for these activational effects of endotoxin. Supported by MRC, MHRC and HSCRF.

424.11

EFFECTS OF LIPOPOLYSACCHARIDE ON OXYTOCIN RELEASE AND ON LOSS OF LOW-AFFINITY THYMIC OXYTOCIN RECEPTOR SITES. J. D. Caldwell*, C. H. Walker, C. A. Pedersen, L. Li, and G. A. Mason. BDRC and Dept. of Psychiatry, Univ. of North Carolina, Chapel Hill, NC 27599-7250

We have previously demonstrated that estrogen increases the affinity of thymic oxytocin (OXT) receptors. This study tested whether an E. coli lipopolysaccharide (LPS) challenge would alter OXT immunoreactive levels or thymic OXT receptors in ovariectomized (OVXed) rats given 5 μg estradiol benzoate (EB) once daily for three days or oil vehicle. On the fourth day rats received iv infusions of 150 μg/kg LPS or saline vehicle 90 min. before decapitation. LPS resulted in a significant increase in blood OXT levels only in estrogen-treated animals. The thymus, MPOA, PVN, septa and pituitaries showed no significant changes in OXT levels with these treatments, although OXT levels in the PVN were correlated with blood levels, while MPOA levels correlated with pituitary levels. Computerized analyses of data from competition binding experiments in which 0.2 to 200 nM OXT were incubated with 0.2 nM ^{125}I-OTA indicated that thymuses from oil-saline treated OVXed matched a two-site model significantly better than a one-site model; demonstrating both high- (Ki = 0.39 $\pm$ .08) and low-affinity (Ki = 9.3 $\pm$ 3.9) OTA binding sites. The EB-saline, oil-LPS and EB-LPS treated animals demonstrated one-site models with only high-affinity sites (Kis were 0.58, 0.42 and 0.42 respectively). In several in vitro experiments 100 nM OXT or 40 μg LPS added to homogenates of thymuses from OVXed oil-saline vehicle treated animals also resulted in the loss of the low-affinity sites (one-site model with Ki = 0.46 for both). OXT release after an immune challenge is estrogen-dependent while LPS directly affects the thymus by eliminating a population of low-affinity OXT receptors.

424.12

HYPOTHALAMIC NEURAL SUBSTRATES CONTRIBUTING TO PROLACTIN (PRL) BUT NOT CORTICOSTERONE (CORT) RELEASE AFTER ENDOTOXIN INJECTION. J.M. Dong, L.T. Chen, R.A. Menzies*, J.M. Oliver, B. Muffly and C.P. Phelps . Depts. of Anatomy and Psychiatry and Behavioral Medicine, Coll. Med., Univ. South Florida, Tampa, FL 33612

Amino acids capable of producing excitation and subsequent small axon-sparing lesions are used as neuroendocrine research tools. Studies of brain regulation of Prl and adrenal hormone release after systemic N-Methyl-D,L,-Aspartic Acid (NMA) administration have demonstrated differing thresholds for the modulation of individual pituitary hormones. The prominent role of Prl and Cort in the modulation of immune function coupled with the possibility that these hormones may also have a differential release in response to immune challenge prompted the following experiments. Male rats received atrial cannulas for blood samples (bs, Oday, AM and PM) before bilateral injections of either artificial CSF (aCSF) or NMA (0.6M in 0.1 and 0.15µl aCSF) at 2 sites in the anterior hypothalamic area (AHA). At 3 and 7d post NMA rats received 1 or 1.5 mg/100g. E. coli endotoxin (Endotx) or saline per cannulas after bs at 0hr(AM) and again at 0.5, 1, 3, 6 and 24 hr post endotoxin. Cort and Prl were assayed by RIA. Endo treatment of control rats resulted in 8X max increase (from basal) in plasma Cort at 0.5 hr and a 5X max increase in Prl at 1.0 hr which returned to baseline at 3 hr. Injection of either aCSF alone or NMA into AHA 7d prior to Endo did not prevent plasma cort increases seen in controls. However, both injection of aCSF alone or with NMA resulted in a respective, progressive reduction in Prl release after Endo, so that NMA rats resembled control rats receiving saline. In summary an increase in Prl and Cort release after Endo occurs with the change the latter occurring sooner and lasting longer. Cort release after endotx is not hampered after progressive damage to the AHA after injection of NMA, whereas Prl release was blocked by small (0.1mm^3) lesions. Supported by MH46808.

424.13

ENDOTOXIN CHALLENGE IN THREE-DAY-OLD RAT PUPS: HYPOTHALAMIC-PITUITARY-ADRENAL ACTIVATION AND DEVELOPMENTAL CONSEQUENCES N. Shanks* & M.J. Meaney. Douglas Hospital Research Centre, Dept. of Psychiatry, McGill University, Montreal, Quebec.H4H 1R3 CANADA

Antigenic challenge with endotoxin (ENDO) is known to activate the hypothalamic-pituitary-adrenal axis (HPA) in adult animals. Recent data have implicated IL-1ß and corticotropin releasing hormone (CRH) as potential mechanisms responsible for HPA activation during the acute phase response to antigenic challenge. However, relatively little is known about HPA and immune interactions during development and whether neonatal immune challenge alters endocrine functioning as an adult. A series of studies were performed assessing the HPA response to *salmonella enteritidis* endotoxin challenge in three-day-old rat pups. It was observed that ENDO at a dose of 0.05mg/kg (ip) provoked marked elevations in both plasma adrenocorticotrophic hormone (ACTH) and corticosterone (CORT), while only slightly altering blood glucose levels. HPA activation peaked between 3 to 4 hours following ENDO administration in both male and female rat pups. However, a marked sex difference was evident with respect to both the dynamics and magnitude of the HPA response. The magnitude of both the CORT and ACTH responses to ENDO were both greater and occurred earlier in female rat pups relative to males, however, hormone levels returned to basal values within 24 hrs in both sexes. Median eminence CRH content decreased 2-4hrs following ENDO in both male and female rat pups, suggesting that the activation of the HPA axis was mediated by ENDO stimulated CRH release. Studies are presently underway to investigate interactions between CRF and IL-1ß in the neonate, and assessing the longterm consequences ENDO challenge during development on HPA function and its relation to gender.

424.14

The Role of Immune Cells in Suppression of EAE Induced by CSF-Infused MBP. C.J. Harling-Berg*, R.A. Sobel, P.M. Knopf, and H.F. Cserr. Physiol. Sec., Brown Univ., Providence, RI 02912 and Dept. of Pathology, Mass. General Hospital, Boston, MA 02114

Pretreatment with a single infusion of guinea pig myelin basic protein (MBP; 90 μg in 9μl) into the CSF of Lewis rats suppresses clinical EAE following an EAE challenge (J. Neuroimmunol., 35:45, 1991). The role of immune cells in this suppression was evaluated by: 1) examining CNS tissues in suppressed rats and 2) determining if immune cells from suppressed rats can transfer suppression. Seven days post-infusion, MBP-pretreated rats were either challenged for EAE (75 μg MBP in CFA s.c) or their spleen (SC) and cervical lymph node cells (LNC) were transferred i.v. (SC 2.0-6.0 X 10^8; LNC 1.9-3.4 X 10^8) to naive recipients who were then challenged for EAE. Sixteen days after EAE challenge, 6 MBP-pretreated rats had fewer CNS inflammatory foci than did 5 non-pretreated controls (167 $\pm$ 35 vs 238 $\pm$ 96, $P<0.05$, Smirnov test). Transferred immune cells, with or without prior Con A stimulation (1.5 μg/ml; 40 h) did not suppress clinical signs in EAE-challenged recipients (mean maximal scores: SC [n=11] 3.7 $\pm$ 1.4; LNC [n=9], 3.2 $\pm$ 1.7; control [n=13] 3.1 $\pm$ 1.6). These results suggest that suppression of clinical EAE by CSF-infused MBP correlates with a reduced amount of CNS inflammation and that this suppression does not appear to be mediated by a transferrable immune cell. (NIH GM 08206, NS 26773, NS 11050, and the RI Foundation)

424.15

BRAIN AND LYMPHOID CELLS BETA-ENDORPHIN AND SUBSTANCE P IN EXPERIMENTAL ALLERGIC ENCEPHALYTIS (EAE). J. Veljic, P. Sacerdote*, G. Monastra^ and A.E. Panerai Dept. Pharmacology, School of Medicine, University of Milano, 20129, Milano; ^Fidia Res. Labs, 35031, Abano Terme, Italy.

Evidence is rapidly cumulating that neuropeptides participate in the modulation of immune responses acting both in the central nervous system, and with autocrine, paracrine or endocrine mechanisms. We measured beta-endorphin (BE) and substance P (SP) concentrations in discrete brain areas and draining lympho nodes cells (LC) of Lewis (EAE sensitive) and Brown Norway (EAE fully resistant) rats before and every other day after treatment with guinea pig spinal cord homogenates (GP) or bovine serum albumin (BSA), together with Freund adjuvant and Bordetella Pertussis. At the same time intervals, also tumor necrosis factor (TNF_α) release from splenocytes was measured. In Lewis rats BE increased after GP or BSA in all the brain areas studied, peaked on days 12/14 and declined by day 21. Increases were significantly higher after GP than BSA. A minor increase with the same time pattern was also present in Brown Norway rats. SP concentrations showed a reverse pattern, with nadir on day 12/14. In LC, BE concentrations increased on day 6 after immunization with GP or BSA and returned to normal values thereafter. TNF_α release from splenocytes increased significantly on days 10 and 12 only in Lewis rats. No correlation was found with clinical scores of the disease, but chronic treatment with the opiate receptor antagonist naltrexone starting on the day of immunization aggravated the clinical scores. The data presented are consistent with an inhibitory role for BE on the immune system and on the development of EAE.

424.16

TRANSACTIVATION OF NEUROPEPTIDE GENE EXPRESSION BY HTLV-1 TAX IS DEPENDENT UPON ENKCRE-2/AP-1 BINDING ELEMENT. K. G. Low*, G. M. Daniels†, M. J. Comb and M.H. Melner†. Laboratory of Molecular Neurobiology, Massachusetts General Hospital, Charlestown, MA 02129. †Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Human T-cell leukemia virus type 1 (HTLV-1) is responsible for adult T-cell leukemia. Transactivation of viral and cellular genes by HTLV-1 is dependent upon interactions between a viral *tax* protein and cellular transcription factors. In transactivation assays using human Jurkat T-lymphocytes, Tax transactivates basal expression of proenkephalin (PENK) and proopiomelanocortin (POMC) genes 3- and 4-fold respectively. Treatment with 7 μg/ml concanavalin A, 500 μM cpt-cAMP or 10 ng/ml TPA enhanced Tax-dependent transactivation of PENK gene transcription between 40- to 70-fold. Deletion analysis of the human PENK gene promoter suggests that a region between -104 to -86 (relative to the start site of transcription initiation), which contains two consensus cAMP-responsive elements (CREs), mediates Tax-dependent trans-activation of PENK gene transcription. Further deletion analysis of this region identifies ENKCRE-2 (-92 to -86)(also a consensus AP-1 binding element) as responsible for mediating the Tax-dependent transactivation. Transactivation assays with CAT reporter constructs comprising multiple copies of ENKCRE-1 and/or ENKCRE-2 confirms that ENKCRE-2 but not ENKCRE-1 is responsible for mediating the effects of Tax. These results implicate an involvement of members of the AP-1 family of transcription factor proteins in mediating the transactivation of PENK gene transcription by Tax during HTLV-1 leukemogenesis.

424.17

MORPHOLOGICICAL CHANGES IN THE HIPPOCAMPUS OF THE CONGENITALLY ATHYMIC (nu /nu) MOUSE. K.C. Prasad, *G.O. Gaufo*, J.A. Reyes, and M.C. Diamond*. Department of Integrative Biology and *Group in Endocrinology*, University of California, Berkeley, CA 94720

Preliminary data in our laboratory show morphological differences in the hippocampus of 120-day old, female homozygous nude (nu /nu) and balb/c (balb/c/balb/c) mice. Measurements of thionine stained sections of the hippocampus corresponding to CA1 and CA2 + CA4 were taken from nude (N=13) and balb/c (N=9) mice.

Our results indicate that the hippocampus in both the right and left hemispheres of the brain was significantly thinner in the nude than in balb/c mice. In the right hemisphere, the CA1 hippocampal region was thinner by 5.4% ($p<0.03$) and the CA2 + CA4 region thinner by 17.6% ($p<0.02$) in the nude mouse compared to the balb/c. In the left hemisphere, the CA1 region was thinner by 5.1% ($p<0.02$) and the CA2 +CA4 region thinner by 11.4% ($p<0.05$) in the nude compared to the balb/c mouse.

We are continuing to investigate cerebral cortical and hippocampal morphology in female nude and balb/c mice with the addition of the heterozygous nude (nu/balb/c) mice as a control. Our purpose is to gain insight on regulatory hormones or factors that may be involved in mediating the deficiencies observed in brain morphology. We are approaching this problem by grafting thymic and/or pituitary tissue in 3-week old, female homozygous nude mice. Using radioimmunoassay, we will obtain serum levels of prolactin, the primary hormone released from extrapituitary graft, corticosterone, and thyroid hormones (T4 and T3). In addition, we will observe changes in CD4 and CD8 lymphocyte population using fluoroscence-activated cell sorter (FACS) in the different experimental groups.

424.18

MITOGEN-STIMULATED SPLENOCYTES ENHANCE EPINEPHRINE (E) RELEASE FROM CHROMAFFIN CELLS S.B.Jones, M.Weber, H. Mathews, J.A. McNulty* and Z. Wang. Loyola University Medical Center, Maywood, IL. 60153.

Enhancement of peripheral catecholamine release from nerve terminals and the adrenal medulla has been suggested to occur during septic shock using whole-animal experiments. Since mononuclear cytokine expression is an important response to sepsis, the present study tested possible neuroendocrine-immune-interactions in vitro by examining the effect of mitogen-stimulated mononuclear cells on catecholamine secretion from chromaffin cells. Chromaffin cells, isolated from bovine adrenals with collagenase digestion, were maintained in 2 cm^2 wells 4-5 days with DMEM and F12 with 10% FCS in 5% CO_2 at 37 C (0.6 x 10^6 cells/well, 1.0 ml). Splenocytes were isolated from bovine spleen and cultured in RPMI with 10%FCS (1.5x10^6 cells/ml) and stimulated with 0.5% phytohemagglutinin (PHA). After 24 hrs. PHA-conditioned media (PHA+) was isolated and frozen. In secretion experiments chromaffin cell growth media was replaced with conditioned (or control) media and E release was measured at variable times. After removal of PHA+ (or control) media, cells underwent nicotinic stimulation (3μM Dimethylphenylpiperazinium) for 10 min. E released was measured and E remaining in cells was determined. Secretion is % of initial E content. After 90 min with PHA+ media, E secretion increased to 19.1$\pm$0.5% of total ($P<0.05$ vs other treatments) compared to 5.2$\pm$0.3% for PHA-extract, 3.3$\pm$0.8% for media + PHA, and 3.7$\pm$0.5% for media alone (N= 4 chromaffin cell preparations). E secretion was 25% by 210 min with PHA+. E release was dose-dependent. Nicotinic stimulation resulted in 5.6$\pm$0.6% release in E after 90 min with PHA+ ($P<0.05$ vs all other treatments) compared to 3.1$\pm$0.3% for PHA-, 2.7$\pm$0.2% for media + PHA and 2.4$\pm$0.4% for media alone (N=4 chromaffin cell preparations). Enhanced nicotinic secretion was also dose-dependent. The results support the concept that sympathetic response to sepsis is in part due to peripheral modulation of catecholamine secretion. (Supported by the Bane Foundation)

424.19

NEURONAL-GLIAL INTERACTIONS: CONDITIONED MEDIUM FROM DEVELOPING GLIA INFLUENCES LHRH NEURON MORPHOLOGY, PROLIFERATION AND SECRETION

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Astrocytes are involved in differentiation , migration and maturation of neurons and establishment of appropriate axonal connections. The immortalized hypothalamic neuronal cell line (GT1-1 subclone) recently developed by genetically targeting the expression of the simian virus-40 large T-antigen in the LHRH neurons (Mellon et al. 1990, Neuron 5: 1-10), was used in the present study to address the question of whether products released by glial cells during development might influence LHRH neuronal activity. For this end, an experimental design in which maturing astroglial cells, in the absence or the presence of growth factors, were maintained in culture and the effect of the conditioned medium (CM) assessed on proliferation and secretion of LHRH neuronal cells. Results of this study show that treatment of GT1-1 cell line with CM from astroglial cell cultures at different stages of differentiation and maturation promotes marked changes in the morphology, proliferative and secretory capacities of the LHRH neuron. At early stages of differentiation and in the absence of growth factors, glial cells release inhibitory signals which produce a 50% inhibition of GT1-1 cell proliferation and a 5-fold decrease of basal LHRH output. At later stages of differentiation , a 2-fold stimulation of proliferation and 70% increase in basal LHRH release were observed, suggesting that paracrine communications between LHRH neurons and glial cells might participate in dynamic regulation of LHRH activity.

424.20

EFFECT OF IONIZING RADIATION ON RAT'S HIPPOCAMPAL NOREPINEPHRINE RELEASE.

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The purpose of this study was to examine the effect of ionizing radiation on hippocampal norepinephrine (NE) release in vitro, stimulated by KCl 0.5 h, 24 h, 48 h, 72 h after irradiation/sham exposure. Rats were irradiated using a ^{60}Co source. The levels of NE were measured by HPLC coupled to electrochemical detection. There was no significant change in NE release between irradiated and nonirradiated rats when the hippocampal NE was determined after 0.5 h irradiation (5-30 Gy at 10 Gy/min) or sham exposure. However, there was a significant decrease in NE release 48 h, and 72 h after irradiation (10 Gy at 10 Gy/min). Pretreating rats with 3-10 μg/kg of recombinant human interleukin-1β (rhIL-1β) or 3-10 μg/kg of corticotropin releasing factor (CRF) administered IP 1 h before sham irradiation enhanced hippocampal NE release and the same treatment prevented the radiation-induced decrease in NE release 48 h after irradiation. These results suggest that ionizing radiation induces decrease in hippocampal NE release 24 h, 48 h, and 72 h after exposure and pretreatment with rhIL-1β or CRF prevents the decrease.

424.21

TIME COURSE OF CENTRAL VARIATIONS OF 5HT AND DA METABOLISM ASSOCIATED WITH A PRIMARY IMMUNE RESPONSE TO SHEEP RED BLOOD CELLS.

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Antigenic challenge is known to influence hypothalamic norepinephrine activity. In the present study, we have investigated the changes of central dopamine (DA) and serotonin (5-HT) activities at 2, 4, 8, 24, 48 and 96 hr following immunization with sheep red blood cell (SRBC) i.v. A plaque forming cell assay was performed at 96 hr which corresponds to the peak of the production of specific antibodies in Fischer 344 male rats. Major central changes were only evident 8 hr after the primary immune response to SRBC. At this time, striatal DOPAC (+43%) and DA (+17%) levels were significantly increased. 24 hr after SRBC inoculation, hypothalamic DA (+30%), 5-HIAA (+16%) and 5-HT (+19%) levels were significantly increased. 48 hr after SRBC inoculation, cortical DOPAC (-24%) and 5-HIAA (-10%) levels were significantly decreased. 96 hr after SRBC inoculation, hypothalamic 5-HT (-14%) and tryptophan (-10%) levels were significantly reduced, but cortical 5-HT (+11%) and 5-HIAA (+14%) levels were increased. In contrast, no changes occurred in the hippocampus and striatum at 96 hr following primary immunization. Thus, peripheral activation of the immune system induced opposite effects on central 5-HT metabolism at 96 hr, i.e., when the splenic immune response is maximal. These data suggest that further *in vivo* microdialysis studies comparing hypothalamic and cortical 5-HT release should be of particular interest.

NEURAL-IMMUNE INTERACTIONS: INTERLEUKIN-1

425.1

BRAIN INTERLEUKIN-1 (IL-1) ENHANCES NOCICEPTION IN THE RAT.

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IL-1 has been shown to play a role in local inflammatory and immune mediated diseases, which are associated with local pain. IL-1β, when given systemically or locally, produces hyperalgesia in rats and rabbits. This hyperalgesia is generally thought to be mediated through increased PGE2, but a prostaglandin-independent mechanism is also pointed out. On the other hand, brain astrocytes and microglia are also known to synthesize IL-1, and such brain-derived IL-1 produces a variety of CNS-mediated responses such as fever.

To determine whether brain IL-1 affects nociceptive function, we investigated the effects of human recombinant IL-1β (rhIL-1β) on nociception in male Wistar rats. The paw lick latency on a hot plate (50±0.5 ℃) was measured before and after LCV injection of rhIL-1β (1pg-1μg/kg). LCV injection of rhIL-1β (1-100pg/kg) dose-dependently reduced the paw lick latency. The hyperalgesic action started 5min after the injection, reached a maximum at 30min and was still observed at 60min. This effect was antagonized by pretreatment with either an IL-1 receptor antagonist, αMSH or salicylate, but not by α helical CRF. An electrophysiological study performed in urethane (1.2g/kg) anesthetized rats revealed that responses of neurons in the spinal trigeminal nucleus to nociceptive stimuli were enhanced by LCV rhIL-1β (10-100pg/kg) with similar time courses as that of the paw lick latency responses. This enhanced responses of nociceptive neurons was also attenuated by αMSH. The results suggest that brain IL-1β induces hyperalgesia through an activation of arachidonate metabolism, but not by that of CRF system.

425.2

INTERLEUKIN-1 DID NOT INDUCE ANALGESIA IN THE RAT HOT-PLATE OR COLD WATER TAIL-FLICK TESTS.

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There are a few reports in the literature that interleukin-1 (IL-1) induces analgesia (Nakamura *et al.*, Eur.J.Pharmacol.149:49,1988; Bianchi *et al.*, Brain Res.546:139,1991). The present study sought to characterize the analgesic effects of centrally administered IL-1 in rats. In the hot-plate test (HP), latency to a rear paw lick or a four-footed jump off a 55°C surface was measured; in the cold water tail-flick test (CWT), latency to tail withdrawal from a ~3°C liquid was timed. Core body temperature was also monitored with a rectal thermistor. After baseline readings, human recombinant IL-1ß (125-2000 U) was injected in 5 μl phosphate-buffered saline with 0.1% bovine serum albumin, and post-drug measures were recorded at intervals from 5-180 min. In the HP, no dose of IL-1 induced greater than 6.1 (±4.4) % maximum possible analgesia (%MPA) at any time point. Similarly, in the CWT, 7.3 (±2.8) %MPA was the largest result. However, dose-related increases in body temperature were observed, with a 1.7 (±0.15) °C rise 120 min after 1000 U IL-1. Since IL-1 alone did not induce analgesia, we tested its capacity to potentiate morphine analgesia. Morphine (5.0 and 10 μg, icv) induced analgesia in the CWT (35.1 and 84.1 %MPA); however, there was no significant effect of IL-1 on morphine-induced analgesia. In summary, we failed to find an analgesic effect of IL-1, alone or in combination with morphine, at doses which clearly had a physiological effect; this is in contrast to the reports cited above.

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425.3

SYSTEMIC ADMINISTRATION OF INTERLEUKIN-1ß RESULTS IN INCREASED LEVELS OF NGFI-B mRNA IN THE ENDOCRINE HYPOTHALAMUS AND ITS MEDULLARY AFFERENTS. Anders Ericsson, D.G. Amaral* and P.E. Sawchenko. The Salk Institute for Biological Studies, La Jolla, CA 92037.

NGFI-B, a protein related to the steroid receptor superfamily, is rapidly and transiently synthesized in neurons and PC 12 cells in response to a number of physiological and pharmacological stimuli. In the present study we have analysed the distribution of NGFI-B mRNA in rat brain at varying intervals following intravenous (iv) injection of interleukin-1ß (IL-1ß) or vehicle alone. Constitutively high levels of NGFI-B mRNA expression were localized primarily to telencephalic structures of both unperturbed and vehichle-injected rats; in the hypothalamus, only the suprachiasmatic nucleus displayed detectable basal levels of NGFI-B expression. In contrast, iv injection of IL-1ß (1.87 µg/kg) resulted in an apparent induction of NGFI-B mRNA in the magnocellular and parvocellular divisions of the paraventricular nucleus (PVN) as well as in the supraoptic (SON) nucleus, with peak levels seen at 1 hr postinjection. Specific, though less marked, increases of NGFI-B mRNA were also detected in a number of cell groups that provide afferent projections to the neurosecretory hypothalamus, including the caudal part of the nucleus of the solitary tract, the rostral and caudal parts of the ventrolateral medulla, the lateral division of the bed nucleus of the stria terminalis, and the central nucleus of the amygdala. Ablation methods were used to assess the potential role of ascending projections in mediating IL-1ß effects on the NGFI-B mRNA in the hypothalamus. Discrete unilateral knife cuts at medullary levels that resulted in a marked depletion of the catecholaminergic innervation of the PVN and SON on the ipsilateral side, also virtually eliminated the hypothalamic NGFI-B mRNA responses on the lesioned side to systemic administration of IL-1ß. These data are congruent with the temporal and spatial patterns of *c-fos* expression that we have observed following iv injection of IL-1, and suggest that the integrity of ascending afferents is necessary for the functional activation of multiple neurosecretory cell types commonly seen in response to systemic cytokine challenges.

425.4

INTERLEUKIN-1 (IL-1) INDUCED EXPRESSION OF INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) IS ANTAGONIZED BY A SOLUBLE IL-1 RECEPTOR. L. Imeri°*, M.R. Opp, L. Hong and J.M. Krueger. Department of Physiology and Biophysics, University of Tennessee, Memphis TN 38163 and °Institute of Human Physiol. II, University of Milan, Milan, Italy.

Intercellular adhesion molecule-1 (ICAM-1) is a cell surface glycoprotein of the immunoglobulin superfamily. It is a specific ligand for lymphocyte function-associated antigen-1. These two molecules are involved in the control of lymphocyte migration. ICAM-1 expression is induced by certain cytokines including IL-1ß. In the present study the effects of a recombinant human soluble IL-1 receptor (sIL-1r) on IL-1ß induced expression of ICAM-1 in a human glioblastoma cell line (HTB 16) were evaluated. Cells were incubated for 4 days with various concentrations of recombinant human IL-1ß (0.1/1/10/100/1000 pg/ml) in the absence or presence of the sIL-1r (1 and 10 µg/ml). An ELISA test was used to quantitate ICAM-1 expression. IL-1ß induced ICAM-1 expression in a dose-related manner. Both doses of sIL-1r significantly attenuated IL-1ß induced expression of ICAM-1; the higher dose was more effective. The present findings suggest that sIL-1r can act as an antagonist to IL-1.

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425.5

POSSIBLE INVOLVEMENT OF INTERLEUKIN-1β IN NEURONAL CELL DEATH AFTER TRANSIENT FOREBRAIN ISCHEMIA. M. Minami, K. Yabuuchi, S. Katsumata, Y. Tomozawa, Y. Kuraishi* and M. Satoh. Dept. of Pharmacol., Fac. of Pharm. Sci., Kyoto Univ., Kyoto 606, Japan.

The expression of interleukin-1β (IL-1β) mRNA in the hippocampus and other brain regions (cerebral cortex, striatum and thalamus) was examined after transient forebrain ischemia using male Wistar rats. IL-1β mRNA was not detected in these regions of sham-operated rats. IL-1β mRNA was induced after transient forebrain ischemia. The induction of IL-1β mRNA had a few peaks, that is, peaks were observed at 30 and 240 min in the four regions examined and an additional peak was observed at 90 min in the striatum.

In addition, the effect of intracerebroventriculer injection of IL-1β on neuronal cell death after transient forebrain ischemia was examined in the hippocampus. Seven days after 10 min of ischemia, the number of neurons in the hippocampal CA1 region of saline-injected rats was decreased to 20-30% but the number of neurons in the hippocampal CA3 region did not change, compared with non-treated rats. Injection of IL-1β (30 ng/rat) during the ischemic insult potentiated the neuronal cell death. The numbers of neurons in the CA1 and CA3 regions decreased to 10-20% and 30-40% of non-treated control rats, respectively.

These results suggest that IL-1β might be produced in the brain after transient forebrain ischemia and was involved in neuronal cell death.

425.6

HYPOTHALAMIC AND HIPPOCAMPAL MONOAMINE ALTERATIONS AFTER PERIPHERAL INTERLEUKIN-1, -2 OR -6 ADMINISTRATION IN MICE. S. Zalcman*, J. Green-Johnson, L. Murray, D. Dyck, H. Anisman and A. Greenberg. Manitoba Institute of Cell Biology, Winnipeg, MB, Canada R3E 0V9.

Central monoamines vary during the course of the immune response to sheep red blood cells. Inasmuch as IL-1 has been shown to induce similar amine variations, we assessed the neurochemical consequences of IL-1 in parallel with the cytokines IL-2 and IL-6, which are released during an ongoing immune response. BALB/c mice received an injection of either recombinant human (rHu)IL-1ß, rHuIL-2 or rHuIL-6 (0.2 ug, ip) and 2 hr later were sacrificed and brains removed for HPLC determinations. IL-1ß increased hypothalamic and dorsal hippocampal MHPG/NE ratios by increasing the utilization and reducing the content of NE in these regions. These effects, however, were evident in about half of the subjects. In contrast, IL-2 enhanced hypothalamic MHPG/NE ratios by profoundly increasing MHPG accumulation without altering NE levels. IL-2 did not appreciably alter hippocampal NE activity and IL-6 did not alter NE activity in either region. Moreover, IL-1ß and IL-6 markedly enhanced accumulation of the serotonin metabolite, 5HIAA in the dorsal hippocampus.

The cytokines differentially influenced plasma corticosterone (CORT). Whereas IL-1ß greatly elevated CORT, IL-2 and IL-6 did not appreciably alter CORT levels. These latter observations coupled with the cytokine-induced NE alterations in the hypothalamus suggest that the IL-1ß induced CORT increases were independent of hypothalamic NE turnover. (supported by NIMH, MRC, NSERC).

425.7

DIFFERENTIAL MODULATION OF DISCHARGE PATTERN IN SYMPATHETIC OUTFLOWS TO THE KIDNEY AND THE BROWN ADIPOSE TISSUE BY INTERLEUKIN 1ß IN CONSCIOUS RATS. H.Kannan[1]*, C-K.Su[3], H.Tanaka[1], Y.Ueta[1], C.Y.Chai[3], Y.Hayashida[2] and H.Yamashita[1]- Dept. Physiol[1]., and Dept. Systems Physiol[2]., Univ. Occup. Environ. Health, Yahatanishiku, Kitakyushu, 807 Japan, and Institute of Biomedical Sciences[3], Academia Sinica, Taipei 115, Republic of China.

We have reported that either i.v. or i.c.v. administration of interleukin 1ß(IL-1ß) elicited an increase in renal sympathetic nerve activity(RSNA) accompanied with increases in blood pressure, heart rate and body temperature in conscious rats (Kannan et al., Soc. Neurosci. Abstr. 17: 1198,1991). The present study was conducted to examine the effects of i.v. injection of IL-1ß on discharge pattern in sympathetic nerves innervating the interscapular brown adipose tissue(IBAT), and compare them with those of RSNA in conscious rats. The IBAT sympathetic nerve activity was increased by IL-1ß with an increase in large grouped discharges synchronous with the cardiac cycle. In contrast, small amplitude activity unrelated to the cardiac cycle increased in RSNA, while large amplitude grouped discharges decreased. Plasma noradrenaline concentration increased in conscious rats after i.v. injection of IL-1ß. The responses were abolished by pretreatment of indomethacin. The results suggest that IL-1ß activates systemic sympathetic outflow via prostaglandins , and modulates the discharge patterns in regional different manner.

425.8

INTERLEUKIN-1 RECEPTOR AND ANTAGONIST IN THE RAT HYPOTHALAMUS AND PITUITARY. M. Schultzberg*, T. Bartfai, J. Bristulf, S. Nobel, A. Simoncsits and S. Tingsborg. Clinical Research Center, Karolinska Institute, Huddinge Hospital and Dept. of Biochem, Stockholm Univ., Stockholm, Sweden.

The cytokine interleukin-1 (IL-1) has been shown to stimulate release of hypothalamic and pituitary hormones, notably with resulting increase in corticosterone production. The present studies investigate the possible occurrence of the endogenous IL-1 receptor antagonist (IL-1ra) in the rat central nervous system, and further analyze the finding of IL-1 receptor (IL-1R) in the rat pituitary gland. Antisera against synthetic peptides of the human monocyte IL-1ra and murine lymphocyte IL-1R were used in immunohistochemistry, and polymerase chain reaction (PCR) technique was used to detect IL-1R and IL-1ra mRNA. In addition to the intense immunoreaction in the cells of the intermediate lobe of the pituitary seen with antisera to the second loop of the extracellular domain of the IL-1R protein, recent bleedings of these antisera stained varicose nerve terminals in the neural lobe. An even denser network was stained with an antiserum to IL-1ra, whereas the cells of the intermediate lobe were negative. In the hypothalamus both IL-1R and IL-1ra immunoreactive neurons were seen in the paraventricular (PVN) and supraoptic nuclei and in nerve terminals in the median eminence. PCR analysis confirmed the synthesis of IL-1ra in the hypothalamus. These findings indicate that IL-1R and IL-1ra may be synthesized in PVN neurons projecting to the neural lobe. Experiments are carried out to verify this, to identify which population(s) of PVN neurons the two proteins occur in, and their functional regulation. This work was supported by grants from the Swedish MRC and Clas Groschinsky's Minnesfond.

425.9

MEASUREMENT OF INTERLEUKIN-1 BETA (IL-1β) IN RODENT BRAIN-ENDOCRINE-IMMUNE TISSUES USING A MODIFIED SANDWICH ELISA. S. G. Culp*, R. C. Newton, T. Takao and E. B. De Souza. CNS Diseases Research & Inflammatory Diseases Research, The Du Pont Merck Pharmaceutical Co., Wilmington, DE 19880.

We have developed a modified sandwich ELISA to detect the cytokine IL-1β in mouse and rat tissues and fluids that utilizes a specific rabbit immunoglobulin (Ig) and a polyclonal antibody to the IL-1β peptide. ELISA sensitivity was ~ 1 pg, the inter- and intra-assay variabilities were < 6% CV, and the range was 1 - 2,000 pg. The ELISA detects both mouse and rat IL-1β, exhibits a lower reactivity to human IL-1β and a human IL-1β clone extended by three amino acids, and shows no cross-reactivity to human IL-1α, human and rat IL-1 receptor antagonist, human tumor necrosis factor α, somatostatin, vasoactive intestinal peptide, and a variety of unrelated peptides. Commercially available ELISA amplification kits did not alter the sensitivity but did increase the resolution at the lower peptide concentrations. IL-1β levels in normal mouse were 7.09 ± 1.65 pg/mg tissue in spleen and 0.36 ± 0.17 pg/mg tissue in testis; IL-1β levels were detectable in plasma, hypothalamus and hippocampus. Robust increases in IL-1β tissue levels were observed in mouse spleen and testis (6 and 15 fold respectively) six hours after injection of lipopolysacharide (LPS) (1 mg/kg, i.p.). Similar, albeit smaller changes, were observed in rat spleen and testis six hours after LPS injection (2 mg/kg; i.p.). In summary, we have developed a sensitive and specific ELISA for detection of rat or mouse IL-1β. This assay should allow the further investigation of the role of IL-1 in regulating brain-endocrine-immune interactions.

425.10

SPECIES DIFFERENCES IN INTERLEUKIN-1 RECEPTORS IN THE BRAIN-ENDOCRINE-IMMUNE AXIS. T. Takao* and E. B. De Souza, CNS Diseases Research, The Du Pont Merck Pharmaceutical Company, Wilmington, DE 19880

Previous studies utilizing ^{125}I-recombinant human interleukin-1α (^{125}I-IL-1α), ^{125}I-IL-1β and ^{125}I-IL-1 receptor antagonist have identified high affinity binding sites for IL-1 in the mouse brain and endocrine tissues with characteristics of Type I receptors in T lymphocytes. In a preliminary study, there were dramatic species differences in the level of ^{125}I-IL-1α binding with high levels of binding present in mouse and rabbit tissues (hippocampus, spleen and testis), while ^{125}I-IL-1α binding to rat and guinea pig tissues was not detectable. In the present study, we further characterized IL-1 binding in mouse and rat tissues and selective immune cell lines. Utilizing 100 pM or 600 pM ^{125}I-IL-1α, moderate to high levels of specific binding were observed in EL-4 6.1 cells (representative of Type I IL-1 receptors) and in mouse tissues, however, no specific ^{125}IL-1α binding was present in Raji cells (representative of Type II IL-1 receptors) and in rat tissues. On the other hand, utilizing 600 pM ^{125}I-IL-1β as a ligand, high specific IL-1 binding was shown in EL-4 6.1 and Raji cells and moderate binding was evident in mouse tissues, whereas specific ^{125}I-IL-1β binding to rat tissues was barely within the range of sensitivity of the assay. These data demonstrate that under optimal conditions for labeling Type I or Type II IL-1 receptors, no specific binding is observed in rat tissues suggesting the presence of novel IL-1 receptor(s) in rat tissues.

425.11

CYCLIC AMP-DEPENDENT MODULATION OF INTERLEUKIN-1 RECEPTORS IN THE MOUSE AtT-20 PITUITARY TUMOR CELL LINE. E. B. De Souza*, D. E. Tracey and T. Takao, The Du Pont Merck Pharmaceutical Company, Wilmington, DE 19880 (E.B.D.S. and T.T.) and The Upjohn Company, Kalamazoo, MI 49007 (D.E.T.)

The cytokine interleukin-1 (IL-1) has a variety of effects in brain involving induction of fever, alteration of slow-wave sleep and alteration of neuroendocrine activity. Previous studies utilizing ^{125}I-recombinant human interleukin-1α (^{125}I-IL-1α) have identified high affinity binding sites for IL-1 in brain and endocrine tissues and in mouse AtT-20 pituitary tumor cells with characteristics of Type I receptors in T lymphocytes. Recently, an upregulation of IL-1 receptors in mouse AtT-20 cells following treatment with corticotropin-releasing factor (CRF) has been demonstrated. In the present study, we utilized various secretagogues to further determine the modulation of IL-1 receptors in mouse AtT-20 pituitary tumor cell culture. CRF, isoproterenol (ISO) and forskolin (FSK) increased ^{125}I-IL-1α binding in a dose-dependent manner in mouse AtT-20 cells; in preliminary studies dexamethasone (DEX; 10^{-7}M) and somatostatin (SMS; 10^{-7}M) did not alter ^{125}I-IL-1α binding. Treatment of CRF (10^{-7}M) + FSK (10^{-5}M) and CRF (10^{-7}M) + ISO (10^{-7}M) showed the synergistic increases in ^{125}I-IL-1α binding, while CRF (10^{-7}M) + DEX (10^{-7}M) and CRF (10^{-7}M) + SMS (10^{-7}M) has no synergistic effects. These data demonstrating effects of agents (CRF, ISO, FSK) that stimulate cAMP production in AtT-20 cells on upregulation of IL-1 receptors suggest the importance of this second messenger in modulating IL-1 receptors.

CARDIOVASCULAR REGULATION: LOWER BRAINSTEM I

426.1

PHYSIOLOGICAL MECHANISMS BY WHICH CENTRAL APPLICATION OF ENDOTHELIN-1 (ET-1) REDUCES CARDIAC OUTPUT (CO). T. R. LaHann*, G. Hematillake and G. Daniell. Dept. Pharm. Sci., Idaho State University, Pocatello, ID 83209

CO in the anesthetized rat is dramatically reduced 5-7 minutes after local application of pmole amounts of ET-1 to the ventral surface of the medulla (VSM). VSM application of vehicle or other pressor agents failed to mimic ET-1's effect on CO, suggesting that the drop in CO was a specific effect of ET-1 and not related to changes in medullary perfusion. Changes in CO might reflect changes in heart rate (HR), afterload, preload or cardiac contractility. Studies were undertaken to determine how VSM application of ET-1 affected each of these parameters. HR and total peripheral resistance (a measure of afterload) were little changed following VSM application of ET-1. Marked decreases in peak stroke output and left ventricular dP/dt after VSM application of ET-1 suggest a decrease in cardiac contractility. Left ventricular end-diastolic pressure and central venous pressure showed little change following VSM application of ET-1. These data are consistent with the hypothesis that central application of ET-1 decreases CO by causing a sustained decrease in cardiac contractility.

426.2

CENTRAL CARDIOVASCULAR EFFECTS OF JOINING PEPTIDE. T.Hamakubo, M.Yoshida, T.Watanabe[1], K.Nakajima[1], R.Mosqueda-Garcia, T.Inagami*, Departments of Biochemistry and Pharmacology, Vanderbilt University, Nashville, TN 37232; Peptide Institute[1], Osaka, Japan

Joining peptide (JP), one of the major products of proopiomelanocortin, is present in the hypothalamus and the pituitary. The biological relevance of this peptide, however, has not been identified. In the present study we characterized the central hemodynamic effects of JP. Rat JP C-terminal amide form (rJP) and bovine JP (bJP) were synthesized according to the deduced amino acid sequence. Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) were anesthetized with urethane and blood pressure (BP) and heart rate (HR) were recorded intraarterially. The animals were placed in a stereotaxic frame and the dorsal surface of the medulla was exposed through a limited occipital craniotomy. In a group of animals, a catheter was placed into the cisterna magna for intracisternal administration of either rJP or bJP (10-50nmols/5μl). In a different group of rats, a glass micropipette was positioned in the nucleus of the solitary tract (NTS) for microinjection of rJP (20 pmoles/30 nl). Intracisternal injections of rJP in SHR increased mean BP and HR in a dose dependent manner. Maximal effects were seen at 50 nmols (22±2 mmHg and 36±10 bpm, n=6). Whereas similar effects were observed with bJP, intravenous administration of either peptide had no effect. In WKY, rJP or bJP were less potent to increase BP and HR. Microinjection of rJP into the caudal region of NTS increased BP by 15±2 mmHg and HR by 37±5 bpm (n=9). Maximal changes in BP occurred at 5 min and recovered within 20 min. The maximal HR response occurred 6 min after microinjection. In 2 of 9 rats the pressor effect was preceded by hypotension and bradycardia. These results suggest that JP is a neuropeptide with important central cardiovascular effects in hypertensive animals.

426.3

AREA POSTREMA STIMULATION INHIBITS RENAL SYMPATHETIC DISCHARGE IN THE ANESTHETIZED RAT. R.B. Felder,* A.L. Pence, L.F. Hayward, College of Medicine, University of Iowa, Iowa City, IA 52242.

Area postrema neurons respond to circulating neurohumoral substances and may play an important role in cardiovascular regulation. We examined the effects of area postrema (AP) stimulation on mean arterial pressure (MAP), heart rate (HR) and renal sympathetic nerve activity (RSNA) in 8 urethane anesthetized Sprague Dawley rats with aortic and carotid sinus nerves sectioned. AP was stimulated at low intensity (40 uA, 0.2 ms, 40 Hz) in the midline along its rostral-caudal axis. Penetrations were separated by .2 mm. Stimulation was carried out on the surface of AP and at .2 mm increments to a depth of .8 mm. Stimulation at the surface (n=9) or at .2 mm depth (n=1) decreased sympathetic discharge (164±10.4 to 138.5±10.2 imp/sec; mean±SE; $p<0.05$) without affecting blood pressure or heart rate. At .2 mm depth (n=8) stimulation elicited significant ($p<0.05$) reductions in blood pressure (100±6 to 90±6 mmHg) and renal nerve activity (155±11 to 109±13 imp/sec). There was no change in heart rate. Similar effects were observed at sites .2 mm lateral to midline. Ventral to .2 mm, a pressor response was usually elicited and became more pronounced at deeper stimulation sites. Prominent depressor responses were also observed at deeper sites. Thus, the predominant effect of selectively stimulating AP neurons is a modest reduction in MAP and RSNA. These findings support a sympathoinhibitory influence of area postrema on cardiovascular reflex control.

426.4

AFFERENT NEURONS TO THE ROSTRAL VENTROLATERAL MEDULLA (RVLM) IN THE RAT: RELATIONSHIP WITH CALBINDIN-D-28K IMMUNOREACTIVITY. A.R. Granata* and H. T. Chang† Departments of Pharmacology*, Anatomy and Neurobiology†, The University of Tennessee, Memphis, College of Medicine, Memphis, TN 38163

RVLM is an important center for cardiovascular control. Although many RVLM neurons express immunoreactivity for calbindin D-28k (CaBP), a Vitamin D-dependent calcium binding protein, the relationships between CaBP and the afferent neurons that project to RVLM, and that between CaBP and the RVLM adrenergic neurons have remained unclear. In this study, FluoroGold (FG) was injected into RVLM to retrogradely label neurons that project to the RVLM, and CaBP+ neurons were revealed by Texas Red immunofluorescence. Many FG labeled neurons were found in the brain, including regions that contain many CaBP+ neurons: the ipsilateral medio-caudal nucleus of the tractus solitarius, the medial vestibular nucleus, the contralateral RVLM, the central gray, the lateral parabrachial nucleus, the paraventricular nucleus of the hypothalamus, the posterior and the lateral hypothalamus, the substantia innominata, and the central amygdala. Only a minority of FG labeled neurons in these regions were CaBP+. On the other hand, the FG labeled neurons in many of these regions were found in close association with terminal fields immunoreactive for phenylethanolamine N-methyl transferase (PNMT), the synthetic enzyme for adrenaline. Our results indicate that while CaBP is not a major marker protein in neurons that project to the RVLM, CaBP+ neurons are found in terminal field of adrenergic fibers, and may be a marker for neurons that are targets of RVLM adrenergic neurons.

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426.5

ROLE OF THE CAUDAL VENTROLATERAL MEDULLA IN REFLEX PRESSOR RESPONSES. I.C. Solomon, A.M. Motekaitis, J.R. Haselton*, and M.P. Kaufman. University of California, Div. of Cardiovascular Med., Davis, CA 95616

Previous studies have shown that the ventrolateral medulla plays a role in the central pathway of the reflex increase in arterial pressure evoked by static muscular contraction, peripheral nerve stimulation, and hypoxia. The role of the caudal ventrolateral medulla (CVLM) in these reflex arcs is not clearly defined. We therefore examined the role of the CVLM in these reflex arcs controlling arterial pressure in chloralose anesthetized cats. Increases in MAP were evoked by three stimuli: static muscular contraction elicited by ventral root stimulation (CONT), electrical stimulation of the sciatic nerve (ScN), and hypoxia (HYP). These changes were compared before and after bilateral microinjection of a broad spectrum glutamate antagonist, kynurenic acid (KYN) (100 mM, 50 nl), into the CVLM. We tested the hypothesis that bilateral microinjection of KYN into the CVLM potentiates the reflex pressor responses to the three stimuli used. The reflex increase in MAP produced by these stimuli were potentiated within 20-30 min following bilateral microinjections of KYN into the CVLM at sites 1.5 mm caudal to 0.8 mm rostral to obex. The reflex increase in MAP either returned to control levels (n=8) or remained augmented (n=10). In two cats, microinjections of KYN into sites very caudal to obex (> 2 mm) elicited either no effect or an attenuation of the reflex increase in MAP. These findings suggest that the reflex arc increasing MAP evoked by static muscular contraction, sciatic nerve stimulation, and hypoxia can be potentiated by blockade of glutamatergic receptors located in the CVLM.

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426.6

VAGAL AND SPINAL MODULATION OF VENTROLATERAL MEDULLA NEURONAL RESPONSES TO RENAL STIMULI. M.A. Vizzard*, A. Standish and W.S. Ammons. Department of Physiology, Thomas Jefferson University, Philadelphia, PA 19107.

Recent experiments from this laboratory demonstrated that cells within the ventrolateral medulla of the cat respond to electrical stimulation of renal nerves and to activation of renal mechanoreceptors and chemoreceptors. The purpose of this study was to determine if these responses are mediated by spinal or vagal afferent pathways. The effect of bilateral cervical vagotomy on spontaneous activity, responses to renal nerve, somatic field and renal receptor stimulation of cells within the ventrolateral medulla was determined. Experiments were performed on 31 alpha-chloralose anesthetized and paralyzed cats (2.5-4.7kg). Bilateral cervical vagotomy decreased the evoked responses of 14/19 cells to electrical stimulation of renal nerves from 3.6±0.6 spikes/s to 2.4±0.4 spikes/s ($p<0.05$). Vagotomy decreased spontaneous activity of 8/14 cells from 5.5±1.8 impulses/s to 0.6±0.0 impulses/s ($p<0.05$), increased spontaneous activity of two cells and did not affect four cells. Bilateral cervical vagotomy increased the evoked response of 5 cells to electrical stimulation of renal nerves from 3.0±0.1 spikes/s to 4.8±1.2 spikes/s ($p<0.05$). Spontaneous activity of each cell also increased from 1.4±0.9 impulses/s to 3.9±0.9 impulses/s ($p<0.05$). Bilateral vagotomy never abolished cell responses to renal nerve stimulation. Five cells (26.3%) were responsive to renal artery occlusion; 1 cell was also responsive to ureteral occlusion. Vagotomy did not alter the responses. The response to renal nerve stimulation for 9 of 9 cells was completely abolished after spinal cord transection. Following spinal cord transection, the spontaneous activity of 7 cells was not significantly affected. Somatic field classification of each cell was unaltered and cell responses to somatic stimulation above the transection level were comparable or greater. The results of these experiments have demonstrated that renal afferent information to the ventrolateral medulla is conveyed via the spinal cord. In addition, the results are consistent with a tonic modulatory effect of the vagus nerve on ventrolateral medulla cell responses to renal stimuli.

426.7

SUPRAMEDULLARY INPUTS TO CARDIOVASCULAR NEURONS OF ROSTRAL VENTROLATERAL MEDULLA IN RATS. S.K. Agarwal+ and F.R. Calaresu* Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada N6A 5C1 and +Playfair Neurosci. Unit, Toronto Hospital, Toronto, Ontario, Canada M5T 2S8

As neuroanatomical and stimulation studies suggest that neurons in the rostral ventrolateral medulla (RVLM) receive and integrate multiple inputs from supramedullary regions of the brain, experiments were done to test the hypothesis that selective activation of cell bodies in the lateral parabrachial nucleus (LPBN), locus coeruleus (LC) and lateral hypothalamic area (LHA) could excite or inhibit the discharge of neurons in the RVLM. We therefore recorded extracellular activity from RVLM units in urethane anaesthetized rats and monitored the changes in firing frequency of these neurons during chemical stimulation of one of LPBN, LC and LHA. Thirtytwo neurons were classified as cardiovascular neurons because their activity was inhibited by baroreceptor activation (1-3 µg phenylephrine i.v.) and displayed a cardiac cycle related rhythmicity. Chemical stimulation with glutamate of arterial pressor sites in the LPBN increased the firing rate (40.3 ± 1.3 %) of 11 (100 %) cardiovascular neurons. Activation of cell bodies in arterial depressor sites in the LC inhibited the firing frequency (59.1 ± 7.1 %) of 10 (91 %) cardiovascular neurons and excited one unit. Activation of cell bodies in arterial depressor sites in the LHA inhibited the discharge rate (25.4 ± 4.7 %) of 6 (60 %) cardiovascular neurons, excited one unit and did not alter the rate of the remaining 3 units. These results provide direct evidence for convergence of excitatory and inhibitory inputs from neurons located in the LPBN, LC and LHA to cardiovascular neurons in the RVLM.

426.8

PARRALLEL AND INDEPENDANT PROCESSING OF SYMPATHETIC BARO- AND CHEMOREFLEXES IN THE MEDULLA OBLONGATA. N. Koshiya*, D. Huangfu and P.G. Guyenet. Dpt. of Pharmacology, University of Virginia, Charlottesville, Va 22908.

Splanchnic sympathetic nerve discharge (SND), phrenic nerve activity (PND) and putative vasomotor sympathetic premotor neurons of the rostral ventrolateral medulla (RVL PMNs) were recorded in urethan- anesthetized vagotomized rats without aortic baroreceptor afferents. Carotid chemoreceptor stimulation (CCSt) with brief N_2 inhalation increased SND by 101 ± 7%, raised MAP and increased the discharge rate of RVL premotor neurons by 46 ± 12% (N=32, range 50% inhibition to 200% activation). During chemoreceptor activation, SND and most RVL neurons displayed pronounced post- inpiratory rhythmicity. These experiments confirm that convergence between peripheral chemoreceptor and baroreceptor inputs to the central network responsible for SND generation occurs at or prior to the RVL premotor neuronal stage. Bilateral microinjection of kynurenic acid (Kyn, 5 nmol in 100 nl) into RVL blocked the sympathetic chemoreflex but left the sympathetic baroreflex intact (N=6). Conversely, bilateral microinjection of Kyn into the caudal ventrolateral medulla at obex level (CVL) blocked the baroreflex but left the sympathetic chemoreflex intact (N=5). These injections also left intact the oscillatory nature of the sympathoactivation produced by CCSt.

The effect of Kyn injection into CVL is attributed to blockade of the baroreceptor input to propriomedullary GABAergic cells of CVL with projections to RVL. These CVL cells are likely to be the last medullary relay of the sympathetic baroreflex before the premotor neuronal stage. Thus, the experiments suggest that the sympathetic chemoreflex and baroreflex utilize two separate and largely independant channels in the medulla until the RVL premotor neuronal stage where algebraic synaptic summation of the two inputs probably occurs.

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426.9

INVESTIGATION INTO THE ROLE OF GLYCINE IN THE PRESSOR RESPONSE TO N-METHYL-D-ASPARTIC ACID (NMDA) INJECTED INTO THE SUBRETROFACIAL NUCLEUS (SRFN). B.Badio and P.J.Gatti*. Dept. Pharmacol., Howard Univ. Coll. of Med., Washington, D.C. 20059.

Recently, it has been shown that there is a glycine binding site associated with the NMDA receptor which facilitates NMDA responses. This site is insensitive to the classical glycine antagonist strychnine. We have shown that NMDA produces a pressor response when injected into the SRFN of the medulla. In this study, we have examined whether blockade of these glycine sites would affect the pressor response to NMDA injected into the SRFN. Adult cats (N=7) were anesthetized with alpha-chloralose 70mg/kg i.v. and a femoral artery was cannulated to measure arterial blood pressure (ABP). The animal was artificially ventilated with room air and the ventral surface of the medulla was exposed. Drugs (50nl) were microinjected into the SRFN of the medulla using glass micropipettes. Stimulation of SRFN with 1mM (N=3) and 10mM (N=4) NMDA produced an increase in ABP of 46±6.6 and 74±6.1 mmHg, respectively. This response could be repeated with no significant change in magnitude. HA-966, an antagonist of the glycine site on the NMDA receptor, was then microinjected into the SRFN and it had no effect on ABP per se. However, after HA-966, the pressor response to NMDA (1 and 10mM) was significantly attenuated to 10±6.9 and 42±6.7 mmHg respectively ($p<0.05$, Student's paired t-test). These data show the importance of the glycine site on the NMDA receptor in the NMDA-induced pressor response in the SRFN.Supported by AHA/NCA.

426.10

Effects of Serotonin on Ionic Currents of Nodose Neurons Co-Cultured with Cardiac Myocytes. **M. Hay* and D. L. Kunze. Dept. of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030.** The effects of serotonin (5-HT) on the cardiopulmonary baroreflex function have been well established however the mechanisms of this action are not fully known. The primary afferent neurons for this reflex are found in the nodose ganglia. The purpose of the present study was to determine the effects of 5-HT on ionic currents recorded from peripheral sensory neurons in the nodose ganglia. One-day old rat nodose neurons were dissociated and plated onto a preformed monolayer of neonatal cardiac myocytes. These neurons were allowed to develop for 2-3 days by which time they appeared to form connections with the spontaneously contracting cardiocytes. Whole cell currents were recorded in response to 200 msec depolarizing voltage steps from a -80 mV holding potential. Initial whole-cell recordings revealed that microejection of 5-HT (200μM) onto nodose neurons results in a decrease in the net outward current when recorded in a physiological bath solution.. The basis for this decrease was determined by isolation of individual ionic currents. Whole-cell inward Ca^{2+} currents were recorded in pipette solution consisting of (in mM) CsCl, 124; EGTA, 2.2; $CaCl_2$, 0.2; $MgCl_2$, 2; and HEPES, 5 and bath solutions of $CaCl_2$, 2.0; Glucose, 10;, and HEPES, 10. Microejections of 5-HT resulted in a 20.7% increase in the peak Ca^{2+} current with no observable effect on threshold voltage or current inactivation. This effect was reversible and repeatable within a given cell. Serotonin also resulted in a moderate decrease in the outward K^+ current. These results suggest the effects of 5-HT may be due, in part, to an increase of the Ca^{2+} current in the sensory neurons of the nodose ganglia. Supported by Grant # HL-36840.

426.11

Three Different Potassium Channels Recorded from Cultured Nodose Ganglia Neurons. **D. L. Kunze* and M. Hay. Dept. of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030.** Presynaptic modulation of potassium (K^+) channel activity has been suggested be involved in the modulation of neurotransmitter release from presynaptic nerve terminals. The purpose of this study was to investigate the properties of the different potassium channels in the peripheral sensory neurons of the nodose ganglia. One-day rat nodose ganglia were dissociated with trypsin and plated on coated coverslips. All recordings were obtained following 1-2 days in culture. Whole cell K^+ currents were recorded in response to 200 msec depolarizing voltage steps from a holding potential of -80mV. Pipette solution consisted of (in mM) KAsp, 145; EGTA, 1.0; $MgCl_2$, 0.5; and HEPES, 10. Bath solution consisted of NMDGCl, 137;, KCl, 5.4; $MgCl_2$, 1.0; Glucose, 10; HEPES, 10. All nodose neurons exhibited a delayed, outwardly rectifying K^+ current that was significantly reduced by extracellular tetraethylammonium (30-50 mM). Approximately 15% of these neurons also exhibited a faster, transient K^+ current which inactivated upon depolarization. This A type K^+ current activated at approximately -35 mV and was blocked by microejected 4-AP. Additional studies in perforated patch whole-cell recordings revealed that microejection of 5mM extracellular Ca^{2+} resulted in a marked increase in the total outward K^+ current. To identify the channels underlying this effect we recorded single channel currents in the inside out patch configuration. Two different channels showed increased open probability when intracellular calcium was raised from 10 nM to 10 uM. The first channel had a slope conductance of approximately 20 pS. A second channel had a slope conductance of 100 pS with a reversal potential near E_k. These data suggest that this channel may be involved in the facilitatory effects of Ca^{2+} on outward K^+ currents in the nodose ganglia. Supported by HL-36840.

426.12

ELECTROPHYSIOLOGICAL EFFECTS OF A 5-HT_2 AGONIST IN THE NUCLEUS TRACTUS SOLITARIUS. **Peter D. Feldman* and Dale K. Paulson. Dept. of Pharmacology, LSU Medical Center, New Orleans, LA 70112.**

Autoradiographic evidence indicates the presence of low densities of 5-HT_2 binding sites in the nucleus tractus solitarius (**nTS**) of the rat (Appel, et al., '90, JPET 255: 843). In view of our recent finding of both excitatory and inhibitory effects of exogenously-applied serotonin on the electrophysiological activity of nTS neurons (The Physiologist 34: 242, 1991), we sought to examine the effect on nTS neuronal activity of a putatively 5-HT_2-selective agonist, α-methyl-5-hydroxytryptamine (**α-Me-5-HT**). Extracellular single unit recordings were made of spontaneous nTS neuronal activity in isolated, superfused slices of the rat medulla. A total of 16 neurons were studied. Ten of the 16 neurons responded to α-Me-5-HT (0.25, 0.5, 1.0, 2.5, 5.0 μM) with depressions of firing rate that were roughly concentration-dependent. In contrast, another 3 of the 16 neurons responded with an increase of firing rate, while the remaining 3 neurons did not respond to α-Me-5-HT at concentrations up to the micromolar range. Analysis of the concentration-dependence of the increases and decreases of activity appears to indicate that the threshold for the excitatory effects of α-Me-5-HT occurred at a lower concentration than that for the inhibitory effects. Further studies involving selective and non-selective serotonergic antagonists are under way to determine the 5-HT receptor subtype(s) mediating these divergent effects of α-Me-5-HT. (Supported by NIH Grant NS-29458).

SUBCORTICAL SOMATOSENSORY PATHWAYS: LEMNISCAL AND SPINOTHALAMIC

427.1

DOUBLE LEBELING OF RAT SPINAL NEURONS PROJECTING TO BOTH SOLITARY TRACT AND DORSAL COLUMN NUCLEI. G.W.LU* and Z.MENG. Dept. of Neurobiology, Captial Institute of Medicine, Beijing 100054, China.

Physiological evidence has been presented for spinal doral horn neurons (SDHN) with branched axons terminating in both the solitary tract and dorsal column nuclei (Lu G.W. et al:Science in China,34:171-183,1991). The present study is aimed at doubly labeling the cells of origin of the double projection SDHN with fluorescent substances.

Propidium (PI) was injected within left solitary tract nucleus (STN) of Wistar rats anesthetized with pentobarbital.Bisbenzimide (Bb) was injected ipsilaterally within the dorsal column nucleus (DCN) 48 hours later.After an additional 8-10 hours of survival, the rats were sacrificed and perfused under deep anesthesia. The spinal cords were sectioned and viewed on a fluoresent microsope.

A total of 282 retrogradely labeled SDHN was found in the left lumbosacral dorsal horn of 10 rats.Of them,51 cells (18%) were doubly labeled by both PI and Bb. One hundred and twenty (42%) cells were labeled by PI only;and 111 (39%) were labeled only by Bb. The PI and Bb labeled cells were triangle or spindle in form and 20-30um in size. These cells were found primarily in segments L4-L5 and mainly in the laminae III-V.

These findings indicate morphologically the existence of SDHN with branched axons ipsilaterally projecting to both the STN and DCN. These neurons were named spino-solitary tract-dorsal column postsynaptic (SST-DCPS) neurons and thought to play an important role in convergence and processing of viscero-somatic inputs.

427.2

CYTOCHROME-OXIDASE (CO) STAINING OF THE CUNEATE NUCLEUS IN THE RAT REVEALS A MODIFIABLE SOMATOTOPIC MAP. D.P. Crockett*, S. Maslany, S.L. Harris and M.D. Egger. Dept. of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854-5635.

Within a rostrocaudally limited region in the middle of the cuneate nucleus (CN), distinctive blotches of intense CO-activity were observed. The CO-staining was maximally differentiated approximately 0.3 - 0.7 mm caudal to the obex, which is located within the previously defined *middle region* (approximately 0.2 - 0.9 mm caudal to the obex) that receives a disproportionately large share of primary afferent terminations (Maslany et al., *Neurosci. Lett.*, in press). No CO-blotches were observed anywhere else in the dorsal column nuclei. Transganglionic labelling (WGA-HRP) demonstrated that several of the CO-blotches in the CN are precisely related to the terminal projection fields of primary afferents from the skin of the forepaws. In contrast, control observations demonstrated that 1) the cytoarchitecture (Nissl-stained sections), 2) the dendritoarchitecture (distribution of microtubule-associated protein 2 (MAP2)) and 3) the organization of retrogradely labelled cuneothalamic cells did **NOT** correspond to the topographical organization of the CO-blotches. Postnatal (up to 11 days postpartum) forepaw deafferentation or removal disrupted the CO-staining pattern in the adult CN.

427.3

DIFFERENTIAL DISTRIBUTION OF CALCIUM-BINDING PROTEINS IN THE DORSAL COLUMN NUCLEI AND SPINAL CORD IN THE RAT. S. Maslany*, D.P. Crockett and M.D. Egger. Dept. of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854-5635.

The distribution of the calcium-binding proteins, calbindin D28k (CaBP) and parvalbumin (PV), were examined in the dorsal column nuclei and spinal cord in the rat by an avidin-biotin peroxidase method. In the cuneate nucleus (CN), both CaBP-immunoreactive (CaBP-IR) and PV-immunoreactive (PV-IR) cells were found throughout the entire rostrocaudal axis, though PV-IR cells were more numerous. However, the density of the PV-IR cells was greatest in the previously defined *middle region* (approximately 0.2 - 0.9 mm caudal to the obex) that receives a disproportionately large share of primary afferent terminations (Maslany et al., *Neurosci. Lett.*, in press) and is similar to the known distribution of thalamic projection cells. The distribution of the CaBP-IR cells appeared uniform throughout the CN. In the gracile nucleus, where the PV-IR cells were also more numerous, the density of both the PV- and CaBP-IR cells was greatest just caudal to the obex. In the spinal cord, PV-IR cells were found in Rexed's laminae II-III, but not in lamina I, while CaBP-IR cells were found in laminae I-II. A few PV-IR and CaBP-IR cells were observed in the ventral horn. While many PV-IR fibers was found in the gracile tract (GT) in the lumbar region, few PV-IR fibers were detected in the GT in the cervical region, suggesting that a large proportion of PV-IR fibers may be proprioceptive in origin.

427.4

THE LOW-AFFINITY RECEPTOR FOR NERVE GROWTH FACTOR IS DIFFERENTIALLY DISTRIBUTED WITHIN THE DORSAL COLUMN NUCLEI OF THE RAT. D.R. Foschini*, D.P. Crockett and M.D. Egger. Dept. of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854-5635.

Nerve growth factor is known to support the survival of many neural-crest-derived primary sensory neurons. We immunocytochemically localized the low-affinity receptor (p75) for nerve growth factor (NGFR) within the dorsal column nuclei of the rat, using a monoclonal antibody (192-IgG; 0.6 - 5.0 μg/ml) and the biotin-avidin method. At the light-microscopic level, most staining appeared to be associated with axons and their terminals. While the gracile nucleus stained intensely and uniformly, within the cuneate nucleus (CN), the most intense NGFR-like immunoreactivity was concentrated in the previously defined *middle region* (approximately 0.2 - 0.9 mm caudal to the obex) that receives a disproportionately large share of primary afferent terminations (Maslany et al., *Neurosci. Lett.*, in press). Within this middle region of the CN, the staining was often patchy, reminiscent of the cytochrome oxidase blotches also observed in this region (Crockett et al., 1992). This pattern of NGFR-like immunoreactivity in the CN was disrupted, and the intensity decreased, in adult rats which had had one forepaw removed perinatally (PD1 - PD6).

427.5

DIFFERENTIAL BRAINSTEM TERMINATIONS OF EXTRINSIC VERSUS INTRINSIC MUSCLE SPINDLE AFFERENTS IN THE MONKEY. C. L. Martin-Elkins*and C. J. Vierck. Department of Neuroscience, University of Florida College of Medicine, Gainesville, FL 32610.

The intrinsic hand muscles, including the lumbricals and the interosseous muscles, appear to play a critical role in fine, coordinated movements of the fingers while the extrinsic hand muscles, located in the forearm, may be more important in gross, powerful movements. Differential effects of dorsal column lesions on these two types of hand movements prompted a comparison of the pattern of central terminations from muscle spindle afferents of intrinsic versus extrinsic muscles of the hand. We have injected cholera toxin conjugated to horseradish peroxidase (CT-HRP) into a variety of intrinsic hand muscles and the superficial digitorum profundus muscle in the forelimb. Prelimary results suggest that the terminal patterns of extrinsic muscle spindle afferents are distinct from those of intrinsic muscle afferents which are not restricted to the triangularis portion of the main cuneate nucleus and the external cuneate nucleus in the brainstem as muscle spindle afferents of extrinsic muscle terminals appear to be. Supported by NS-17474 and NS-07261.

427.6

EFFECTS OF A DORSAL COLUMN LESION ON SOMATOSENSORY EVOKED POTENTIALS IN PRIMATES. J.C. Makous*, R.F. Friedman and C.J. Vierck,Jr. Department of Neuroscience, University of Florida School of Medicine, Gainesville, FL 32610.

Previous work has shown that an intact dorsal column (DC) pathway is necessary for normal frequency and duration discriminations. This study addressed the issue of temporal coding in the absence of dorsal columns through evoked potential recordings. Gross potentials were recorded from both awake and anesthetized macaques in response to non-noxious electrocutaneous and mechanical stimulation of the foot at various frequencies. Recordings of compound action potentials from the white matter tracts of the spinal cord in anesthetized macaques indicate that anterolateral, dorsolateral and DC pathways respond reliably at frequencies of up to 10 Hz. However, epidural awake recordings over primary somatosensory cortex indicate that a chronic lesion of the DC results in a greater than 70% reduction in the amplitude of the evoked potential at 1.5 Hz, whereas at 10 Hz the potential can not be distinguished from the noise. These results are consistent with the idea that the DC is important for temporal discriminations made at frequencies of 10 Hz or greater.

427.7

OVERLAYING OF NERVE-RELATED AND SOMATOTOPIC ORGANIZATION IN THE CUNEATE NUCLEUS OF PRIMATES. S.K. Rasey and J.T. Wall*. Department of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Sensory fibers from individual fingers, palmar pads, and other somatotopic divisions of the hand terminate in rod-like clusters in the cuneate nucleus. The hand innervation territories of the median, ulnar, and radial nerves do not always coincide with the borders of the somatotopic divisions. How are sensory fiber terminations of these nerves organized in the cuneate nucleus? Afferent terminal labeling was reconstructed in the cuneate nucleus of squirrel monkeys following wrist-level nerve injections of horseradish peroxidase conjugates. The termination field of each nerve is elongated rostrocaudally, and spans multiple somatotopic clusters in the mediolateral and dorsoventral dimensions. Label density varies within the field from "core" regions containing dense, continuous label to "fringe" regions containing lower density label. These nerve-related termination patterns presumably contribute to subcortical substrates of nerve dominance columns in the cortical hand map, and provide images of the presynaptic neuropil which loses normal sensory signals after injury of a hand nerve. Supported by NS21105.

427.8

ULTRASTRUCTURAL CHARACTERIZATION OF SYNAPTIC TERMINALS IN THE RAT CUNEATE NUCLEUS

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In six adult rats WGA-HRP was injected in dorsal root ganglia to label primary afferent terminals (PATs), or in the cervical spinal cord (6 days after unilateral cervical rhizotomy and lesion of the dorsal quadrant at T1) to label post-synaptic dorsal column terminals in the cuneate nucleus. The anterograde tracer was revealed by TMB histochemistry. Post-embedding immunogold staining was then performed on thin sections, using antisera for GABA and for glutamate. PATs are large (mean area= 3.14 μm^2, n= 177), contain many mitochondria and small round vesicles. They are GABA-; some are glutamate +; in the plane of the section they contact two or more dendrites of various caliber, and receive synapses from small boutons, some GABA+. Terminals containing HRP reaction product after injection of the tracer in the spinal cord are small (mean area=1.34 μm^2, n= 185) and contact a single thin dendrite or a cell body. They may be GABA+, or glutamate+ or negative for either antiserum. GABA+ terminals are the smallest of the three types (mean area= 0.9 μm^2, n= 288), and, besides contacting dendrites and cell bodies, they are often involved in complex synaptic arrangements with other terminals. (NIH NS 27827 and MURST 40%)

427.9

ULTRASTRUCTURAL INVESTIGATION ON THE DORSAL THALAMUS OF GUINEA PIG

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Recent anatomical investigations provided evidence that GABAergic neurons are present in some thalamic nuclei of the guinea pig (Asanuma, 1991). Aim of the present study was to investigate the intrinsic synaptic organization of selected thalamic nuclei such as the ventrobasal complex (VB) and the ventrolateral nucleus (VL), characterized respectively by the presence or absence of GABAergic neurons. Adult guinea pigs were perfused under anesthesia with 2.5% glutaraldehyde and 0.5% paraformaldehyde. Vibratome sections from the thalamus were either processed for immunohistochemical detection of GABA or osmicated and wafer-embedded in Epoxy plastic for ultrastructural investigation. LM confirms that GABA+ neurons are present in VB but not in VL. At EM, several complex synaptic arrangements, similar to those found in the cat, are observed in VB, whereas VL contains only simple axo-dendritic and axo-somatic synapses, as in the rat. The differences in the intrinsic synaptic organization between the two examined nuclei are also confirmed by post-embedding GABA immunogold labeling. (NIH NS 27827)

427.10

ULTRASTRUCTURAL EXAMINATION OF THE ISOLATED GUINEA PIG BRAIN PERFUSED *IN VITRO*

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The present work investigates at electron microscopic level the preservation of different areas of the adult isolated guinea pig brain maintained *in vitro* by arterial perfusion (de Curtis et al.1991: Hippocampus 1, 341). After characterization of the electrophysiological viability of the preparation via extracellular field potential recordings, the brains were fixed at different times after isolation (one to seven hours) with a mixed aldehyde solution (4% paraformaldehyde and 0.1 % glutaraldehyde in PB, pH 7.2) perfused through the cannula inserted in the vertebral artery. Samples of neocortex, thalamus and hippocampus were trimmed out from 100 um coronal sections, were osmicated and embedded in Epon Spur resin. Semithin and ultrathin sections were then cut, observed at LM and EM respectively and compared to control samples obtained from deeply anesthetized animals directely perfused with the same fixative through the aorta. In all regions explored the tissue was well preserved during the early hours after dissection and showed only minimal and localized alterations after longer incubation times. Cells shape (somatic and dendritic), mielyn sheaths, cytoplasmatic membranes and synaptic clefts in the thalamus and superficial layers of the neocortex showed no major alterations after several hours. Different degrees of extracellular vacuolization (probably due to swelling) and cell damage (membrane shrinkage, cytoplasm vacuolization, darkly stained dendrites, etc.,) were observed in the hippocampus and in the deep neocortical layers after five hours of perfusion. These anatomical evidences add new data to the demonstration of the long-term preservetion of the isolated *in vitro* brain.

427.11

MEDIAL LEMNISCAL TERMINALS IN *M. FASCICULARIS* : 3-DIMENSIONAL COMPUTER-ASSISTED RECONSTRUCTIONS OF SYNAPTIC RELATIONSHIPS.

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Medial lemniscal afferents (ML) to the somatosensory thalamus of *M.fascicularis* have been labeled by the anterograde axonal transport of wheatgerm agglutinin-horseradish peroxidase (WGA-HRP) following injection of the tracer into the dorsal column nuclei of anaesthetized animals. The tissue was prepared for post-embedding GABA immunocytochemistry and studied by electron microscopy. Computer-assisted reconstruction analysis of serial thin sections was utilized to determine the nature of the morphology of the ML terminals, the distribution and spatial organization of the projections to the dendritic arbors of thalamocortical relay cells (TCR's) and GABAergic local circuit neurons (LCN's) and the 3-dimensional features of the synaptic relationships. ML terminals form axodendritic contacts upon the proximal dendritic arbors of TCR cells, usually terminating on the first 100µm of their dendrites. In addition, the ML terminals synapse upon the GABAergic dendritic shafts and appendages of LCN's. Counts of contacts of ML afferents upon thalamic neurons demonstrate that more than 90% of the ML terminals contact GABAergic profiles in addition to TCR dendrites. In contrast, the synaptic terminals of STT afferents (STT - adjacent poster) form simple axo-dendritic synapses upon TCR dendrites but are rarely seen to contact GABAergic profiles. 3-D reconstruction analysis of ML profiles reveals that they form eleborate synaptic arrays with multiple GABAergic dendrites and dendritic appendages of LCN's, the latter in turn, synapsing upon the same element of the TCR dendrite as does the ML terminal. A given TCR dendritic segment receives ML (and no STT) afferent terminals. We conclude that the ML synaptic input upon TCR cells also elicits substantial GABAergic feed-forward modulation of the ML synaptic effect. This support the hypothesis that the transfer of non-noxious information by primate thalamic neurons is markedly influenced by GABAergic LCN processing, in contrast to the lack of GABAergic modulation of noxious information. Supported by NS-23347.

427.12

SPINOTHALAMIC TRACT TERMINALS IN *M. FASCICULARIS*: 3-DIMENSIONAL COMPUTER-ASSISTED RECONSTRUCTIONS OF SYNAPTIC RELATIONSHIPS.

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Spinal afferents (STT) to the somatosensory thalamus of *M.fascicularis* have been labeled by the anterograde axonal transport of wheatgerm agglutinin-horseradish peroxidase (WGA-HRP), following injection of the tracer into the spinal cord of anaesthetized animals. The tissue was prepared for post-embedding GABA immunocytochemistry and studied by electron microscopy. Computer-assisted reconstruction analysis of serial thin sections was utilized to determine the nature of the morphology of the STT terminals, the distribution and spatial organization of projections to the dendritic arbors of thalamocortical relay cells (TCR's) and GABAergic local circuit neurons (LCN's) and the three dimensional features of these synaptic relationships. STT terminals form axodendritic contacts upon the proximal dendritic arbors of TCR cells, terminating on the first 100µm of their dendrites. STT terminals are rarely seen to contact the GABAergic dendritic shafts or appendages of LCN's. Counts of contacts of STT afferents upon thalamic neurons demonstrate that more than 85% contact the TCR's without interacting with GABAergic profiles. In contrast, the synaptic terminals of medial lemniscal afferents (ML - adjacent poster) form elaborate synaptic interactions with GABAergic profiles and TCR dendrites, suggesting a major role for GABAergic modulation of medial lemniscal projections to primate thalamic neurons. 3-D reconstruction analysis of the STT profiles confirms the finding that they primarily form simple axodendritic contacts with TCR cells. A given TCR cell dendritic segment receives only STT (and no ML) afferent terminals. We conclude that the STT synaptic input upon TCR cells is not subject to GABAergic modulation. This supports the hypothesis that the transfer of noxious information by primate thalamic neurons is less influenced by GABAergic LCN processing than is non-noxious information. Supported by NS-21445

427.13

IMMUNOCYTOCHEMICAL COMPARTMENTS IN THE LEMNISCAL AND SPINOTHALAMIC PATHWAYS IN MONKEY BRAINSTEM.

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Cytochrome oxidase (CO) staining reveals a histochemical parcellation of the principal trigeminal (PrV) and dorsal column nuclei (DCN) in monkeys (Noriega and Wall, Brain Res.,565:188,1992). CO-rich and CO-weak compartments, also defined by immunoreactivity for calcium binding proteins parvalbumin (PV) and 28kd- calbindin (CB) respectively, are also present in the thalamic ventral posterior nucleus (Rausell and Jones, J Neurosci.,11:210,1991). Lemniscal and spinothalamic fibers terminate preferentially in these compartments. Compartmentalization based on CO- and immunocytochemical staining is also a common feature of relay nuclei at other levels of lemniscal and spinothalamic systems.

In *M. fasciculatis* and *M. fuscata* CO-rich compartments in PrV are made up of clusters of neurons that show PV immunoreactivity and stain with SMI-32, an antibody to nonphosphorylated neurofilament protein. PV and SMI-32-positive axons also leave the nucleus. CB immunoreactivity occurs in the lateral periphery of PrV and in other CO-weak regions that alternate with the CO-rich zones. CB immunoreactive neurons are more numerous in the dorsal part of PrV, and no CB axons are observed. DCN show similar CO, PV and SMI-32 staining patterns but few CB neurons are present. PV and SMI-32 staining are present in the medial lemniscus. In the spinal trigeminal nucleus, CO staining is weaker overall and absent in the dorsal-most portion. PV and SMI-32 staining are confined to the CO-positive and CB staining to the CO-negative regions.

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427.14

MODELLING SPINDLE-LIKE OSCILLATION IN THALAMOCORTICAL NEURONES.

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Recent experimental studies have shown that thalamocortical neurones are capable of displaying a variety of oscillatory states that do not require synaptic currents (Leresche et al., 1991). The pacemaker oscillation (i.e. low-frequency oscillation associated with high-frequency bursts of action potentials) has successfully been reproduced by models (McCormick et al., 1992; Tóth and Crunelli, 1992) based on the properties of a low threshold Ca^{++} current, I_T and an inward Na^+/K^+ current, I_h that were identified in voltage clamp experiments.

Using the same set of system parameters, however, it has not been possible to reproduce the spindle-like oscillation (SLO), a type of activity where low-frequency oscillation occurs intermittently every 5-25 sec. Our model has been extended by taking into account that an increase in intracellular $[Ca^{++}]$ is known to shift the activation curve of I_h, towards more positive potentials. Thus we have been able to obtain an oscillatory behaviour that closely resembles SLO and to mimic some of the qualitative properties of SLO observed in vitro.

We conclude therefore that I_T, I_h, and the leakage current, I_L are sufficient to describe SLO but the dependence of I_h on the intracellular $[Ca^{++}]$ must be taken into account when dealing with this oscillatory state.

427.15

THE CHARACTERIZATION OF THE INPUT FROM THE NUCLEUS RETICULARIS THALAMI TO THE VENTRO-BASAL THALAMUS OF THE RAT. J.P. Turner* and V. Crunelli. Dept. Physiology, Univ. Wales, Coll. Cardiff, Cardiff, CF1 1SS, U.K.

The GABA containing cells in the thalamus consist of local circuit neurones and those of the nucleus reticularis thalami (NRT), which project to most thalamic nuclei. In the rat ventro-basal complex (VB), local circuit neurones make up $<0.5\%$ of the total neuronal number. When recording intracellularly from VB thalamo-cortical cells in a slice preparation containing rat VB and NRT, single shock, low-frequency stimulation of the NRT resulted in an EPSP (due to activation of cortico-fugal fibres passing through the NRT) and a hyperpolarization. The co-application of 20μM CNQX and 100μM DL-AP5 blocked the EPSP to reveal a fast IPSP, which had a time to peak of 10-30msec, reversed at -70mV, and was blocked by bicuculline (10-50μM). A slow IPSP (time to peak of 200-300 msec), sensitive to the $GABA_B$ antagonist CGP 35348 (100-300μM), was also observed. In the presence of this antagonist, at membrane potentials in the -55 to -65mV range, the decay of the $GABA_A$ IPSP was able to evoke a low-threshold Ca^{2+} potential, indicating that $GABA_A$ receptors may underlie sleep spindle activity in the thalamus. We conclude that the input from the NRT to the VB is mediated via both $GABA_A$ and $GABA_B$ receptors.

PAIN MODULATION: SPINAL I

428.1

THE EFFECTS OF MICROINJECTIONS OF SEROTONIN ON DORSAL HORN NEURONS IN THE CAT. **A.R.Evans* and R.W.Blair, Dept. Physiol. & Biophysics, Univ. Okla. Health Sci. Ctr., Oklahoma City, OK. 73190.**

Serotonin (5-HT) is known to be a neurotransmitter in descending pathways. The purpose of this study was to determine the effects of 5-HT on feline dorsal horn neurons. Three cats were anesthetized with sodium pentobarbital (35 mg/kg). Extracellular potentials were recorded from 7 dorsal horn neurons in the thoracic spinal cord (T_2-T_3). The axons of 3 of the cells were antidromically activated from the medial reticular formation. Seven barrelled glass micropipettes were used to pressure eject (100 msec, 50 psi) small volumes (65 pL) of 3 concentrations (10 μM, 100 μM, 1mM) of 5-HT (pH 7.3), vehicle (phosphate buffered saline, pH 7.4), and 1 mM D-L-homocysteic acid (DLH; pH 7.3). Microinjections of 5-HT increased the spontaneous activity of all 7 cells and the change occurred within 1 sec. The mean changes in activity for 10 μM, 100 μM and 1 mM 5-HT were -0.8, 2.7 and 10.5 spikes/s, respectively. The excitatory responses to 1 mM 5-HT were significantly different from the responses to 10 μM and 100 μM 5-HT. There was no difference in the change in spontaneous activity between the 2 lower concentrations of 5-HT. Five cells were tested for responses to 1 mM DLH; 4 were excited and 1 had no effect. No responses to vehicle were found. Using the pressure ejection technique, 5-HT has been found to excite dorsal horn neurons, some of which are spinoreticular tract neurons. (supported by NIH grant HL29618 and OCAST grant HR9-089).

428.2

MODULATION OF SEROTONIN (5-HT) RELEASE FROM SPINAL CORD TISSUE BY OPIOIDS AND KETAMINE. B.K. Kradel, E.H. Stullken, Jr.*, D.L. Smith, P.J. Monroe, D.J. Smith. Dept. of Anesthesiology, WVU Health Sciences Center., Morgantown WV 26506

Antinociception from some intrathecally administered drugs appears to be mediated in part by spinal serotonergic processes (Pain 12: 57,82; Eur.J.Pharm. 160: 211,89 & 194: 167,91; Neuropharm. 28: 1047,89). In this study, several opioids and ketamine (each in a concentration of 1 μM) were evaluated for their ability to alter the release of ^{3}H-5-HT from superfused rat spinal cord tissue. Using synaptosomes, it was found that neither ß-endorphin nor [D-Pen^{2,5}] enkephalin (DPDPE) affected basal or evoked release (15mM K^+) of ^{3}H-5-HT. These results expand an earlier report (Neuropharm. 25: 261,86) demonstrating that ketamine, morphine and ethylketocyclazocine (EKC) were also ineffective in this preparation, and provide further evidence that these drugs do not release 5-HT directly from spinal serotonergic terminals. On the other hand, when a spinal cord slice preparation was used (neuronal connectivity is less compromised), 5-HT efflux was modified by some of these agents. Ketamine, DPDPE and Tyr-D-Ala-Gly-MePhe-Gly-ol (DAMGO) enhanced the K^+-evoked release of total ^{3}H to 153 ± 13, 190 ± 18 and 178 ± 26 % of control, respectively. In contrast, EKC and U50,488 reduced K^+-evoked release to 63 ± 14 and 63 ± 13 %, respectively. No changes were resolved with either morphine (70 ± 22) or ß-endorphin (115 ± 14 %). Thus, these drugs appear to affect neuronal processes that converge on spinal serotonergic terminals. Interestingly, the effect of the various agents on 5-HT release was not always consistent with what would be proposed from the antinociceptive studies that have implicated 5-HT in their local spinal actions.

428.3

RELEASE OF ADENOSINE FROM THE SPINAL CORD BY 5-HYDROXYTRYPTAMINE (5-HT) AGONISTS: CHARACTERIZATION OF RECEPTOR SUBTYPES. J. Sawynok*, D. Leeson, A. Reid, G. Doak and T. White. Dept. Pharmacology, Dalhousie University, Halifax, NS B3H 4H7.

Release of adenosine by 5-HT may mediate a component of the spinal antinociceptive action of 5-HT (Brain Res. 426: 346, 1988). In this study, the 5-HT receptor subtypes involved in adenosine release were characterized using behavioral and neurochemical approaches. 8-Phenyltheophylline, a methylxanthine adenosine receptor antagonist, reduced spinal antinociception by 5-HT and CGS 12066B (5-HT_{1B} agonist) but not TFMPP (5-HT_{1C} agonist) or DOI (5-HT_2 agonist) in the hot plate test. In neurochemical experiments, 5-HT, CGS 12066B, TFMPP and DOI (but not 8-OH-DPAT, a 5-HT_{1A} agonist, or 2-Me-5-HT, a 5-HT_3 agonist) induced release of adenosine from dorsal spinal cord synaptosomes. In all cases, the adenosine originated from released nucleotide rather than as adenosine per se, as release was reduced by inhibition of 5'-nucleotidase activity. Release of adenosine by 5-HT and CGS 12066B was Ca^{2+}-dependent, while that induced by TFMPP and DOI was not. Release of adenosine from the spinal cord appears to be due to activation of a 5-HT_{1B} receptor subtype; the Ca^{2+}-dependent component of release appears to contribute to behavioral effects of 5-HT agonists. (*Supported by MRC Canada*)

428.4

DESIPRAMINE REDUCES NMDA-INDUCED NOCICEPTIVE BEHAVIOUR MEDIATED THROUGH THE SPINAL 5HT_{1A} RECEPTOR. N.Mjellem, A.Lund and K.Hole*. Departement of Physiology, University of Bergen, N-5009 Bergen, Norway.

The mechanism of the antinociceptive effect of desipramine (DMI) is unclear. It is generally accepted that excitatory amino acids (EAA) act as neurotransmitters in primary nociceptive fibers, and in vitro studies have shown that tricyclic antidepressants may influence the NMDA receptor complex.

The modulatory effect of DMI on the nociceptive behaviour induced by intrathecal (i.th.) NMDA (0.4 nmol, 7µl) was studied in mice. DMI was administered either i.th. (7µg, 7µl) 5 min prior to administration of NMDA, intraperitoneally (i.p.) (10 mg/kg) 90 min prior to NMDA, or in the drinking water during 3 weeks. The nociceptive behaviour (biting and scratching) produced by i.th. NMDA was significantly reduced when the animals were pretreated with DMI, either acutely or chronically.

The 5HT_{1A} agonist 8-OH-DPAT inhibits NMDA-induced behaviour. A functional upregulation of the 5HT_{1A} receptor was found after chronic administration of DMI. We therefore investigated if blocking of the 5HT_{1A} receptor would reduce the effects of DMI.

A selective 5HT_{1A} antagonist, NAN-190 Hydrobromide (10 µg), was injected i.th. 5 min prior to NMDA in animals pretreated with DMI. The 5HT_{1A} antagonist reversed the reduction of NMDA-induced behaviour produced by DMI.

These findings indicate that both acute and chronic administration of DMI reduce the NMDA-induced nociceptive behaviour, and that this may be mediated at least partly through the 5HT_{1A} receptor in the spinal cord.

428.5

DIFFERENTIAL EFFECTS OF 5-HT AND NE UPTAKE BLOCKADE ON OPIOID RECEPTOR SUBTYPE SELECTIVE ANALGESIA. D. Paul* and A.R. Fowler. Department of Pharmacology, Lousiana State University Medical Center, New Orleans, LA 70112.

Selective stimulation of mu_1, mu_2, delta, $kappa_1$ or $kappa_3$ opioid receptors produces analgesia through neuroanatomically and neuropharmacologically distinct mechanisms. Blockade of 5-HT or NE uptake potentiates morphine analgeia. To determine whether selective 5-HT and NE uptake blockade will potentiate analgesia produced through each of the opioid receptor subtypes, we assesed the analgesic effects of morphine, i.c.v. DAMGO (mu_1), i.t. DAMGO (mu_2), i.c.v. and i.t. DPDPE (delta), U50,488H ($kappa_1$), and nalorphine ($kappa_3$) in mice treated with zimelidine (10 mg/kg), desipramine (5 mg/kg) or saline using the tail-flick assay. Zimelidine produced a leftward shift in the dose-response curves for morphine, i.t. DAMGO (5-fold), and U50,488 (2-fold). Desipramine produced a lefward shift in the dose-response curves for morphine and i.t. DPDPE (2-fold). These results indicate that a 5-HT may modulate mu_2 and κ_1 analgesia, whereas NE may modulate spinal delta analgesia.

428.6

SPINAL 5HT1B BUT NOT 5HT1A RECEPTOR SUBTYPES DEPRESS RESPONSES OF DORSAL HORN CELLS TO CUTANEOUS NOXIOUS THERMAL STIMULATION IN THE RAT. Z. Ali, G. Wu, S. Barasi, S. R. Williams and V. Crunelli.* Dept. of Physiology, U.W.C.C., Cardiff. U.K. CF1 1SS.

Intrathecally applied 5HT1B agonists depress and 5HT1A agonists facilitate spinal nociceptive reflexes. To further investigate the actions of 5HT1A and 1B agonists we combined the intrathecal route of drug delivery with extracellular recordings from single lumbar dorsal horn neurons responding to noxious thermal stimulation.

Male Wistar rats were anaesthetised with halothane. Responses in all neurons were depressed by the 5HT1-like agonist 5-CT (0.3nmol., n=6; 3nmol., n=2) and antagonised by cyanopindolol (50nmol., n=4). TFMPP (300nmol.), a 5HT1B agonist, reduced responses in 6 of 7 neurons. However, in 2 of 4 cells 8-OH DPAT (150nmol.), a 5HT1A agonist, increased nociceptive responses.

These data suggest the antinociceptive actions of intrathecally applied 5HT1B agonists in behavioural tests may be mediated by a reduction in responses of dorsal horn neurons to noxious thermal stimuli. In contrast, facilitation of spinal reflexes by 5HT1A agonists may be mediated by increased responses of dorsal horn neurons.

428.7

NOCICEPTION MEDIATED BY THE ACTIVATION OF POLYMODAL NOCICEPTORS MAY BE SELECTIVELY ATTENUATED BY SEROTONIN$_{1A}$ AGONISTS. V. Pirec,* D.C. Yeomans, H.K. Proudfit. Dept. of Pharmacology, Univ. of Illinois College of Medicine at Chicago, Chicago, IL 60612

Experiments were designed to determine whether different rates of noxious skin heating activate different types of nociceptors and if the antinociceptive effects of 5-HT$_{1a}$ agonists are dependant on the nature of the thermal stimulus used for nociceptive testing.

The first group of experiments was designed to determine whether high and low rates of thermal stimulation activated different nociceptor types. Capsaicin was topically applied on the skin of rat's hindpaw to sensitize polymodal nociceptors. Capsaicin sensitized nociceptive responses to low (0.6°C/sec), but not to high (6.5°C/sec) heating rates. As capsaicin selectively sensitizes polymodal nociceptors, low heating rates appear to preferentially activate polymodal nociceptors.

The second group of experiments was designed to determine whether 5-HT$_{1a}$ agonists would selectively reduce nociceptive responses produced by low heating rates. Intrathecal administration of the 5-HT$_{1a}$ selective agonists 8-OH-DPAT and buspirone, in both capsaicin sensitized and non-sensitized rats, produced significant antinociception only when low rates of skin heating were used. These antinociceptive effects of 5-HT$_{1a}$ agonists were significantly attenuated by intrathecal injection of the 5-HT$_{1a}$ antagonists spiroxatrine or NAN-190.

These results indicate that 5-HT$_{1a}$ receptors in the spinal cord selectively modulate the nociception that is mediated by polymodal nociceptors that are activated by low rates of skin heating. (Supported by USPHS Grant DA03980 from the National Institute on Drug Abuse).

428.8

INTRATHECAL (I.T.) METHOXAMINE POTENTIATES DEXMEDETOMIDINE SPINAL ANTINOCICEPTION IN THE RAT: EVIDENCE OF A ROLE FOR α_1-ADRENOCEPTORS AND ENDOGENOUS OPIOIDS. C.W. Loomis, K.D. Arunachalam, D. Vyas* and B. Milne, School of Pharmacy, Memorial University, St. John's, Nfld, Canada, A1B 3V6

I.t. α-agonists induce selective antinociception (ANT) in animals via spinal α_2-adrenoceptors. The role of spinal α_1-adrenoceptors is less clear. The objectives of this study were to determine: 1) if i.t. methoxamine (MX; α_1-agonist) potentiates ANT induced by i.t. dexmedetomidine (DX; α_2-agonist) in the rat; and 2) if endogenous spinal Met-Enk is involved in this interaction. Male, Sprague-Dawley rats (250-400 g) were implanted with i.t. catheters. Rats whose baseline responses and motor function were normal were used in the tail-flick and paw pressure withdrawal tests. All drugs were given i.t. The addition of a fixed (10 µg) threshold (<10% MPE) dose of MX to DX yielded an 8-fold leftward shift of the DX dose-response curve (DRC). MX potentiation of DX was: not due to a prolonged duration of DX action; and was significantly antagonized by each of the following i.t. pretreatments: prazosin (10 µg), Wyeth 27127 (0.5 µg), naloxone (30 µg), or antiserum to Met-Enk (but not by antiserum preabsorbed with Met-Enk). Pretreatment with i.t. SCH 32615 (NEP inhibitor) shifted the MX+DX DRC to the left 6-fold compared to vehicle pretreated rats but had little effect on the DX DRC (no effect on MX). Light microscopy of spinal cords from rats given MX+DX (twice daily for 3 days) revealed no inflammation, haemorrhage or necrosis, and no changes in substance P- or CGRP-like IR compared to vehicle controls. The data indicate that the MX potentiates DX spinal ANT without neurotoxicity. The effect appears to be mediated, at least in part, by spinal Met-Enk, consistent with the known ANT interaction between opioid- and α_2-agonists. (Supported by MRC Canada)

428.9

ALPHA-2-ADRENOCEPTOR AGONIST MEDETOMIDINE IS HIGHLY EFFECTIVE FOR SUPPRESSION OF FORMALIN-INDUCED IMMEDIATE EARLY-GENES IN SPINAL BUT NOT IN THALAMIC NEURONS. A.Pertovaara*, T.Herdegen[2], R.Bravo[3] and M.Zimmermann[2]. Dept.Physiol.Univ. Helsinki, Finland, [2]II.Physiol.Inst.Univ.Heidelberg, Germany and [3]Bristol-Myers Squibb Res.Inst.,Princeton,NJ, USA

The expression of c-JUN, JUN B, c-FOS, FOS B and KROX-24 proteins were used to study the antinociceptive effect of ip administered medetomidine (MED), a selective alpha-2-adrenoceptor agonist, in the rat CNS. Proteins were detected by immunocytochemistry. MED (100 or 300 ug/kg) was injected 12 min before the injection of formalin (FOR; 5% 50 ul) in the plantar skin of the hindfoot. The rats were killed and perfused 90 min later. Atipamezole (ATI; 1.5 mg/kg ip) was used to reverse the effects of MED.

FOR induced expression of all studied proteins in the spinal dorsal horn ipsilaterally, and in the medial thalamus. Both MED doses strongly (80-90%) suppressed the expression of all proteins in spinal but not in thalamic neurons. ATI completely reversed the effect of MED.

Thus, the expression of immediate-early gene (IEG) encoded proteins is under powerful control of alpha-2-adrenoceptors in spinal but not in thalamic neurons. These IEG data dissociate from recent behavioral and electrophysiological findings (Pertovaara et al. Neurosci.1991.44.705) indicating that in the thalamus also the low but at the spinal level only the high MED dose produced antinociception. Supported by DFG

428.10

REINFORCEMENT OF SPINAL NORADRENERGIC NEUROTRANSMISSION INDUCES ANTINOCICEPTION IN THE RAT TAIL FLICK TEST - ONE ADDITIONAL MODE OF ACTION OF THE CENTRAL ANALGESIC TRAMADOL. W.S. Reimann*, H. Schlütz and F. Schneider. Dept. Pharmacology, Grünenthal GmbH, W-5100 Aachen, FRG.

Tramadol has low affinity for opioid receptors and shows norepinephrine (NE) and serotonin uptake inhibition in standard assays. We tested whether NE uptake blockade occurred at spinal sites and whether it can effect antinociception. Slices of the rat dorsal spinal cord were preincubated with ^{3}H-NE then superfused and stimulated electrically twice. Tramadol enhanced the stimulation-evoked overflow of tritium, starting at 1 μM, qualitatively similar to the NE uptake inhibitor desipramine (DMI). The effects were mainly due to the (-)-enantiomer of tramadol. When uptake sites were blocked by DMI, tramadol was virtually ineffective, suggesting NE uptake inhibition is responsible for tramadol's effects. I.t. injection site of 12 μg tramadol at the lumbar produced antinociception in the rat tail-flick test; both enantiomers were roughly equally potent. Antinociception was also observed with i.t. DMI, starting with 6 μg. Effects of DMI and tramadol were antagonized by i.p. injection of 1 mg/kg yohimbine. The results from in vitro and in vivo experiments provide evidence that tramadol reinforces NE neurotransmission at the spinal site and that this mode of action in addition to opioid-like activity is involved in spinal antinociception.

428.11

CENTRAL ACTION OF SYSTEMIC LIDOCAINE MEDIATED BY GLYCINE SPINAL RECEPTORS: AN IONTOPHORETIC STUDY IN RATS. G.Biella and M.L.Sotgiu.*Dept.Physiopathol.and Therapy of Pain, Milan University, and I.F.C.N.-C.N.R. Milan, ITALY.

Some antinociceptive properties displayed by systemic lidocaine (lido) result from centrally played effects.In the effort to identify the central target of lido, single neurons in the spinal cord dorsal horn of anesthetized rats were recorded. The microejection of various drugs was used as explorative tool. Concurrent recording to iontophoretic and micropressure ejections of drug solutions (glutamic acid 100mM, strychnine sulphate 1mM, glycine 10mM) were performed. Non- and noxious stimuli were delivered to somatotopically competent areas of the lumbar cord and wide dynamic range neurons (WDR) were selected. Intravenous lido inhibited, for 15-25 min, the excitatory response induced by centrally iontophoresized glutamate (27.3 ± 1.3 to 4.7 ± 0.9 spikes/s). During the inhibitory period,the iontophoretic ejection of strychnine partially counteracted the lido inhibitory effects (4.7 ± 0.9 to 22.0 ± 1.1 spikes/s). Systemic lido had no effect on concurrent glutamate and strychnine ejection; micropressurized lido showed no influence on the excitatory response of the recorded neurons. Strychnine effects were evident also in the case of glycine inhibitions of glutamate induced excitations. We suggest that some of the central effects of lido act upon spinal glycine strychnine-sensitive receptors, possibly by glycine residue bearing metabolites of lido itself.

428.12

MUSCARINIC CHOLINERGIC SPINAL ANTINOCICEPTION. E.T. Iwamoto*, L. Marion, N.W. Pedigo, and R.D. Guarino. Department of Pharmacology, University of Kentucky College of Medicine, Lexington, KY 40536

Intrathecal (i.t.) administration of the muscarinic cholinergic agonist, (+)-*cis*-methyldioxolane (CD), into the lumbar spinal cord of male Sprague-Dawley rats produced antinociception as assessed by the 52° C hot-plate and tail-flick (7 sec control latency) assays. I.t. CD induced antinociception within 5 min for up to 3 hr without altering four other motor reflexes. The median effective i.t. dose of CD was ~10 nmol in both antinociceptive assays. Tissue content of CD 30 min after an i.t. injection of 37.5 nmol ^{3}H-CD was estimated at 1 μM. The antinociception induced by 37.5 nmol of CD was antagonized by 5 min i.t. pretreatment with pirenzepine or methoctramine at IC50 doses of approximately 1 and 7 nmol, respectively. Five min i.t. pretreatment with 20 nmol LY-53857, 25 nmol S-(-)-zacopride, or 25 nmol idazoxan (doses which were inactive alone) each partially antagonized 37.5 nmol CD antinociception, whereas buffer, mecamylamine or naloxone had no effect. CD inhibited ^{3}H-QNB binding in spinal cord homogenates with μmolar affinity. The data suggest that i.t. CD antinociception may be mediated via M1 and/or M2 muscarinic, 5-HT2 and/or 5-HT3 serotonergic, and/or α2 adrenergic lumbar spinal receptor sites. (Supported in part by NS 28847 and KTRB.)

428.13

MULTIPLE SPINAL MEDIATORS IN NICOTINE ANTINOCICEPTION. D. T. Rogers* and E. T. Iwamoto, Dept. of Pharmacol., Univ. of Kentucky Coll. of Med., Lexington, KY 40536.

The antinociceptive effects of s.c. nicotine were evaluated in male Sprague-Dawley rats using the hot-plate and tail-flick tests. Nicotine (0.125, 0.25, 0.375, or 0.5 mg/kg s.c.) produced a dose-related inhibition of nociception in both tests. No significant alteration of other motor reflexes was observed with 0.375 mg/kg s.c. nicotine. The effect of 0.1 μmol of various receptor antagonists intrathecally administered 12 minutes before 0.375 mg/kg s.c. nicotine was examined. The order of potency for inhibition of nicotine antinociception in the hot-plate test for 0.1 μmol antagonists was: methysergide ≥ scopolamine > yohimbine > mecamylamine> idazoxan > atropine > naloxone = 0. In the tail-flick test, the order of potency for inhibition of nicotine antinociception was: scopolamine > methysergide > atropine > idazoxan > yohimbine > mecamylamine > naloxone = 0. These data suggest that the antinociceptive effects of s.c. nicotine are mediated via a number of sites in the spinal cord, including noradrenergic, serotonergic, and muscarinic cholinergic. Spinal nicotinic and opioid receptors appear to have less of an involvement. (Supported by NS 28847 and the KTRB.)

428.14

THE EFFECTS OF AGING ON SPINAL DAMPGO-INDUCED ANTINOCICEPTION. T. Crisp*, J.L. Stafinsky, M. Uram and V.C. Perni. Department of Pharmacology, N.E. Ohio Univs. Coll. of Med., Rootstown, OH 44272.

This study investigated the effects of aging on the antinociceptive efficacy of the μ opiate agonist DAMPGO. Young, mature and aged Fischer 344 rats (6, 16 and 26 months old, respectively) were injected intrathecally (i.t.) with various doses of DAMPGO. Opiate-induced changes in tail-flick latency (TFL) and hot plate latency (HPL) were recorded 5, 15, 30, 60 and 120 min post-injection. The results demonstrated that DAMPGO dose-dependently elevated TFL and HPL in each of the three age cohorts. When the tail-flick test was used as the nociceptive measure, the potency ratio of DAMPGO was significantly different between the 16 and 26 month old rats. No significant age-related differences were observed in the potency ratio for DAMPGO when the hot plate was used as the nociceptive test. Thus, while higher i.t. doses of DAMPGO are apparently needed to produce antinociception in older rats, the μ opioid receptor site continues to mediate spinal DAMPGO-induced antinociception throughout the life span of the rat.

428.15

SELECTIVE INHIBITION OF NOCICEPTIVE RESPONSES OF SPINAL DORSAL HORN UNITS BY COCAINE. J.A. Kiritsy-Roy*, P.J. Danneman, B. Shyu, T.J. Morrow, and K.L. Casey. Depts. of Neurol. and Physiol., Univ. of Michigan and VAMC, Ann Arbor, MI 48105.

Cocaine produces a dopaminergic dose dependent antinociceptive effect in the rat hot plate and formalin tests (Lin et al., Brain Res. 479:306, 1989). To investigate spinal mechanisms mediating the antinociceptive action of cocaine, the inhibitory effect of cocaine on the responses of spinal dorsal horn (SDH) neurons to several natural somatic stimuli was determined in anesthetized rats. Units in the SDH at T13-L1 with receptive fields (RFs) on the ipsilateral hindpaw were identified by their responses to non-noxious (tap) or noxious (pinch) stimulation. SDH units at L2-L3 with RFs on the tail increased firing rate in response to stimulation with an infrared CO_2 laser at intensities that produced a brisk tail flick response in the awake animal (10 W, 45 msec pulses). Stimulation induced unit firing rate was determined before and after intravenous administration of cocaine. Cocaine inhibited the responses of 11 units to noxious pinch of the ipsilateral hindpaw and of 7 units to laser stimulation of the tail. This effect of cocaine was dose related in the range of 0.1 to 3.1 mg/kg iv. Units that responded only to non-noxious stimulation of the hindpaw were not inhibited by the same doses of cocaine. Cocaine failed to attenuate unit firing in response to noxious stimulation of the hindpaw in rats with complete spinal cord transection at the upper thoracic level. These results indicate that cocaine produces a selective reduction of *noxious* somatic input at the level of the SDH. The effect of spinalization suggests that cocaine antinociception is mediated at least in part via supraspinal descending inhibitory pathways. (Supported by the Veterans Administration and Bristol-Myers Squibb Award)

428.16

EFFECTS OF INTRATHECALLY INJECTED NON-PEPTIDE SUBSTANCE P ANTAGONIST CP-96,345 ON NOCICEPTION IN RATS Y.I. Garces[1], S.F. Rabito[2], R. Minshall[2], R.S. Cohen*[1] and J. Sagen[1]. Departments of Anatomy and Cell Biology[1] and Anesthesiology[2], University of Illinois College of Medicine, Chicago, IL 60612.

Substance P (SP) may mediate nociceptive transmission via the NK-1 receptor in the spinal cord. Studies in this field have been limited by the lack of specific antagonists. A recently developed non-peptide SP antagonist, CP-96,345, is specific for the NK-1 receptor. The purpose of this study was to assess the effects of this antagonist on nociception. Nociception was determined using tail flick, paw pinch and hot plate tests in rats. The rats received intrathecal injections of various doses of CP-96,345 and pain sensitivity was assessed at several time intervals up to two hours following injection. In addition, the ability of CP-96,345 to inhibit SP-induced biting and scratching was assessed. Results from analgesiometric testing indicated that doses up to 240 μg had no significant effect on either tail flick latency or paw pinch threshold. Hot plate latency was only elevated by high dose of the antagonist. In addition, the SP antagonist failed to inhibit the SP induced biting and scratching. The intrathecal injection of CP-96,345 produced a dose-related decrease in tail skin temperature which could be mediated by the NK-1 receptor. Preliminary binding studies using ^{125}I-SP were also performed. Assessment of the ability of CP-96,345 to inhibit the binding of ^{125}I-SP to the rat spinal cord and rat salivary tissue indicated that CP-96,345 and SP behave similarly at the NK-1 receptor. The results of this study suggest that, while CP-96,345 binds the SP site at the NK-1 receptor in the spinal cord, this receptor is most likely not involved in mediating some types of acute pain at the spinal cord level. (Supported in part by NS25054.)

428.17

Evaluation of the Effect of Nitric Oxide on the Release of iCGRP from the Dorsal Horn of the Spinal Cord in Rats.

M.G. Garry*, H.E. Geier, and K.M. Hargreaves, Univ. of Minnesota, Dept. of Restorative Sciences, and Dept. of Pharmacology, Minneapolis, MN.

Nitric oxide (NO) is a labile gas, previously known to be produced by bacteria. More recently NO has been localized to macrophages, endothelium, and to neurons where it stimulates the formation of cyclic GMP. It has been proposed that NO plays a role in peripheral nociceptive mechanisms (Brain Res. 531:342, 1990). We have previously shown that enhanced capsaicin-evoked *in vitro* release of iCGRP from the dorsal horn may serve as a biochemical marker for hyperalgesia during inflammation (Brain Res., in press). Whether NO plays a role in the processing of nociceptive information at the level of the spinal cord, however, has not been evaluated. Therefore, the goal of this study was to evaluate the effect of NO on the *in vitro* release of iCGRP from certain primary afferent neurons in the dorsal horn of rats. Spinal cords from male Holtzman rats (175-199g) were ejected by hydraulic extrusion. The dorsal horn of the lumbar enlargement was isolated and chopped into 200μ cubes and placed into chambers which were superfused with oxygenated Krebs (pH 7.4) for 51 min. Following this period, one group of chambers (n=3) was superfused for 21 min with 1mM sodium nitroprusside (a liberator of NO) after which the chambers were superfused with capsaicin (10μM) for 6 min to selectively depolarize certain primary afferent neurons in the preparation. Another group (n=3), which was superfused with capsaicin but not with nitroprusside served as the control. The amount of iCGRP in each fraction was assessed by radioimmunoassay. Our results indicate that NO enhances capsaicin-evoked release of iCGRP from the dorsal horn. Preliminary results indicate that there is a significant increase in the capsaicin-evoked release of iCGRP occurs following pretreatment with nitroprusside when compared to controls (5638±511 vs. 2149±736 fmol/gm tissue/6 min, respectively [$p<0.05$]). In contrast, basal release of iCGRP was unchanged following nitroprusside. These results suggest that NO may play a role in the processing of nociceptive information at the level of the spinal cord.

428.18

REGULATION OF TACHYKININ RECEPTOR mRNA EXPRESSION IN RAT DORSAL HORN DURING NOCICEPTION. K. E. McCarson* and J. E. Krause. Dept of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

The tachykinin peptides substance P (SP) and neurokinin B (NKB) are both contained in the dorsal horn of the spinal cord. SP is contained in both primary afferent and intrinsic neurons and has been shown to be a mediator of nociception, whereas NKB appears to produce antinociception when applied to the dorsal horn. Binding sites for both peptides exist in the dorsal horn. We used the formalin test as a model of nociception to examine changes in steady state levels of SP, SP receptor (SPR) and NKB receptor (NKBR) mRNA in the dorsal horn of the rat during formalin-induced nociception. Rats were injected s.c. with 100μl of 5% formalin in the plantar aspect of the right hind paw. Sham injected animals served as controls. Either 2 or 6 hr after formalin injection, the rats were decapitated, the spinal cords removed, and RNA extracted from the lumbar dorsal horns. Control tissues were removed 2 hr after sham injection. Solution hybridization-nuclease protection assays were used to quantitate SPR and NKBR mRNA levels and Northern blot analysis was used to quantitate total SP-encoding mRNA levels. SPR mRNA levels were increased by 80% 2 hr after formalin injection and NKBR mRNA levels were increased by 50% 6 hr after injection. Dorsal horn SP mRNA levels were not altered. From these studies we suggest that acute alterations in tachykinin receptor systems occur during formalin-induced nociception.

PAIN MODULATION: SPINAL II

429.1

ACTIVITY OF SPINAL DORSAL HORN NEURONS FOLLOWING UV-INDUCED HYPERALGESIA IN THE RAT. **L. Urban,* A. Dray, E.A. Campbell, M.N. Perkins and I. Patel, Sandoz Institute for Medical Research, 5 Gower Place London, WC1E 6BN, UK.**

Activation of nociceptors during peripheral inflammation or neuropathy increases excitability of spinal wide dynamic range (WDR) neurones. We have characterised the activity of WDR cells during the thermal and mechanical hyperalgesia induced by UV irradiation in the rat (Perkins et al This Meeting).

Female Wistar rats with UV-induced hyperalgesia of the glabrous skin of one hind paw were anaesthetised (urethane, 1 g/kg) and an L2-4 laminectomy performed. Activity of spinal WDR cells was recorded extracellularly in response to stimulation of their peripheral receptive fields by noxious heat (52°C) and pinch in a) control animals, b) 1-3 and 5-7 days after UV treatment.

There was an increase in the proportion of spontaneously active cells in hyperalgesic animals (control, 15% n=39; days 1-3, 47% n=19; days 5-7, 20% n=20). The duration of the response and the total number of action potentials evoked by a thermal stimulus was significantly increased in UV treated animals. In addition, the threshold for noxious heat activation was decreased in the hyperalgesic groups (control, 51.8±1.1°C, n=8; days 1-3, 46.5±1.1°C, n=11; days 5-7, 47.1±0.8°C, n=8) and there was an expansion of the receptive fields of WDR cells.

These data show a lowering of the nociceptive heat threshold and hyper-excitability of spinal WDR cells following UV-irradiation of their receptive fields. These changes may underlie the behavioural hyperalgesia seen in this model.

429.2

USE OF FOS-LIKE IMMUNOREACTIVITY TO STUDY THE EFFECTS OF ANALGESIC COMPOUNDS IN SPINAL CORD NEURONS OF POLYARTHRTIC RATS. C. Abbadie and J.-M. Besson*. INSERM U161 and EPHE, 2 rue d'Alésia, 75014 Paris, France.

We have recently shown that during the development of adjuvant-induced arthritis (AIA) in the rat, the number of Fos-like immunoreactive (FLI) neurons in lumbar spinal cord was maximal 3 weeks after induction of the disease, which is the acute phase of hyperalgesia. Moreover, the number of FLI neurons induced by repeated pressure to the ankle was greater in arthritic animals than in healthy ones. In non-stimulated arthritic rats, FLI neurons were mainly present in the neck (laminae V-VI) of the dorsal horn and in the ventral horn of L3-L5, whereas following stimulation they were numerous in the superficial laminae. Thus, this model was used to gauge the effects of various analgesic compounds.

1. We administered morphine or naloxone in non-stimulated arthritic and in stimulated arthritic animals before ankle stimulation. In non-stimulated arthritic rats, morphine (1-9 mg/kg i.v.) or naloxone (1-3 mg/kg i.v.) induced no change in the FLI present in lumbar spinal neurons. But in stimulated arthritic animals, pretreatment with morphine at 0.5 or 1 mg/kg reduced by more than 50% the number of FLI neurons in the superficial laminae and at 3 mg/kg quite abolished the labeling, whereas in the neck of the dorsal horn and in the ventral horn the decrease of FLI was about 55% at 3 mg/kg i.v. This decrease was reversed by naloxone. Pretreatment with naloxone alone (1mg/kg i.v.) did not change FLI.

2. A 2 week chronic treatment with aspirin or acetaminophen (respectively 300 and 500 mg/kg/day) clearly reduced FLI when administered at the same time as the inoculation of AIA, while there was no clear effect when administered 2-3 weeks after the inoculation.

These results question the sensitivity of FLI to test minor analgesic compounds.

429.3

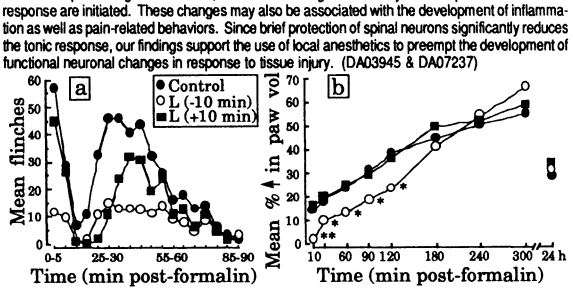
MONOARTHRITIS INDUCES COMPLEX CHANGES IN μ, δ AND k OPIOID BINDING SITES IN THE SUPERFICIAL DORSAL HORN OF THE RAT SPINAL CORD. M-C. Lombard*, D. Besse, J. Weil-Fugazza, S.H. Butler and J-M. Besson, INSERM, U. 161 and EPHE, 2 rue d'Alésia 75014 Paris, France.

Recently, a model of monoarthritis was described in the rat after inoculation by Freund's adjuvant of the tibio-tarsal joint of one hindlimb. Clinical signs of arthritis are visible from 2 to at least 6 weeks post-inoculation (PI). Our aim was to study the regulation of μ, δ and k opioid binding sites in the superficial layers (laminae I-II) of the lumbar and cervical enlargements of the spinal cord 2, 4 and 6 weeks PI. Using quantitative autoradiography and highly selective opioid ligands, we have shown complex changes in binding for the 3 types of sites. They mainly consist of 1) a bilateral increase in [^{3}H]DAMGO and [^{3}H]pCl-DPDPE specific binding at 2 weeks PI when behavioral contralateral hypoalgesia but no significant ipsilateral change in nociceptive threshold (Randall-Sellito) are measured, 2) a bilateral return to control values for [^{3}H]DAMGO and [^{3}H]pCl-DPDPE specific binding and a decrease in [^{3}H]U.69593 specific binding at 4 weeks PI, concomitantly with behavioral contralateral return to control nociceptive threshold and ipsilateral hyperalgesia. These changes are limited to the lumbar level. At 6 weeks PI, there is a bilateral increase in [^{3}H]pCl-DPDPE specific binding at both lumbar and cervical levels. Our results suggest that, following probable local changes in endogenous opioid peptides, which need to be investigated in the present model, the 3 types of opioid binding sites are differentially involved in the development of the pathological process. These data contrast with the lack of significant modification in μ, δ and k opioid binding classically reported at various levels of the spinal cord in polyarthritic rats at 3 weeks PI and verified for 2, 4 and 6 weeks PI in the present study.

429.4

THE ROLE OF ACUTE ACTIVATION OF SPINAL NEURONS IN TONIC PAIN AND EDEMA DEVELOPMENT AFTER FORMALIN-INDUCED TISSUE INJURY. A. Cowan* and H. Wheeler-Aceto. Dept. of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140.

Behavioral and electrophysiological studies of the biphasic response to formalin (F) in rats suggest that neuronal changes associated with the early phase may be required for full expression of the tonic nociceptive response. Here, saline (20 μl) or lidocaine (L, 20 μl, 1%) was injected i.th. into 70-90 g male SD rats at either -10 min or +10 min after F (50 μl, 5% into the right hind paw; n=6). F-induced flinching behavior (Fig a) and paw volume (Fig b) were recorded. I.th. L affects hind limb function and increases 50°C hot-water foot-flick latency only transiently (~ 15 min), but when it is given before F significant and prolonged depression of both phases of the flinching response occurs. In contrast, post-F L attenuates late phase for only slightly longer than it increases foot-flick latencies, after which flinching gradually returns to control levels. Although pre-F L initially retards development of edema, at 2-3 h edema rapidly reaches control levels. Post-F L had no effect. These data suggest that, if immediately after F the barrage of primary afferent firing impinging on spinal neurons surpasses a given threshold, then central changes necessary for full expression of the tonic response are initiated. These changes may also be associated with the development of inflammation as well as pain-related behaviors. Since brief protection of spinal neurons significantly reduces the tonic response, our findings support the use of local anesthetics to preempt the development of functional neuronal changes in response to tissue injury. (DA03945 & DA07237)



429.5

DORSAL HORN NEURONS SERVING THE LOW BACK: ACTIVATION BY SYMPATHETIC STIMULATION. R.G. Gillette, R.C. Kramis, and W.J. Roberts*, Dept. of Neurosurg. & R.S. Dow Neurol. Sciences Inst., Good Samaritan Hospital & Medical Center, Portland, OR

Most somatosensory neurons in the extreme lateral dorsal horn of L4/5 in cats have receptive fields in the lumbar region and/or hip and proximal hindleg. Many are **hyperconvergent** WDRs which receive input from all or most of the following tissues: paraspinal muscles, facet joint capsules, vertebral periost, spinal dura, disc, hip and proximal leg muscles, and skin. Such neurons are likely to subserve low back pain.

Clinical reports indicate that sympathetic blocks reduce low back pain in some patients, suggesting a causal sympathetic role. We therefore tested somatosensory neurons in the lateral lumbar spinal cord for responding to electrical stimulation of the lumbar sympathetic trunk. Single unit recordings were made from L4/5 in adult pentobarbital anesthetized cats.

Of 82 neurons with cutaneous and/or deep receptive fields in the lumbar region, 70% were responsive to sympathetic stimulation (SS). Excitatory responses to SS were differentiable into; a) short-latency, entrained responses; and b) longer-latency, non-entrained responses. The former were resistant to systemic alpha-adrenergic antagonists and are thought to result from activation of afferent fibers in the sympathetic trunk, some of which may innervate paraspinal tissues. The non-entrained responses were attenuated by alpha antagonists and are thought to result from direct or indirect sympathetic activation of afferents. The results indicate that both somatosensory afferent and sympathetic efferent activity in the lumbar sympathetic trunk can contribute to low back pain.

429.6

GALANIN-MEDIATED INHIBITION OF NOCICEPTION: ENHANCED ROLE AFTER NERVE INJURY. **Z. Wiesenfeld-Hallin[1], X.-J. Xu*,[1], V.M.K. Verge[2], Ü. Langel[3], K. Bedecs[3], T. Hökfelt[2] and T. Bartfai[3]. Depts. of [1]Clin. Neurophysiol., Karolinska Institute, Huddinge, [2]Histology and Neurobiology, Karolinska Institute, and [3]Biochemistry, University of Stockholm, Stockholm, Sweden.**

We have studied electrophysiologically and behaviorally the endogenous role of the neuropeptide galanin (GAL) in spinal nociceptive mechanisms in rats with intact and sectioned sciatic nerves with M-35 [GAL(1-13)-bradykinin(2-9)-amide], a newly developed high affinity GAL receptor antagonist.

I.t. M-35 potentiated the facilitation of the flexor reflex by conditioning stimulation (CS) of C-afferents in rats with intact and sectioned sciatic nerves, an effect that was strongly enhanced after axotomy. In a behavioral study, i.t. infusion of M-35 on the lumbar enlargement via an osmotic minipump for 10 days after axotomy dramatically increased the incidence and severity of self-mutilation (autotomy), a behavioral sign of neuropathic pain. I.t. infusion of the GAL antagonist did not prevent the increase in GAL mRNA in sensory neurons after axotomy.

GAL synthesis is dramatically up-regulated in primary sensory afferents after axotomy. The present results demonstrate that endogenous GAL plays an inhibitory role in the mediation of spinal cord excitability and this function of GAL is remarkably enhanced after peripheral nerve injury. From behavioral and electrophysiological data it is suggested that GAL or GAL receptor agonists may be useful in treating neuropathic pain.

429.7

APPLICATION OF TRANS-ACPD OR PHORBOL ESTERS BY MICRODIALYSIS MODIFIES THE BACKGROUND ACTIVITY AND THE RESPONSES OF PRIMATE STT NEURONS TO SENSORY STIMULI AND TO EXCITATORY AMINO ACIDS. P.M. Dougherty*, J. Palecek, V. Paleckova, J. Ragland, and W.D. Willis, Department of Neuroscience and Anatomy, 200 University Blvd., University of Texas Medical Branch, Galveston, Texas 77550-2772.

We explored the effects of two second messenger systems, the inositol phosphate cascade (activated by trans-ACPD) and the protein kinase C pathway (activated by phorbol tri-acetate, TPA) on the responses of STT cells to cutaneous and chemical stimuli. The experiments were conducted in 5 young adult monkeys (*Macaca fascicularis*). STT neurons were identifed by antidromic activation and recorded using a carbon filament electrode in the center of a multibarrel array, the outer barrels of which contained excitatory amino acids (EAA's). The responses of the STT cells to cutaneous stimulation (BRUSH, PRESS and PINCH) and to iontophoretically released EAA's were recorded. Either ACPD or TPA was then infused for one hour by microdialysis and the responses to the sensory and chemical stimuli were again recorded. Both ACPD and TPA resulted in an initial increase followed by a prolonged decrease in background activity. After the spontaneous activity had become reduced, initially, TPA and ACPD produced a decrease in the responses to the mechanical stimuli. However, following a washout period of one to two hours the responses to the mechanical stimuli, particularly BRUSH and PRESS, doubled. Variable changes were observed in the responses to the EAA's. These results suggest that activation of either the inositol phosphate cascade or the protein kinase C pathway may enhance the reponses of primate STT cells. This work was supported by grants NS09743, NS11255, NS08660, and an unrestricted grant from the Bristol-Myers Squibb Corp.

429.8

NEUROKININ RECEPTOR ANTAGONISTS MODIFY THE RESPONSES OF PRIMATE STT NEURONS TO CUTANEOUS STIMULI. W.D. Willis*, J. Palecek, V. Paleckova, J. Ragland, and P.M. Dougherty, Department of Neuroscience and Anatomy, 200 University Blvd., University of Texas Medical Branch, Galveston, Texas 77550-2772.

The effects of neurokinin receptor antagonists on the responses of STT neurons to cutaneous sensory stimuli were studied. Nine experiments were performed on anesthetized young adult monkeys (*Macaca fascicularis*). STT neurons were identified by antidromic activation and recorded using a carbon filament microelectrode in the center of a multibarrel array, the outer barrels of which were used to release excitatory amino acids (EAA's) or neurokinin receptor agonists into the vicinity of the cells under study by iontophoresis. The responses of the STT cells to cutaneous stimuli (BRUSH, PRESS and PINCH; heat and cold; and intradermal injection of capsaicin, i.d. CAP) and to EAA's were recorded before and then after a one-hour infusion of NK-1 antagonists (CP96345, 0.1mM, GR82338, 0.05 mM). CP96345 was used on 15 cells and resulted in reduced responses to noxious PINCH, heat and i.d. CAP. In addition, CP96345 prevented the development of sensitization of STT cells which normally follows CAP. The inactive isomer CP96344 had no effects in ten neurons. The results using GR82337 are still preliminary; however, this compound also appears to modify the responses of at least some STT neurons to sensory stimuli in a manner very similar to CP96345. In conclusion, our results support the contention that neurokinin-1 receptors participate in nociceptive transmission to STT neurons and in the generation of sensitization of the responses of STT neurons that may underlie the development of hyperalgesia. This work was supported by NIH grants NS09743, NS11255, National Service Award NS08660, and an unrestricted grant from the Bristol-Myers Squibb Corp.

429.9

EFFECTS OF SYSTEMIC MORPHINE ON LAMINA I NEURONS IN THE CAT. L.P. Serrano* and A.D. Craig. Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013

The action of morphine on lamina I spinothalamic tract (STT) cells, which form half of the STT, has not been determined. Previous work demonstrated that morphine enhances the activity of "cold"-sensitive lamina I STT cells. This study further tests the hypothesis that morphine's actions are organized in a functionally significant manner by examining its effects on nociceptive lamina I cells. Units recorded with tungsten microelectrodes in L7 in barbiturate-anesthetized cats were characterized with natural stimuli and with antidromic activation from the thalamic lamina I termination sites identified earlier with PHA-L. Quantitative heat and pressure stimuli were used to measure the effects of increasing doses of systemic morphine (0.1-2 mg/kg iv) on the responses of 10 nociceptive lamina I cells (6 STT, 3 non-STT, 1 unclassifiable) that had receptive fields on the ventral hindpaw. All cells had graded sensitivity to noxious heat. Morphine inhibited the responses of 9 cells (mean=45% of control), including all 6 STT cells, 7 in a dose-dependent manner. One non-STT lamina I cell was not inhibited and 2/3 non-STT cells were enhanced by low doses (145%). Morphine in higher doses suppressed the responses of 2 non-STT WDR neurons to heat but not to pinch. Naloxone reversed the effects in 7/8 cells. These observations are consistent with the concept that lamina I STT cells form an integral component of the central representation of pain, since all such cells were inhibited by morphine. However, these results also suggest that non-STT lamina I neurons are differentially responsive to morphine, which supports the hypothesis that opiatergic modulation of lamina I neurons is functionally organized. (Supported by DA 07402)

429.10

RELEASE OF AMINO ACIDS AND PGE_2 INTO THE SPINAL CORD OF LIGHTLY ANESTHETIZED RATS DURING DEVELOPMENT OF AN EXPERIMENTAL ARTHRITIS: ENHANCEMENT OF C-FIBER EVOKED RELEASE. L.S. Sorkin*, UCSD, Anesth. Res. Lab., La Jolla, CA 92093

Glutamate (Glu) and other amino acids (AA) are thought to participate at the spinal level in the development of hyperalgesia associated with tissue inflammation. Prostaglandins also contribute to inflammatory pain, but until recently were thought to be involved only in the periphery. The present study measures the spinal release of AAs and PGE_2 in response to C-fiber stimulation of the sciatic nerve in normal animals and potentiation of that release after acute joint inflammation initiated by intra-articular injection with kaolin/carrageenan.

Extracellular concentrations of AAs and PGE_2 were measured from the dorsal spinal cords of rats lightly anesthetized with halothane. Half-hour consecutive samples were obtained via microdialysis and assayed by HPLC with UV detection and RIA, respectively. C-fiber intensity (200*minimum motor threshold) stimulation of the sciatic nerve elicited release of Glu and glycine, but not aspartate, taurine or PGE_2. C-fiber stimulation 4 hrs after initiation of the inflammation elicited release of Glu and glycine as well as aspartate, taurine and PGE_2. This release was superimposed upon elevated extracellular concentrations of Glu, glycine, taurine and PGE_2 induced by the inflammation.

These results are consistent with the hypothesis that spinal release of excitatory AAs and prostanoids participate in the generation of arthritis pain. Enhanced stimulus evoked release of some of these substances may mediate the hyperalgesia that occurs during inflammation.

(This work was supported by G.D.Searle & Co.)

429.11

INTRATHECAL ADMINISTRATION OF EXCITATORY AMINO ACID RECEPTOR ANTAGONISTS SELECTIVELY ATTENUATES CARRAGEENAN-INDUCED BEHAVIORAL HYPERALGESIA. G.M. Williams*, K. Ren, J.L.K. Hylden, M.A. Ruda and R. Dubner, NAB, NIDR, NIH, Bethesda, MD 20892.

Dizocilpine maleate (MK-801), a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, has been shown to reduce thermal hyperalgesia in rats with hindpaw inflammation. Other excitatory amino acid (EAA) receptor antagonists were examined to further elucidate the involvement of NMDA receptors in the behavioral hyperalgesia associated with inflammation. Hyperalgesia, assessed by paw withdrawal latency (PWL) to a heat stimulus, was induced by a single injection of carrageenan (CARRA) (6.0 mg) into the hindpaw of rats. The effects of NMDA receptors were examined by intrathecal (I.T.) injection of the EAA receptor antagonists: (±)-2-amino-5-phosphono-pentanoic acid (AP-5), (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), MK-801, ketamine hydrochloride (KETA), 7-chlorokynurenic acid (7-CK), and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Whereas PWLs of non-injected paws and those of naive rats were not significantly affected, PWLs of injected paws were dose-dependently elevated. The rank order of potency of these agents to reduce hyperalgesia was: MK-801 ≥ AP-5 ≥ CPP = 7-CK = KETA >> CNQX > 0. In contrast, I.T. injection of the opioid receptor agonist, [D-Ala^2,$MePhe^4$,$Gly\text{-}ol^5$] enkephalin (DAMGO, μ-selective) produced antinociception in both injected and non-injected paws. DAMGO was about 66 times more potent than MK-801. The dose-dependent effects of various NMDA receptor antagonists provide additional evidence that NMDA receptors are involved in CARRA-induced thermal hyperalgesia.

429.12

DIFFERENTIAL ROLES OF NMDA AND NON-NMDA RECEPTOR ACTIVATION IN INDUCTION AND MAINTENANCE OF THERMAL HYPERALGESIA IN RATS WITH PAINFUL PERIPHERAL MONONEUROPATHY D.J. Mayer[1]*, J. Mao[1], R.L. Hayes[2], J. Lu[1] and D.D. Price[1]. [1]Dept. of Anesthesiology, Medical College of Virginia, Richmond, Virginia 23298, [2]Division of Neurosurgery, University of Texas, Houston, Texas 77030

Excitatory amino acid (EAA) receptor activation within the spinal cord has been implicated in neuropathic pain following nerve injury. Using a rat model of painful peripheral mononeuropathy (Bennett and Xie, Pain, 1988, 33:87), we compared the effects of intrathecal treatment with NMDA receptor antagonists (MK801 or HA966) and a non-NMDA receptor antagonist (CNQX) on induction and maintenance of thermal hyperalgesia induced by chronic constrictive injury (CCI) of the ligated sciatic nerve. Four daily single treatments with 20 nmol HA966 or CNQX beginning 15 min prior to nerve ligation (pre-injury treatment) reliably reduced thermal hyperalgesia in CCI rats on days 3, 5, 7, and 10 after nerve ligation. Thermal hyperalgesia also was reduced in CCI rats receiving a single post-injury treatment with HA966 (20 or 80 nmol) or MK 801 (5 or 20 nmol) on Day 3 after nerve ligation when thermal hyperalgesia was well developed. In contrast, a single post-injury CNQX (20 or 80 nmol) treatment failed to reduce thermal hyperalgesia or to potentiate effects of HA966 or MK 801 on thermal hyperalgesia. Moreover, multiple post-injury CNQX treatments utilizing the same dose regimen as employed for the pre-injury treatment attenuated thermal hyperalgesia only when the treatment began 1 or 24 hrs (but not 72 hrs) after nerve ligation. The results suggest that NMDA and non-NMDA receptor activation may have differential roles in induction and maintenance of thermal hyperalgesia following constrictive nerve injury and that mechanisms underlying post-injury neuropathic pain may be associated with EAA-mediated central alterations.

Supported by PHS grants NS 24009.

429.13

NMDA RECEPTOR-MEDIATED BIPHASIC EFFECTS ON THERMAL NOCICEPTIVE SPINAL TRANSMISSION. R. Kolhekar,* S.T. Meller and G.F. Gebhart. Dept. of Pharmacology, The University of Iowa, Iowa City, IA, 52242

NMDA (1 fmol-1 nmol) was administered through an 8.5 cm intrathecal (i.t.) catheter to the lumbar spinal cord of awake Sprague-Dawley rats. Changes in tail-flick (TF) latency in response to noxious heat were monitored 0.5, 1, 2, 5 and 10 min post-drug. Lesser doses of NMDA (100 fmol-10 pmol) produced a dose-dependent facilitation of the TF reflex (maximal at 0.5-1 min) while greater doses (0.25 nmol-1 nmol) inhibited the TF reflex (maximal at 2-5 min), and also produced a caudally-directed scratching and biting behavior and vocalizations (CBSV). Maximal facilitation was produced by 10 pmol NMDA while maximal inhibition and CBSV was produced by 1 nmol NMDA. Pre-treatment with D-APV (1 fmol-1 pmol, i.t.), which produced no change in baseline TF latency, blocked all NMDA-produced effects in a dose-dependent manner (100 fmol D-APV produced maximal blockade for about 40 min). D-serine (10 pmol-1 μmol, i.t.), but not L-serine (10 pmol-1 μmol, i.t.) or glycine (10 pmol-10 μmol, i.t.), produced a dose-dependent facilitation of the TF reflex which was comparable to the facilitation produced by NMDA (10 pmol); the facilitatory effects of NMDA and D-serine were blocked by 7-chlorokynurenic acid (10 fmol-10 pmol, i.t.). The NMDA-produced biphasic effects on TF latency, but not the CBSV were similar in lightly pentobarbital-anesthetized rats. Reversible spinalization at T_8-T_{10} by cold-block in lightly anesthetized rats completely abolished the inhibition of the TF reflex produced by 1 nmol NMDA while the facilitation produced by 10 pmol NMDA remained unchanged.

In summary, these data suggest that while NMDA-produced facilitation of the TF reflex may be a local phenomenon, inhibition of the TF reflex and CBSV may be produced by activation of ascending pathways that in turn engage a descending inhibitory pathway to produce inhibition of the TF reflex.

429.14

HYPERESTHESIA INDUCED BY SPINAL GLUTAMATE AND NK-1 TACHYKININ RECEPTORS IS REDUCED BY INTRATHECAL NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs). A.B. Malmberg and T. L. Yaksh*, Dept. of Anesthesiology, UCSD, La Jolla, CA 92093-0818

Spinal administration of NSAIDs can significantly diminish the second phase, behavior induced by subcutaneous injection of formalin in the paw. Based on the growing appreciation of the involvement of excitatory amino acids (EAA) and substance P in the initiation of a facilitatory state of spinal processing, as observed in the phase 2 of the formalin test, we injected NMDA, AMPA and substance P directly into the spinal canal. Intrathecal injection of NMDA (7 nmol), AMPA (1 nmol) and substance P (7 nmol) each resulted in a dose dependent decrease in the latency of the response evoked by a thermal stimulus applied to the hind paw. The selective antagonists MK-801 (29 nmol), CNQX (27 nmol) and CP96,345 (330 nmol), respectively, given 10 min before the agonists, blocked the observed thermal hyperesthesia for the respective agonists. Importantly, injection of the antagonists 10 min after the agonists failed to block the decreased paw withdrawal. To assess the potential role of prostanoids released by the spinal activation of the respective receptors, we examined the effects of several NSAIDs on the evoked thermal hyperesthesia. We found that acetylsalicylic acid (100 nmol), ketorolac (27 nmol) and S(+)ibuprofen (27 nmol) resulted in a complete reversal of the induced thermal hyperesthesia. Importantly, the intrathecal NSAIDs were equally effective in reducing the thermal hyperesthesia whether administered before or after the agonists. Additionally, the inactive stereoisomer R(-)ibuprofen failed to show any effect at doses 10 times higher than the active isomer S(+)ibuprofen. This emphasizes that the NSAIDs action were mediated by an effect upon cyclooxygenase. These data provide evidence that activation of spinal glutamate and NK-1 receptors results, at least in part, in local generation and release of prostanoids, which mediate a spinal facilitation of the animal's response to noxious stimuli. (Supported by NIH DA 02110, TLY)

429.15

EFFECTS OF INTRATHECAL PROSTAGLANDINS ON BEHAVIORAL RESPONSES TO NON-NOXIOUS AND NOXIOUS STIMULI. M.Kaneko[1], Y. Saito*[1], Y.Kirihara[1], J.G.Collins[2] and Y.Kosaka.[1] Dept. of Anesth., [1]Shimane Med.Univ., Izumo, 693 Japan and [2]Sch.of Med., Yale Univ., New Haven, CT 06511

Prostaglandins(PGs) influence the development of hyperalgesia in the periphery. However, centrally their role is not yet well defined. This experiment was designed to investigate the influences of intrathecally administered PGE_1 and $PGF_{2\alpha}$ on behavioral responses to non-noxious, and noxious somatic and visceral stimuli in rats.

Intrathecal catheter was implanted at the level of L2-L3 in rat. The tail flick (TF) test and colorectal distension (CD) test were employed to measure the responses to noxious somatic and visceral stimuli, respectively. Agitation score (AS) to mechanical pressure produced by three kinds of Semmes-Weinstein monofilaments (SWMs) ranging 0.08 g to 2.35 g was measured to assess the hypersensitive state to non-noxious stimulus. After measuring baseline value measurements were repeated for 3 hours after intrathecal injection of 500 ng of PGE_1, 100 ng of $PGF_{2\alpha}$ or saline. ASs were also evaluated for 2 days after administration.

PGE_1 produced a significant decrease for 20 min in both TF latency and CD threshold compared to intrathecal saline which caused no changes. $PGF_{2\alpha}$ produced a slight decrease in CD threshold but no significant change in TF latency. ASs produced by 2.35 g SWM were significantly increased following administration of both PGE_1 and $PGF_{2\alpha}$ and the effects lasted for at least 2 days, while in the saline group there were no significant changes in ASs produced by any of SWM. This result suggests that PGs may act on hyperalgesic processing in the spinal cord.

430.1

Handedness is Predicted by Cerebral Blood Flow Asymmetry. R.A. Stea*, N.M. Szeverenyi, S.H. Manglos, J. Brueggemann, F.D. Thomas, A.V. Apkarian. Depts. of Neurosurgery and Radiology, SUNY-HSC, Syracuse, NY 13210.

Handedness and speech are highly lateralized in the human cerebral cortex. However, earlier imaging studies have not shown consistent correlations between handedness and regional cerebral blood flow (rCBF) hemispheric asymmetries. In this study, left versus right parietal lobe activity in 6 subjects was evaluated and used to predict handedness. Handedness was determined by a manual dexterity test and Annett's questionnaire. Blood flow studies were done by Single Photon Emission Computed Tomography (SPECT) with the retainable tracer ^{99m}Tc-HMPAO. Baseline studies were done with the subject's right hand immersed in neutral temperature water (control condition for the pain study, see below). Data was analyzed by the region of interest method; parietal lobe was directly localized from the patient's co-registered MRI scan. Five subjects had one SPECT scan; one subject had four.

Hemispheric Asymmetry in parietal lobe activity was predictive of handedness in all subjects; greater parietal activity was contralateral to the dominant hand. In four right handed subjects, left parietal activity was greater than right parietal activity (mean: 5.4%). In one mixed-handed subject, left and right activity were equal. In the left handed subject, the right parietal activity was greater by 5.0%. In the right handed subject with four scans (normalized), the left versus right differences were consistent across studies and significant by student's t-test (p=0.01). The results suggest that handedness correlates with parietal cortical activity.

This study is part of a larger ongoing investigation of the cortical representation of pain. In some of the above subjects, thermal pain was induced in either the hand or the foot in order to reveal the nociceptive somatotopy in the cortex. These results will be presented at the conference.

430.2

RESPONSES IN THE RAT TO NOXIOUS COLORECTAL DISTENSION IN THE PRESENCE OF ACETIC ACID. M. Burton* and G.F. Gebhart. Department of Pharmacology, University of Iowa, Iowa City, IA 52242.

The mechanisms of visceral pain have recently been a major focus of investigation. Distension of hollow organs has been reported to be noxious. Clinically, there is usually some pathology associated with pain from visceral organs; however, this has not been extensively studied in animals. Therefore, the aim of this study was to determine the effects of acetic acid irritation on the responses to noxious colorectal distension (CRD).

Pressor and motor (EMG) responses to CRD (80 mmHg, 20 sec, 3 min apart) were examined in unanesthetized, chronically instrumented, male Sprague-Dawley rats before and 6 and 24 hours after 1 ml intracolonic 5% acetic acid (or vehicle). The colons of a separate group of rats were examined 4, 6, 8 or 24 hours after 1 ml intracolonic acetic acid for evidence of leukocyte infiltration. Acetic acid did not produce any change in resting mean arterial pressure (MAP), or in the ΔMAP in response to CRD at any time tested. In contrast, the magnitude of both baseline EMG and ΔEMG produced by CRD were significantly increased at 6 and 24 hours after acetic acid, as noted in the table. Acetic acid had no effect on the number of leukocytes in colonic tissue at any time tested.

These results suggest that irritation, but not inflammation, of the colon produced by acetic acid results in an increase in the magnitude of nociceptive responses to CRD.

	control	6 hr	24 hr
Baseline EMG (%)	100	170 ± 66	255 ± 99
ΔEMG (%)	100	187 ± 49	216 ± 34

430.3

EFFECTS OF PARADOXICAL SLEEP (PS) DEPRIVATION ON PAIN-RELATED BEHAVIORS AND NEUROGENIC INFLAMMATION IN RATS. C.A. Landis*. Dept. of Physiological Nursing, University of Washington, Seattle, WA, 98195.

PS deprivation (PSD) is known to increase central nervous system excitability and behavioral activation. PSD rats show a reduction both in the threshold for reflex withdrawal to noxious stimuli and in antinociceptive responses to opiates. Increased excitability of somatic sensory nerves could facilitate mechanisms of peripheral and/or central sensitization to noxious stimuli.

Compared to baseline, after 96 h of PSD by the small platform method, 5 μl of mustard oil (50% in 95% ethanol) applied to the dorsum of one hind paw produced a quantifiable increase in pain-related behaviors. This same solution produced an increase in Evans blue extravasation in 4mm skin biopsies from the dorsum of the left hind paw in PSD rats, compared both to large platform and to normal control rats. Ethanol applied to the contralateral paw was without effect in all rats. These observations suggest neurogenic inflammatory mechanisms contribute to heightened pain sensitivity in PSD rats. Supported by School of Nursing BRSG and a GSF grant.

430.4

EFFECTS OF MANIPULATING INTRACELLULAR CALCIUM ION CONCENTRATION USING VOLTAGE SENSITIVE CALCIUM CHANNEL BLOCKERS AND DANTROLENE SODIUM ON PERSISTENT HINDLIMB FLEXION IN SPINALIZED RATS. M.F. Anderson* and J.T. Earnhardt Department of Psychiatry, St. Elizabeth's Hospital of Boston, Boston, MA 02135 & Department of Pharmacology, University of New England, Biddeford, ME 04005.

Stimulation (2mA, 7ms, 100Hz, 60min) across the musculature of the upper hindlimb in the spinalized rat produces a persistent hindlimb flexion. Previously, we have shown that NMDA receptor antagonists interfere with the induction of this flexion. Given NMDA receptor stimulation indirectly may activate voltage sensitive calcium L-channels, the dose response of pretreatment with nimodipine, nifedipine and diltiazem on induction of a persistent hindlimb flexion was explored.

Following spinalization of adult Long Evans rats under halothane anesthesia, animals were pretreated with one dose of a particular test agent (nimodipine: 0.03, 0.1, 0.3, and 1.0mg/kg, i.p., nifedipine: 0.1, 0.3, 1.0, and 3.0mg/kg, i.p., diltiazem: 0.03, 0.1, 0.3, and 1.0mg/kg, i.p.) and wound clips were applied to the musculature of the right upper hindlimb. After a 30min waiting period, current (2mA, 7ms, 100 Hz, 1 hr) was delivered across the clips. In all groups, flexion was reduced in a dose dependent manner. Since 1) the systemic administration of calcium L-channel blockers may promote skeletal muscle relaxation and 2) NMDA receptor activation may effect release of intraneuronal calcium ion stores, the dose response of pretreatment with dantrolene sodium which theorectically inhibits the release of both skeletal muscle and neuronal calcium ion stores was studied. Following the above protocol, dantrolene sodium (0.1, 0.3, 1.0, and 3.0mg/kg, i.p.) pretreatment reduced poststimulation flexion in a dose dependent manner. These data suggest that induction of a persistent hindlimb flexion in the spinalized rat depends on both adequate 1)neuronal and skeletal muscle intracellular calcium ion levels and 2) activation of skeletal muscle afferents.(Support: Mead Johnson & St.Elizabeth's)

430.5

THE CENTRAL DELAY OF THE LASER-ACTIVATED RAT TAIL FLICK REFLEX. P.J. Danneman, J.A. Kiritsy-Roy , T.J. Morrow, M. Mata* and K.L. Casey. Unit for Lab. Animal Med. and Depts. of Neurol. and Physiol., Univ. of Michigan and VAMC, Ann Arbor, MI 48105.

Latency of the rat tail flick reflex is dependent upon: 1) nociceptor activation; 2) afferent conduction to the dorsal horn (DH); 3) central delay; and 4) conduction to and activation of tail muscles. Knowledge of the central delay of the rat tail flick reflex would provide a framework in which to evaluate the temporal constraints on neuronal modulation of this reflex. Using a CO_2 infrared laser (10 W, 45 msec) to produce synchronous activation of tail nociceptors, we measured tail flick latency in awake rats (N=9). Shifts in reflex latency from two points of stimulation on the tail provided an estimate of conduction velocity in the afferent limb of the reflex (0.8 ± 0.1 m/s), indicating that the response is mediated by C-fibers. Animals were then anesthetized with pentobarbital (55 mg/kg), and multiple unit activity and evoked potentials (EPs) were recorded from lamina I DH at L2-L3 during stimulation of the tail using laser or high intensity electrical stimulation (10 mA, 1 msec). Unit activity or EPs elicited by either stimulus consisted of two distinct components, corresponding to activation of A-δ and C-fibers. The difference in latency between laser and electrical evoked activity indicated that an average of 64 ± 7 msec is required for laser activation of nociceptors in the tail. Electrical stimulation of the ventral horn (VH) produced a tail flick response with a latency of 4 msec. Average central delay, calculated as the difference between total tail flick reflex latency and the time required for nociceptor activation, afferent conduction to the DH and efferent conduction from VH, was 72 ± 10 msec. Thus, any pain-activated intrinsic analgesia system must exert its modulatory influence during this interval in order to alter tail flick reflex latency. (Supported by #RR00052, the VA and a Bristol-Myers Squibb Award)

430.6

ENDOGENOUS ANTI-ANALGESIA: EFFECTS OF AMYGDALA, DORSAL RAPHE & SPINAL LESIONS. L.R. Watkins*, E.P. Wiertelak, K. Mooney-Heiberger, R. Grahn & S.F. Maier. Dept. Psychology, University of Colorado at Boulder, Boulder, CO 80309.

We have shown that rats can learn to activate neural circuitry that blocks opiate analgesias (Wiertelak et al., Science, 1992). Using classical conditioning procedures, "danger" signals (cues predicting shock) come to elicit conditioned analgesia & "safety" signals (a light cue predicting that shock will NOT occur for some period) come to elicit conditioned anti-analgesia.

To begin defining anti-analgesia circuitry, we tested the effect of lesions of central nucleus of the amygdala (CeA; bilateral), dorsal raphe nucleus (DRN) & spinal dorsolateral funiculus (DLF; bilateral), vs. sham controls. Each of these areas has been previously implicated in fear &/or analgesia.

After surgery, rats received training to contextual "danger" signals & the discrete light "safety" signal (see Wiertelak et al., Science, 1992). Tail flick (TF) latencies were recorded during each of the 4 days of conditioning to assess the effect of the various lesions on acquisition of conditioned analgesia & anti-analgesia. Conditioned analgesia was then extinguished across 4 days. All rats were injected with morphine the next day to test whether any lesion would prevent reversal of morphine analgesia by the safety signal.

The results were as follows. CeA lesions (a) blocked development of conditioned analgesia, yet (b) did NOT block safety signal reversal of 5 mg/kg s.c. morphine analgesia. Similarly, DLF lesions (a) blocked development of conditioned analgesia, yet (b) did NOT block safety signal reversal of intrathecal morphine analgesia. In contrast, DRN lesions (a) did NOT block development of conditioned analgesia yet (b) prevented reversal by the safety signal of both conditioned analgesia & 5 mg/kg s.c. morphine analgesia. Taken together, these data provide strong evidence that anti-analgesia is mediated by neural circuitry distinct from that previously defined for analgesia systems. NIMH 5T32MH14617-15; K.M.-H. supported by the Hughes Fdn.

430.7

INTERNAL AVERSIVE STIMULI ELICIT ACUTE & CONDITIONED HYPERALGESIA. E.P. Wiertelak*, K. Mooney-Heiberger, W. Van Wyhe, G.R. FitzPatrick II, S.F. Maier & L.R. Watkins. Department of Psychology, University of Colorado, Boulder, CO 80309.

Hypoalgesia aversive to external stimuli promotes effective defense/escape behaviors. Perhaps, then, the adaptive response to aversive internal stimuli is hyperalgesia, to promote recuperative behaviors.

An emetic agent (0.15 M lithium chloride [LiCl] i.p.) was used to produce an aversive internal state (illness). Indeed, LiCl produced profound hyperalgesia (tail flick), compared to controls. This appeared specific for nociception, as reactivity to non-painful stimuli (vonFrey hairs) was unchanged. LiCl-induced hyperalgesia involves activation of a centrifugal pathway since it is blocked by spinal transection. Further, this effect appears to be mediated by cholecystokinin (CCK) since it is abolished by proglumide (0.4 mg/kg s.c.), a CCK antagonist. Paradoxically, 7 mg/kg naltrexone produced profound analgesia in LiCl injected rats, but not in vehicle controls.

Since illness clearly could produce hyperalgesia, could hyperalgesia be conditioned to cues associated with illness? To address this question, 2 groups were tested using a taste aversion paradigm. The first received saccharine paired with LiCl. The second (control) received equal numbers of LiCl & saccharine exposures, but in an unpaired fashion. On test day, all rats received saccharine. The rats that previously had saccharine paired with LiCl showed profound hyperalgesia, compared to controls. Conditioned hyperalgesia was again abolished by proglumide, indicating a role of CCK. Again, paradoxically, naltrexone produced profound analgesia in the rats that previously had saccharine paired with LiCl.

These data clearly demonstrate that conditioned effects on pain sensitivity are bidirectional. External danger cues can produce analgesia. The present data demonstrate that aversive internal stimuli can produce hyperalgesia as can learned cues that signal such events. Supported by NIMH 5T32MH14617-15; K.M.-H. & W.V.W. supported by the Hughes Fdn.

430.8

CONTROLLABLE, BUT NOT UNCONTROLLABLE SHOCK ELICITS AN ANTINOCICEPTION IN SPINALIZED RATS. C.W. Parker III., P.A. Illich, J.W. Grau & M.W. Meagher*. Dept. of Psychology, Texas A&M Univ. College Station, TX 77843.

Prior work has shown that exposure to inescapable shock produces both short- and long-term analgesia. These effects are not observed in rats that experienced the same amount of shock, but can control its termination. It has been generally assumed that such effects are mediated by forebrain systems. However, we have recently shown that other antinociceptive phenomena thought to involve forebrain systems can be obtained in spinalized subjects. Here, we assess whether having control influences nociception in spinalized subjects. A day after rats received a spinal transection at T2, they were placed in restraining tubes and tested for baseline levels of nociception. Nociceptive thresholds were defined as the shock intensity needed to produce a leg flexion. Subjects were then given a 30 min training session in which they received 1.0mA footshocks. Subjects were run in pairs. One rat could terminate the shock by flexing its leg (Exp). The other rat received the same shock irrespective of leg position (Yok). Exp, but not Yok subjects spent increasingly more time with their legs in a flexed position. Interestingly, after training, Exp subjects exhibited an increase in nociceptive threshold (antinociception), while Yok subjects showed a decrease in nociceptive threshold(hyperalgesia) ($p < .01$). The results suggest that controllability influences nociception in spinalized rats.

430.9

MILD SHOCK PRODUCES AN UNCONDITIONED, NALTREXONE-INSENSITIVE INCREASE IN REACTIVITY ON THE VOCALIZATION MAGNITUDE AND THRESHOLD TESTS. P.A. Illich*, C. W. Parker III., K.D. Burks, & J.W. Grau. Dept. of Psychology, Texas A&M Univ. College Station, TX 77843.

Prior work suggests that an aversive event can produce either an increase (hyperalgesia) or decrease(hypoalgesia) in pain reactivity. Although stress-induced hypoalgesia has been extensively examined both at the behavioral and neural levels, relatively little is known about stress-induced hyperalgesia. Here, we first establish a paradigm that produces a robust increase in responding to pain. Subjects experienced either mild shock (3, 0.75-s, 1.0mA) or nothing, and were then given a single test shock (0.75-s, 1.0mA). Previously shocked subjects vocalized more to the test shock relative to unshocked controls. A second set of subjects were treated the same except that vocalization threshold rather than magnitude was assessed. Shocked subjects exhibited a substantial decrease in vocalization threshold (hyperreactivity) that decayed over time (Experiment 2), irrespective of whether they remained in the shock-context (Experiment 3). Experiment 4 showed that this effect was naltrexone (14 mg/kg) insensitive. The results suggest that mild shock can produce an unconditioned, naltrexone-independent, increase in responding on the shock-induced vocalization and vocalization threshold tests. We are currently exploring the neural mechanisms that underlie this effect. Supported by BRSG 2S07RR07090 to J.W.G.

430.10

EFFECTS OF CAPSAICIN DERIVATIVES ON TAIL-FLICK REFLEX,NEURAL CONDUCTION AND CONTENT OF SUBSTANCE P. J.H.Lee*1, S.S. Lee2, J.S.Kim1, and K.N.Kim1. Department of Physiology, Seoul National University, School of Dentistryl and School of Pharmacy2, Seoul, Korea, 110-460.

This experiments was performed to compare the effects of capsaicin and its new derivatives on the tail-flick and jaw opening reflex evoked by noxious stimuli and content of substanceP in spinal dorsal horn and trigeminal sensory nucleus. Tail-flick reflex was tested by hot water immersion method in rat after subcutaneous injection of capsaicin, paradol, shogaol and demethoxy-NE(DM-NE). Neural conduction of peripheral nerve was determined in saphenous nerve. Saphenous nerve was exposed and action potential was evoked by noxious electrical stimulation. Capsaicin and its derivatives were dissolved in vehicle and applied to saphenous nerve between stimulating and recording site for 30 minutes. Amplitude and conduction velocity of action potential was compared before and after application of drugs. SubstanceP of spinal cord and trigeminal sensory nucleus was determined by radioimmunoassay. Capsaicin inhibited neural conduction of mainly C-fiber but paradol and shogaol had inhibitory effect on both A- and C-fiber. Capsaicin and DM-NE significantly increased latent period of tail-flick reflex at first day after injection. Capsaicin decreased the substance P in spinal dorsal horn and caudal part of trigeminal sensory nucleus.

430.11

A COMPARISON OF NOCICEPTIVE RESPONSES USING TWO METHODS OF HOT-PLATE MEASUREMENTS: ABSOLUTE TEMPERATURE THRESHOLD VERSUS REFLEX LATENCY. Przemyslaw Marek[1]*, Anthony L. Vaccarino[2], Bogdan Sadowski[3] and John C. Liebeskind[1], [1] Dept. of Psychology, UCLA, CA 90024, [2]Dept. of Psychology, University of New Orleans, LA 70148 and [3]Institute of Genetics and Animal Breeding, Jastrzebiec, Poland.

Mice bred for high (HA) and low (LA) stress-induced analgesia display dramatic differences in baseline hot-plate latencies. Interpretation of baseline sensitivity in the hot-plate test, however, is confounded by the stressful nature of this test and it is unclear whether such differences are due to stress-induced analgesia (produced by the test itself) or due to differences in tonic pain inhibition. We have recently developed a method of measuring hot-plate pain sensitivity which reduces the stressful component. HA and LA mice were habituated for 20 minutes to a hot-plate maintained at $37\pm1^{o}C$. The temperature of the hot-plate was then gradually increased and the temperature to hind-paw lick was recorded. No differences were found in baseline temperature threshold between HA and LA mice. In contrast, HA mice showed higher baseline responses than LA mice when the conventional hot-plate test was used (i.e. plate maintained at $56\pm1^{o}C$). Morphine produced a dose-dependent analgesia in both tests. These results suggest that this method of measuring absolute tempurature threshold may provide an alternative method of assessing pain sensitivity, which greatly reduces the confounding effects of stress. Supported by NIH Grant NS07628 and an Unrestricted Pain Research Grant from the Bristol-Myers Squibb Company.

430.12

EXPOSURE TO A HEAT STRESSOR INDUCES AN OPIOID CONDITIONED HYPOALGESIA IN RATS TESTED FOR NOCICEPTION TO FORMALIN. H. Foo* and R.F. Westbrook. School of Psychology, Univ. of N.S.W. Sydney, Australia.

Rats exposed to a heated floor in a distinctive environment (E1) and subsequently tested there on that floor are hypoalgesic. This conditioned hypoalgesia is naloxone irreversible and not cross-tolerant with morphine. However, morphine-tolerant rats come to acquire the conditioned hypoalgesia and naloxone given in combination with the initial exposure to the heated floor enhances the acquisition of the conditioned hypoalgesia.

The present study examined whether these hypoalgesic effects are observed when a chronic pain measure is used to assess nociception. Rats exposed to a heat stressor in E1 were hypoalgesic when subsequently tested there for pain sensitivity to formalin. This conditioned hypoalgesia was naloxone-reversible and cross-tolerant with morphine. The unconditioned hypoalgesia induced by formalin was also naloxone-reversible and cross-tolerant with morphine. These results, thus, suggest that the nature of the conditioned hypoalgesia associated with pre-exposure to a heat stressor might depend upon the type of pain elicited on test. Although acquisition of the conditioned hypoalgesia was facilitated by pairing exposure to the heated floor with naloxone, morphine-tolerant rats were hyperalgesic in the formalin test. However, morphine-tolerant rats were just as hypoalgesic as morphine-naive ones when tested with the opioid in the formalin test. Thus, the capacity of morphine tolerance to block the acquisition of conditioned hypoalgesia appears to be independent of opioid pain mechanisms.

430.13

NOCICEPTIVE RESPONSES TO HIGH AND LOW RATES OF FOOT SKIN HEATING IN RATS MAY BE MEDIATED BY DIFFERENT NOCICEPTORS.
D.C. Yeomans*, V. Pirec, and H.K. Proudfit. Department of Pharmacology, U.I.C., Chicago, IL 60612.

The selective actions of capsaicin or morphine to increase or decrease nociceptive responses mediated by polymodal nociceptors were used to test the hypothesis that heating the dorsal hairy surface of the hindpaw at low rates elicits withdrawal responses evoked by polymodal nociceptor activation, whereas high rates of heating evoke responses mediated by other nociceptors. Capsaicin was applied topically to the hairy foot skin of rats to selectively sensitize polymodal nociceptors. Capsaicin reduced the latency of the reflexive withdrawal response evoked by low heating rates, but not by high heating rates. In addition, the skin temperature at which the response to low heating rates occurred was reduced from 46 to 41°C, but the temperature at which the response to high heating rates occurred (51°C) was not affected.

Responses mediated by the activation of C polymodal nociceptors have been demonstrated to be preferentially attenuated by low doses of systemic morphine (Cooper et al., Pain, 24:93-116, 1986). In the present experiments, low doses of morphine (0.01 to 1.0 mg/kg i.p.) selectively attenuated nociceptive responses to low heating rates in normal rats, and to an even greater extent after capsaicin treatment. The same doses of morphine did not attenuate nociceptive responses to high heating rates in either normal rats or those with capsaicin sensitized skin. Higher doses of morphine (2.0 to 10.0 mg/kg i.p.) attenuated responses to both types of stimulation. These results support the conclusion that low rates of heating elicit responses elicited by the activation of polymodal nociceptors. In addition, the potency of analgesic drugs, determined using thermal methods, may depend on the rate of skin heating. Supported by USPHS Grants DA03980 (HKP) and DA05406 (DCY).

430.14

C-FIBER CONDITIONING CAUSES FACILITATION OF TOUCH-EVOKED RESPONSES IN RAT FLEXOR MOTONEURONS IN VIVO
L.G. Sivilotti* and C.J. Woolf, Anatomy Dept., University College London, Gower St., London WC1E 6BT, U.K.

Touch-evoked pain, mediated by Aβ fibers, can be produced in humans following C-fiber activation by intradermal capsaicin (Torebjörk et al. (1992), J.Physiol., 448, 765). In the rat, pharmacological blockade of inhibition in the spinal cord results in touch-evoked agitation (Yaksh (1989), Pain, 37, 117). We set out to examine the effects of C-fiber conditioning and central disinhibition on flexor motoneuron responses in the decerebrate spinal rat. The response of hamstring motoneurons to noxious and non-noxious stimuli applied to the receptive field was tested. In control conditions tactile or Aβ stimulation caused little or no activity. Responses to repeated standardized touches of the hindpaw or electrical activation of Aβ afferents were greatly enhanced after C-fibers were activated either by cutaneous application of mustard oil or by conditioning the sural nerve (1 Hz, 20 s; +1118 ± 248 and +279 ± 48%, respectively). A similar facilitation of touch-evoked responses was observed following a subconvulsant dose (3 μg) of strychnine or bicuculline i.t. (+1042 ± 499 and + 1239 ± 377%, respectively). These changes were associated with a decrease in the mechanical threshold of the flexor reflex and an increase in the response to noxious stimuli. Sural conditioning at Aβ strength (1-10 Hz, 20 s) had no effect. These results suggest that C-fiber induced central sensitization results in allodynia as well as hyperalgesia and that disinhibition may be involved.

RETINA AND PHOTORECEPTORS: AMACRINE, GANGLION AND GLIAL CELLS

431.1

SYNAPTIC ORGANIZATION OF DOPAMINERGIC AMACRINE CELLS IN THE LARVAL TIGER SALAMANDER RETINA.
P.A. Glazebrook, K.R. Fry* and C.B. Watt. Alice R. McPherson Laboratory of Retina Research, Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX. 77380.

Immunocytochemistry of tyrosine hydroxylase (TH) was used to visualize tiger salamander dopaminergic amacrine cells. The avidin-biotin immunoperoxidase method was used to immunostain TH immunoreactive cells in vibratome-prepared sections that were routinely processed for ultrastructural examination. TH-positive somas exhibited an evenly distributed peroxidase reaction product throughout their cytoplasm. Their nuclei were generally unstained and possessed indented nuclear membranes. TH-positive processes were generally stained throughout and were characterized by an occasional dense-core vesicle in addition to a generally homogeneous population of small round clear synaptic vesicles. They formed conventional synaptic contacts that were characterized by symmetrical synaptic membrane densities.

A total of 112 synaptic relationships were observed in the inner plexiform layer that involved TH immunoreactive processes. TH-positive processes were presynaptic to amacrine cell processes (33.0%) and to processes that lacked synaptic vesicles (26.8%). Synapses onto amacrine cell processes were observed only in sublayer 1. Synaptic contacts onto processes that lacked synaptic vesicles were observed primarily in sublayer 1 (93.4%), but also in sublayers 3 (3.3%) and 5 (3.3%). As postsynaptic elements, they received synaptic input from both amacrine (39.3%) and bipolar (0.9%) cells. Bipolar input was observed only in sublayer 1, while amacrine cell input was found in sublayers 1 (59.1%), 3 (25.0%) and 5 (15.9%). Supported by grants from the NIH (EYO5622) and the Retina Research Foundation (Houston).

431.2

COEXISTING RELATIONSHIPS OF SUBSTANCE P-AMACRINE CELLS IN THE LARVAL TIGER SALAMANDER RETINA.
C.B. Watt*, V.J. Florack and R.B. Walker. Alice R. McPherson Laboratory of Retina Research, Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX. 77380.

Substance P (SP) immunocytochemistry was combined with either immunocytochemistry of gamma-aminobutyric acid (GABA) or autoradiography of high-affinity GABA uptake to examine for the presence of GABA in SP-amacrine cells of the larval tiger salamander retina. Three thousand SP-like immunoreactive cells were visualized in double-label preparations. Double-label analyses revealed two populations of SP-amacrine cells that express each marker of GABA activity. The majority of these cells were situated in the innermost cell row of the inner plexiform layer and were designated as Type 1 amacrine cells. The other population was displaced to the ganglion cell layer. No SP immunoreactive Type 2 amacrine cells co-labelled for markers of GABA activity. Approximately 10% of SP-amacrine cells in the amacrine and ganglion cell layers co-labelled for markers of GABAergic activity. Double-labelled cells accounted for less than 1% of GABA-labelled cells observed in these layers. SP immunocytochemistry was combined with autoradiography of glycine high-affinity uptake to examine for the presence of glycine in SP-amacrine cells of the tiger salamander retina. Preliminary results reveal that approximately 90% of SP-cells exhibit high-affinity accumulation of glycine.

This study was supported by grants from the NIH (EYO5622) and the Retina Research Foundation (Houston).

431.3

INTRACELLULAR RECORDING, STAINING WITH BIOCYTIN AND IMMUNOHISTOCHEMISTRY OF AMACRINE CELL NETWORKS IN CARP RETINA.
J.C. Goddard[1], E.M. Fitzgerald[1]*, U.D. Behrens[2], H.-J. Wagner[2] and M.B.A Djamgoz[1]. [1]Dept. of Biology, Imperial College, London, SW7 2BB, UK; [2]Inst. Anatomy and Cell Biology, Philipps University of Marburg, D-355 Marburg, FRG.

Correlation of electrophysiological, morphological, neurochemical characteristics and connectivity of neurones is important for understanding the functional organization of neuronal systems. The amacrine cell of the carp retina has been chosen to show how the above characteristics can be brought together in a model system. Amacrine cells were electrophysiological recorded and then injected with a solution of 4% biocytin in 0.5 M KCl.

Biocytin diffused through gap junctions and often up to 25 coupled amacrine cells were observed from a 2 min injection at 1-10 nA. From the analysis of the stained networks, it appeared that not all coupled amacrine cells were morphologically the same. Some cell groups were cryosectioned and processed for GABA immunohistochemistry. Several cells generating on-off transient responses have yielded positive results. However, it was not clear whether all coupled cells within a network were GABAergic. Present research is further analysing the morphological and neurochemical characteristics of amacrine cell networks.

431.4

MORPHOLOGIES AND MOSAICS OF AXON-BEARING AMACRINE CELLS IN THE RABBIT RETINA: A NEUROBIOTIN TRACER-COUPLING STUDY.
L.L. Wright and D.I. Vaney*. Vision, Touch and Hearing Research Centre, University of Queensland, Brisbane 4072, Australia.

Many types of retinal amacrine cells show homologous coupling when injected with the biotinylated tracers, biocytin or Neurobiotin, thus revealing their somatic array (Vaney, 1991). Moreover, these small tracers are readily transported along the thin processes that characterize "axon-bearing" amacrine cells: visualization of these long processes is greatly aided by photochromic intensification of the DAB reaction product in the presence of tetrazolium salts (Vaney, 1992). We have thus used Neurobiotin injections of microscopically identified cells in the superfused rabbit retina to characterize the complete morphology and the somatic mosaic of several types of low density, axon-bearing amacrine cells. They include the long-range cell (LR; Vaney et al., 1988), the type 1 and type 2 catecholamine-accumulating cells (CA1 & CA2; Tauchi et al., 1990), the type 1 NADPH-diaphorase cell (ND1; Sagar, 1990), the interstitial amacrine cell (PA1; Famiglietti, 1992a), and the presumptive somatostatin cell (PA2; Famiglietti, 1992b). Whereas the LR, ND1 and PA1 cells showed strong homologous coupling, the PA2 and CA1 cells were only weakly coupled, and the CA2 cell showed no coupling. The LR and PA1 cells showed homologous coupling to somata located in either the inner nuclear, inner plexiform or ganglion cell layers. Both the LR and ND1 cells also showed heterologous coupling to other types of presumptive amacrine cells. The distal processes of the LR cells and the axons of the ND1 cells could be traced for up to 5 mm from the soma: this greatly exceeds the extent of other retinal interneurons that have been intracellularly labelled by previous methods.

431.5

COMPARISON OF RETINAL GANGLION CELL MORPHOLOGY IN NORMAL AND TRANSGENIC MICROPHTHALMIC MICE. **S.D. Schlussman and S.C. Sharma*** Depts. Cell Biol. and Anatomy and Opthalmology, New York Medical College, Valhalla, N.Y. 10595.

Genetic ablation of gamma-2 crystallin expressing cells leads to a microphthalmic phenotype. We have previously demonstrated a decreased density of neurons in the Ganglion Cell Layer in transgenic microphthalmic mice as compared to controls. This observation prompted a consideration of the morphology of RGC in microphthalmic retinae. HRP labelled RGC were analyzed in normal and transgenic microphthalmic mice on the basis of 1) soma diameter, 2) dendritic field diameter and 3) dendritic morphology. The diameter of RGC somata in normal CD-1 mice ranged from a low of 3.0 μm to a high of 22.4 μm with an average of 13.1 ± 3.9 μm. The dendritic field diameters of normal RGC ranged from a low of 11.168 μm to a high of 145.6 μm and had an average of 70.7 ± 27.96 μm. Transgenic microphthalmic RGC somata ranged in size from 10.24 μm to 26.3 μm and had an average of 16.3 ± 3.8 μm. The dendritic field diameters in these animals ranged from a low of 63.7 μm to a high of 234.9 μm and had an average of 136.8 ± 50.0 μm. These correspond to an average increase of approximately 24% and 93% respectively. These data indicate that RGC dendritic fields are capable of altering their morphology in response to changes in their local environment. Supported by NEI 01426

431.6

SPATIO-TEMPORAL RESPONSE PROFILES OF CAT RETINAL W-CELLS M.H. Rowe* and J.F. Cox, Neurobiology Program, Ohio Univ., Athens, OH 45701.

We have used a one dimensional version of the reverse correlation procedure of Jones and Palmer (J. Neurophysiol., 58: 1187-1211, 1987) to examine the spatio-temporal (ST) response profiles of cat retinal W-cells and compare them to those of X- and Y-cells. The stimuli consist of stationary bright or dark bars which are presented for a fixed duration, typically 50 msec, at one of 16 positions spanning the entire receptive field along one spatial dimension. A series of stimuli are presented successively with no interstimulus interval, and both the position and contrast (bright or dark) of each stimulus are varied randomly. The times of occurrence of all action potentials occurring during the presentation sequence are recorded and the reverse correlation procedure quantifies the relationship between the time of occurrence of each spike and the position, contrast and time of onset of each stimulus in the sequence. The resulting ST profiles thus represent one dimension of space and one of time, and provide an estimate of the cell's spatio-temporal impulse response. Most tonic W-cells have ST profiles similar to those of X- or Y-cells, i.e. a spatial center-surround organization at short latencies, which is space-time inseparable due principally to the longer latency of the surround response. Some phasic W-cells also conform to this pattern, but some have profiles that are much simpler, consisting of a single central zone, not flanked in space or time by an antagonistic region, while others have much more complicated profiles, often with central zones which are bimodal in the space domain, flanked by antagonistic regions that are offset in both space and time. Supported by NIH EY08038.

431.7

CHARACTERIZATION OF LIGHT-EVOKED NMDA RECEPTOR-MEDIATED INPUT TO GANGLION CELLS IN DARK-ADAPTED RETINAS. J. S. Diamond and D. R. Copenhagen*. Bioengineering Graduate Group, Departments of Physiology and Ophthalmology, University of California, San Francisco, CA 94143.

Light-evoked excitatory synaptic input to ganglion cells has been shown to be mediated by both NMDA and AMPA-type glutamate receptors (Mittman et al. (1990) *J. Physiol.* **428:** 175).

We have used standard whole-cell patch clamp techniques in the tiger salamander retinal slice preparation to investigate the time course and magnitude of the light-evoked glutamate receptor-mediated inputs to ganglion cells in dark-adapted retinas. In voltage-clamp recordings, the reversible effect of D-2-amino-7-phosphonoheptanoate (AP7), a competitive NMDA antagonist, on the response to a two-second flash of light was greatest at -40 mV, a potential at which NMDA-mediated current is thought to be near maximal; the AP7 effect was least at -90 mV, where the NMDA receptor channel is blocked by external Mg^{2+} (1 mM). The relative strength of the NMDA-mediated input with respect to the AMPA input was determined by taking the ratio of the light response at -40 mV to that at -90 mV. AP7 (30 μM) caused as much as a five-fold reduction in this ratio.

Mittman et al. showed that cells in light-adapted preparations displayed a relatively fast response to light and that the excitatory inputs could be distinguished temporally, because the NMDA input had a much slower time course (time to peak = 100 msec) than the AMPA input (time to peak = 28 msec). In dark-adapted retinas the ganglion cell response to dim light had a much slower time course (time to peak ≈ 500 msec). When AP7 was applied, the magnitude of the light-evoked current at -40 mV decreased, but no significant change in time course was seen. Neither the magnitude nor the time course of the dim flash responses at -90 mV was affected by AP7. Consequently, there is considerably more temporal overlap of the AMPA- and NMDA-mediated light responses in dark-adapted than in light-adapted retinas. Any nonlinear interactions between the AMPA and NMDA conductances would, therefore, be enhanced under dark-adapted conditions.

431.8

CHANGES IN THE MORPHOLOGY AND SURVIVAL OF THE CELLS OF THE GANGLION CELL LAYER OF THE RETINA FOLLOWING CORTICAL LESIONS AND OPTIC NERVE SECTION IN THE FERRET. T.J. DROLSUM AND E.H. POLLEY*. Dept. of Anatomy & Cell Biology, Univ. of Ill., Chicago, Ill. 60612.

The morphological responses of retinal ganglion cells (RGCs) to axonal section and loss of postsynaptic targets was studied in 13 male ferrets (Mustela putorius furo). Experiments included: 1) ablation of the visual cortex (with retrograde degeneration of cells in the lateral geniculate nucleus (LGN); 2) a lesion of LGN (to include projecting optic nerve terminals); and 3) intracranial section of one optic nerve. Survival times ranged from 5 mos. to 1 yr. Retinal flatmounts were embedded in glycol methacrylate, sectioned en face at 5 microns, and stained with 0.1% Thionin. An unbiased counting frame (Gundersen et al., 1988) was used to measure cell density and cell size distributions from serial sections of the RGC population in a defined area, 2.5 mm. temporal to the optic disc. In addition, changes in the frequency distribution of surviving small, medium and large RGCs were obtained from a larger population of RGCs with the aid of a digitizing computer program by Sigma Scan (Jandel). Only those cells demonstrating a nucleus, nucleolus, and Nissl material were included in the study.

Data analysis indicates that there is a decreased frequency in the numbers of medium sized cells among the RGCs surviving a lesion of visual cortex. While the frequency of the large cells in the RGC layer remained unchanged, the relative numbers of the small cells increased. Following lesions of the LGN or after transection of the optic nerve, the frequency distribution of both large and medium sized cells decreased while that of the small cells increased. The latter procedures also resulted in a marked decrease in the overall cell density in the RGC layer, whereas cell density remained unchanged following the cortical lesions.

These results suggest that the retrograde transneuronal effects of visual cortical lesions on retinal ganglion cells is limited to a modification of normal soma size of the medium sized cells. This is in contrast to the degenerative effects of a lesion to optic nerve terminals or optic nerve fibers which broadens the retrograde effects to include a degeneration and shrinkage of large, medium and small RGCs. We believe that the cortical lesion demonstrates a dependency of a subset of RGCs on an intact postsynaptic target for specific maintenance of soma size. The retrograde degenerative effects of lesion of the optic nerve axon or its terminals demonstrates a dependency of the RGCs that is associated with the integrity of these processes.

431.9

Circuitry of Midget Ganglion Cells In Primate Fovea Distinguishes Two Classes of Cone. D.J. Calkins, K. Klug[1], S.J. Schein[1], Y. Tsukamoto[2]*, P. Sterling. U. of Penn., Phila., Pa 19104, [1]UCLA, 90024, [2]Hyogo Col. Med., 663 Japan.

Middle (M) and long wavelength sensitive (L) cones, which together constitute about 90% of the foveal cone population, can be distinguished from short wavelength sensitive (S) cones by differences in immunoreactivity to antigenic markers, dye uptake, and ultrastructure. However, M and L cones have not so far been distinguished from each other. Consequently, neither their relative numbers nor their circuitry have been determined. We reasoned that if the synaptic circuits that link each cone to its pair of midget ganglion cells (on- and off-) differ for M and L cones, then by reconstructing these connections we might distinguish the two classes. In electron micrographs of serial sections, we identified, for a small patch of cones (outer segments at 1.5° nasal), the pair of midget bipolar cells to which each cone connected and also the corresponding pair of midget ganglion cells. For one set of cones (N=17), the number (mean ± s.d.) of synaptic outputs (ribbons) per midget bipolar cell was 29 ± 2, and the number of inputs (at ribbons) per midget ganglion cell was 15 ± 2. For another set of cones (N=9), the number of synaptic outputs per midget bipolar cell was 51 ± 3, and the number of inputs per midget ganglion cell was 26 ± 3. Thus, in the parametric space defined by this circuitry, the cone array was partitioned into two distinct groups. The ratio of the two classes was 1.9, and within the patch studied they were completely interspersed. Psychophysical results[1] suggest that the ratio of L to M cones is about 2, so conceivably the two classes identified here represent, respectively, L and M cones. Supported by EY08124.
We thank P. Masarachia, A. Meyers, and L. Chukoskie for their help.
[1]Nerger, J.L. and Cicerone, C.M. (1992) The ratio of L to M cones in the human parafoveal retina. *Vision Res.* **32**, 879-888.

431.10

HYPEROSMOTIC ACTIVATION OF TRANSMITTER RELEASE FROM PRESYNAPTIC TERMINALS ONTO RETINAL GANGLION CELLS. W. Yu and R. F. Miller*. Department of Physiology, University of Minnesota Medical School, Minneapolis, MN 55455.

We studied neurotransmission from 2nd order neurons to ganglion cells, using micro hyperosmotic stimulation (μHS) to activate transmitter release from presynaptic terminals in the inner plexiform layer. These experiments were carried out in a perfused retinal slice preparation of the tiger salamander, using direct visualization under an upright binocular microscope. A micropipette, filled with hyperosmotic sucrose (0.5 M + Ringer) was placed in the IPL and whole-cell recordings were obtained from single ganglion cells. Single cells were studied under current and voltage-clamp conditions, and many cells were stained with 5 and 6 carboxyfluoroscein added to the whole-cell pipette and visualized with fluorescence microscopy.

Brief positive pressure pulses (0.5-2 bar), applied to the sucrose pipette, activated a postsynaptic response in ganglion cells which was graded in magnitude, based on the duration of the pressure pulse. Voltage-clamp analysis and pharmacological studies indicate that the μHS response usually consists of both excitatory and inhibitory components. When the inhibitory components are blocked with picrotoxin (100 μM/strychnine (10 μM)), the postsynaptic current can be studied as a relatively pure excitatory postsynaptic event which typically consists of NMDA and non-NMDA contributions based on the use of dAP7, an NMDA receptor antagonist and NBQX (2,3 Dihydroxy-6-nitro-7-sulfamoylbenzo (F) quinoxaline), a non-NMDA receptor antagonist. This approach permits the analysis of relative NMDA/non-NMDA contributions into identified cell types and at different spatial positions along the soma-dendritic tree. Supported by NIH grants RO1 NEI-07376 and NS-17763 awarded to RFM.

431.11

STRUCTURE AND FUNCTION OF GANGLION CELLS PROJECTING TO THE CAT'S GENICULATE WING: AN *IN VITRO* STUDY. M. Pu, T. Pan and D.M. Berson*. Div. Biology & Medicine, Brown Univ., Providence, RI, 02912

The cat's geniculate wing (retinorecipient zone of the pulvinar) is thought to receive its retinal input from a single morphological class of presumed W-cells termed epsilon cells (Leventhal, et al., JCN 194:'80; Rodieck and Watanabe, Neurosci. Abst. 12:'86). We labeled wing-projecting cells by retrograde transport of fluorescent beads and studied their morphology and physiology in vitro. Intracellular staining with Lucifer Yellow and biocytin showed that nearly all of these cells (147/150; 98%) had the medium-sized somas (17-32 μm dia., mean 24 μm) and large, simple, radiate dendritic fields typical of epsilon cells. Dendritic-field diameters increased with eccentricity in the central retina (200-400 μm near the a.c.; 500-800 μm at 3 mm eccentricity) but exhibited little further increase more peripherally (500-1000 μm). Many dendritic profiles were elliptical with long axes pointing toward the a.c. Dendrites stratified narrowly in the inner IPL. Axons were as thick as any among presumed W-cells and similar to those of beta cells (~ 1 μm dia.). The morphology of these epsilon cells closely matches that of a wide-field type prominent among cells we have labeled by retrograde transport from the medial interlaminar nucleus (Neurosci. Abstr. 17:'91).

Epsilon-cell anatomy suggests certain physiological traits: large receptive field (large dendritic profile); ON-center (inner IPL ramification); tonic subtype of W-cell (thick axon, large soma, and uncrossed projections from temporal retina). We have begun testing these hypotheses by recording extracellularly from identified wing-projecting cells in a flattened superfused eyecup preparation. To date, all cells in our small sample (n=5) had the predicted large ON-center, OFF-surround receptive fields and tonic light responses.

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431.12

IMAGING OF LABELLED RETINAL GANGLION CELLS IN THE LIVING MAMMALIAN EYE. R.W. Rodieck*, B. Lia, & P. Kuthan. Department of Ophthalmology, RJ-10, University of Washington, Seattle WA 98195.

At present, it is difficult to correlate the ganglion cell types distinguished by function with those distinguished in terms of morphology and central projections. However, straightforward techniques *are* available to:
1. determine functional properties by means of extracellular recording from ganglion cells *in vivo*, and 2. determine morphology and central destinations, by means of a retrograde label and intracellular injection (e.g. HRP, Neurobiotin) of ganglion cells *in vitro*. We have developed an approach that combines these two techniques with *in vivo* imaging so as to allow the receptive-field properties, dendritic morphology, and central destinations of individual ganglion cells to be characterized.

Near-infrared fluorescent microspheres ('Crimson', Molecular Probes) are used as the retrograde label, in order to preserve rod sensitivity. Labelled ganglion cells are visualized by what is, in effect, an epifluorescence ophthalmoscope. This device makes use of a barrier filter, a 135 mm imaging lens, and a CCD array cooled to -45° C in order to allow the slow collection of the image of the ganglion-cell layer, using a relatively dim, monochromatic excitation beam. Spatial resolution is 2 µm/pixel; intensity resolution is 12 bit (0.025%). Image quality is good, with contrast between labelled cells and background as large as 3:1. Images may be saved on disk. Tungsten-in-glass recording electrodes with Crimson microspheres in the tip can be advanced onto selected labelled cells, and the apparatus swung out of the way in order to investigate receptive-field properties. Later, the same cells can be located and intracellularly injected via the *in vitro* technique. Supported in part by NIH grants EY02923 and EY01730, and by the E.K. Bishop Foundation.

431.13

NEURAL CIRCUITRY OF FOVEAL GANGLION CELLS IN THE HUMAN RETINA. Helga Kolb* and Jill Crooks, Physiology Dept., University of Utah, Salt Lake City, U.S.A.

While the relationship of midget bipolar cells and midget ganglion cells is well established (Kolb and DeKorver, '91) little is known of the synaptic circuitry involving amacrine cells to ganglion cells in the primate fovea. Thus in this study we have concentrated on understanding the neurochemical signature of the input amacrine cells to ganglion cells using techniques of postembedding immuncytochemistry for GABA and glycine on EM serial sections.

Both midget and parasol ganglion cells and their respective input bipolar cells have been included in the analysis. GABAergic amacrine cells provided about 27% of the amacrine input to ON-center ganglion cell dendrites while glycinergic amacrines provided only 6% of the synapses. OFF-center cells received about the same proportion of GABAergic synapses (23%) as ON-center cells but a higher proportion of glycinergic synapses (23%). 50 to 60% of the amacrine synapses to all types of ganglion cells were of unknown neurotransmitter species.

GABAergic amacrine cell synapses upon bipolar axon terminals formed about 50% of all the amacrine input for both ON-center and OFF-center variety. Only in a minority of cases was the same GABAergic amacrine postsynaptic to the bipolar presynaptic upon the ganglion cell dendrite. In contrast, GABAergic amacrine cells were frequently reciprocal upon the bipolar cell without making a synapse upon the ganglion cell. There is evidently a complex synaptic circuitry concerning bipolar/ganglion/amacrine interactions even to construct the most "simple" foveal ganglion cell receptive fields. (Supported by grant EY03323)

431.14

EXCITATORY SYNAPTIC INPUTS TO SALAMANDER RETINAL GANGLION CELLS ARE MODULATED BY POLYAMINES AND GLYCINE. Peter D. Lukasiewicz* & Carmelo Romano. Department of Ophthalmology & Visual Science, Washington Univ. Sch. of Med, St. Louis, MO 63110.

Recent biochemical and electrophysiological studies have shown that both polyamines and glycine can enhance the activity of the NMDA receptor. This work was undertaken to determine if polyamines and glycine can modulate the strength of the excitatory synaptic inputs to retinal ganglion cells.

Excitatory synaptic currents were recorded from ganglion cells in the tiger salamander retinal slice preparation using whole-cell patch recording. Synaptic inputs were elicited by puffing K^+ onto the dendrites of bipolar cells presynaptic to the ganglion cell. The NMDA component of the input was isolated by including 1-5μM CNQX, 1μM strychnine and 100 μM picrotoxin in the bath. We investigated whether or not a polyamine site was associated with NMDA receptors on ganglion cells using the polyamine inverse agonist diaminodecane (DA10). DA10, at 10-50 μM, reversibly reduced the amplitude of the NMDA component of the synaptic input. The competitive polyamine antagonist diethylenetriamine reversed the suppressive action of DA10, consistent with DA10 acting specifically at the polyamine site. These results suggest that polyamines can modulate excitatory synaptic inputs to ganglion cells.

We used the glycine modulatory site antagonist 7-Cl-kynurenate to determine if glycine could modulate the NMDA component of synaptic input. 7-Cl-kynurenate (20 μM) reversibly reduced the puff-elicited NMDA component of the synaptic input to ganglion cells. These results suggest that a glycine site associated with the NMDA receptor complex on ganglion cells can modulate the strength of their excitatory synaptic inputs.

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431.15

EFFECTS OF VASOACTIVE INTESTINAL PEPTIDE ON GANGLION CELLS IN THE RABBIT RETINA. Ralph J. Jensen.* Southern College of Optometry, Memphis, TN 38104.

The putative neurotransmitter vasoactive intestinal peptide (VIP) has been shown to be present in a population of amacrine cells. Like the neurotransmitter dopamine, VIP stimulates adenylate cyclase activity in the rabbit retina. In the present study, I examined the effects of bath-applied VIP on ganglion cells recorded extracellularly in a superfused, rabbit retinal preparation.

Most OFF-center and ON-center ganglion cells responded with an increase in spontaneous activity when VIP was applied to the retina. The light responses of these cells to spots and annuli were however only minimally affected. When applied to retinas bathed with the dopamine antagonist (+)-SCH 23390, VIP enhanced the light responses of most OFF-center ganglion cells. VIP brought out both the center and surround responses that were reduced by (+)-SCH 23390. The effects of (+)-SCH 23390 on ON-center ganglion cells were not reversed by VIP. It is proposed that VIP counteracts the effects of (+)-SCH 23390 on OFF-center ganglion cells by stimulating adenylate cyclase activity in dopamine-receptive cells of the retina (Supported by NIH grant EY07318).

431.16

Prenatal Development of Neuropeptide Y Immunoreactivity in the Ganglion Cell Layer of the Cat Retina Jeffrey J. Hutsler* and Leo M. Chalupa, Psychology Department and the Center for Neurobiology, University of California, Davis.

Previously we have shown that immunoreactivity for neuropeptide Y (NPY-IR) identifies a subgroup of gamma type ganglion cells in the adult cat retina which project to the superior colliculus (Hutsler et al., 1991). In the adult, these cells (approximately 2,000 per retina) are clustered along the horizontal streak with the highest density at the area centralis. Amacrine cells within the inner nuclear layer (INL) are also NPY-IR in the adult cat, and these are distributed in a regular array across the entire retinal surface (Hutsler et al., in preparation). We have now examined the development of NPY-IR in these two population of cells. At embryonic day (E) 38 NPY-IR profiles are not present within the GCL or the INL. By E46 such cells appear in a densely packed region of the central retina within the GCL, and by E50 NPY-IR cells within the GCL have extended out to the retinal periphery. At this age their overall distribution is similar to that seen in the adult. NPY-IR cells within the INL do not appear until E50, when they are largely confined to the central retina. About one week later the NPY-IR amacrine cells are first seen in the periphery, with the overall population characterized by a regularly spaced array. During fetal development the number of cells that are NPY-IR was not found to be appreciably greater than in the adult retina. These results indicate that NPY-IR can be utilized during prenatal development to identify specific populations of amacrine and ganglion cells in the cat retina . (Supported by EY03991 from the NEI.)

431.17

RETINAL GANGLION CELLS ARE NOT FRACTAL IN THE BOX COUNTING MEASURE. J. Panico and P. Sterling*, Dept. of Neuroscience, Univ. Penn., Phila., PA 19104.

Fractal geometry can be used to characterize patterns that are statistically self-similar, i.e., for which pieces of the pattern, when magnified, resemble the whole. The degree of fractalness is measured by the degree of self-similarity over a certain range of scales. Many physical systems which have been modelled with fractal geometry display a high degree of self-similarity over at least 3 decades of scale. Though retinal ganglion cells also appear fractal, their reported self-similarity spans at most 1 decade of scale and has not been rigorously evaluated.

Images of HRP-filled retinal ganglion cells (from this and other labs) were digitized, then "skeletonized", and overlaid with a square grid of mesh size d. The number of boxes intersected by the skeleton was counted. In a plot of log(#boxes intersected) vs. log(d), fractalness is assessed by the degree of linearity over a designated portion of the abscissa. As a control, "box counting" was applied to patterns generated to express self-similarity over specified range of scales. Also, we manipulated the spatial struture of neural and control skeletons according to various randomizing algorithms and observed the effects on the log-log plots.

The log-log plots for the controls have linear regions spanning more than one decade of scale. However, plots for the neural patterns have no significant linear portion; instead they are truly sigmoidal: not fractal. We then established an operational criterion: we designated a pattern "fractal" if arbitrarily deforming the pattern made its log-log plot less linear. The plots for control patterns lost most of their linearity, but the plots for neural patterns were unaltered by the deformation. Thus we conclude that, in the box counting measure, ganglion cells are not measurably fractal. Supported by EY08124.

431.18

RAB3A IS EXPRESSED IN THE PRIMARY VISUAL SYSTEM. K.L. Moya*, O. Stettler, A. Zahraoui, L. DiGiamberardino and B. Tavitian. INSERM U334 and CNRS URA 1285, C.E.A., S.F.H.J., Orsay and INSERM U248, Paris, France.

Rab3A is a small GTP-binding synaptic vesicle protein preferentially expressed in the brain and implicated in exocytosis. Our previous studies have shown that rab3A is present in specific neuronal circuits, expressed in a subset of neurons in the brain and is developmentally regulated. In the normal adult rat brain, the superficial layers of the superior colliculus (SC) show considerable rab3A immunoreactivity. Excitotoxic lesions of primary visual cortex, while diminishing rab3A in the dorsal lateral geniculate nucleus (LGNd), did not appear to affect the SC. These experiments suggested that the corticogeniculate projection from layer 6 contained considerable rab3A, whereas the descending corticotectal projection from neurons in layer 5 did not account for the observed staining in the SC. This raised the possibility that rab3A is synthesized in other neurons that project to the SC such as retinal ganglion cells which send a massive input to the tectum.

We used in situ hybridization of the retina and immunohistochemistry of the midbrain after eye enucleation to assess whether rab3A was synthesized in retinal ganglion cells and if their terminals in the SC contained this protein. In the retina, silver grains were observed over the nuclei of retinal ganglion cells after hybridization with a labeled 30-mer oligonucleotide that was derived from the hypervariable C-terminal coding region and specific for rab3A mRNA. Immunohistochemical studies showed that rab3A was reduced in the superficial layers of the contralateral SC 6 (N=2) and 14 (N=2) days after unilateral eye enucleation. In addition, immunoreactivity was also reduced in the contralateral pretectal nucleus, however, there were no obvious changes in the LGNd.

These results show that rab3A is expressed in retinal ganglion cells and localized to their terminals in the SC. The presence of this small G protein in the primary visual pathway suggests that rab3A can participate in the transmission of visual information.

431.19

TRANS-ACPD EVOKES INCREASES IN INTRACELLULAR FREE CALCIUM CONCENTRATION IN ISOLATED RETINAL GLIAL (MULLER) CELLS S.A. Keirstead* and R. F. Miller. Department of Physiology, University of Minnesota Medical School, Minneapolis, MN 55455.

Electrophysiological studies have demonstrated that retinal glial (Muller) cells do not respond to application of ionotropic glutamate receptor agonists (NMDA, quisqualatic acid, kainic acid). Rather, these cells respond only to application of glutamate with an inward current generated by an electrogenic glutamate uptake mechanism (Henshel and Miller, 1986, Soc. Neurosci. Abstr. 12: 169; Brew and Attwell, 1987, Nature 327: 707-709). The calcium imaging studies that we report here revealed that the metabotropic gluatamate receptor agonist 1S,3R-1-aminocyclopentane-1,3-dicarboxylic acid (trans-ACPD) evokes increases the intracellular free calcium concentration ($[Ca^{2+}]_i$) of isolated Muller cells .

Muller cells were acutely isolated from the retina of the larval tiger salamander and were loaded with the calcium sensitive dye Fura-2 AM. A commercially available imaging system was used to observe changes in $[Ca^{2+}]_i$ evoked by bath application of trans-ACPD (50-200 uM) in calcium-free Amphibian Ringer (2mM EGTA). Nine of eleven Muller cells tested responded to the application of this glutamate agonist with an increase in $[Ca^{2+}]_i$. In six of the responsive cells the increase in $[Ca^{2+}]_i$ ocurred later in the endfoot region of the cell. In one cell the $[Ca^{2+}]_i$ the region of the nucleus was lower at rest than that in the other regions of the cell, and the response to trans-ACPD in the nucleus occured later than the other regions, and was of a larger amplitude. Further experiments include the pharmacological characterization of these responses to determine if they are due to specific activation of the metabotropic glutamate receptor and if glutamate itself also evokes increases in $[Ca^{2+}]_i$ in Muller cells. Supported by NIH grant EY03014 to R.F. M.

431.20

TRYPAN BLUE STAINING ON RETINAL GANGLION CELL OF RAT INSULTED IN EXPERIMENTAL HIGH INTRAOCULAR PRESSURE. Z.H. Liu, Q.L. Liu, D.H. Lu, J.Z.Shuai and X.G. Luo*. Dept. of Neurobiology, Hunan Medical University, Changsha, Hunan 410078, P.R.C.

We have continued the study on damaged retinal ganglion cell (RGC) resulting from ischemia-reperfusion (I/R) in experimental high intraocular pressure. The experimental high intraocular model was used as before (Liu at el., Soc. Neurosci. Abst. 17:1567 '91). In 10 albino rats, two eyes of each rat were enucleated and fresh whole-mounted retinae were prepared for dye-exclusion test. Trypan blue (TB) was used as a marker to determine the viablity of RGC and was applied according to the method of Taylor and Hunt (Brit. J. Ophthal. 65:815 '81). The nucleus and perikaryon of RGC, which lost its viablity, were full of TB substance. For statistics, the stained RGCs within the four quadrants of each retina was counted at a microscopic magnification of 200-fold with the help of fixing a scored reticle on the slide. The area of whole retina and its four quadrants were digitalized respectively with an image analysis system (PC VISION Plus). The data were analyzed for statistical significance utilizing Student's t-test by Macintosh computer. As comparing these two kinds of retinae, far more RGCs of the I/R retina were stainable than those of the SI (simple ischemia) retina ($p<0.001$). The results indicated that the disintegration of RGCs occurred predominatly since the artificial pressure has been removed. The increased permeablity of membrane of RGCs may be related to the attack of the free radical produced in the reperfusing periods as reported by us. Supported by NSFC 3870621 to Z.H..Liu.

SUBCORTICAL VISUAL PATHWAYS: CORTICO-TECTAL, ACCESSORY OPTIC SYSTEM, CENTRIFUGALS

432.1

OCCIPITOTEMPORAL AND PARAHIPPOCAMPAL GYRUS PROJECTIONS TO THE BASIS PONTIS IN RHESUS MONKEY. *J.D. Schmahmann,*D.N. Pandya, Massachusetts General Hospital, Boston, MA, and ENRM Veterans Hospital, Bedford, MA 02130.*

We used tritiated amino acids to study projections to the basilar pons from parastriate cortices in thirteen rhesus monkeys to determine how connectional and functional heterogeneity of these regions are reflected in corticopontine circuitry. Labelled fibers travelled with those from other parasensory associative cortices as they arched over the dome of the lateral geniculate nucleus and entered the cerebral peduncle. Pontine projections were derived from area 19 at the medial convexity, from the dorsal part of the prelunate gyrus, and from the caudal part of the parahippocampal gyrus (area TF). No pontine projections arose from the ventral prelunate gyrus or from the inferotemporal region. Terminations in the pons were observed in the dorsolateral and lateral nuclei, and the lateral part of the peripeduncular nucleus. Medial convexity injections produced more extensive rostrocaudal labelling, as well as terminations in the extreme dorsolateral nucleus. Dorsal prelunate injections had additional terminations in the ventral pontine nucleus. Posterior parahippocampal gyrus injections had discrete label in the lateral and dorsolateral nuclei. These results suggest that the dorsal visual stream communicates with the pons whereas the ventral visual stream does not. The posterior parahippocampal gyrus implicated in visual spatial memory also sends efferents to the pons. These anatomical results have implications for cerebellar function. (Supported in part by NIH 16841 and the Veterans Administration).

432.2

CORTICOSTRIATAL AND CORTICOTECTAL PROJECTIONS FROM THE VISUAL CORTICAL AREA 17, 18 OR 18a IN THE RAT: AN ANTEROGRADE TRACING STUDY Masahiro Serizawa, Kaeko Hoshino and Masao Norita* Dept. Anatomy, Niigata Univ. Sch of Med., Niigata 951, Japan

The neurons in the deep laminae of the superior colliculus (SC) send descending efferents to a variety of regions of brainstem and spinal cord involved in orienting the eyes, head and limbs indicative of important role of the collicular neurons for "visually guided orientation behavior." In an attempt to detail the corticostriatal and corticotectal projections from the visual cortex in the rat, we injected the tracers such as biocytin or WGA-HRP into visual cortex of Long Evans rat and observed the terminal distributions of the cortical efferents. After injection of the tracer into area 17, numerous labeled axons and terminals were observed in the superficial laminae of SC. In this case, only a few, if any, labelings were found in the caudate putamen. On the other hand, when the tracer was injected into area 18a, a number of labelings were found in the dorsocaudal region of caudate putamen as well as in the deep laminae of SC. Following the tracer injection into area 18, some labelings were observed in the dorsocaudal region of caudate putamen as well as in the deep laminae of SC. The present results suggested that these corticofugal projections from area 18a are important in controlling the visually guided orientation behavior as those found in the cat's corticostriatal and corticotectal projections from the lateral suprasylvian visual area (Norita et al., Neuroscience Res. 10: 149-155, 1991).

432.3

CORTICAL DEACTIVATION DISRUPTS MULTISENSORY INTEGRATION. L.K. Wilkinson[1]*, M.A. Meredith[2], and B.E. Stein[3]. Departments of Psychology[1], Anatomy[2], and Physiology[3], Medical College of Virginia, Richmond, VA, 23298

The cortex of the anterior ectosylvian sulcus (AES) contains unimodal visual, somatosensory and auditory neurons whose projections converge, along with those from lateral suprasylvian cortex (LS), on multisensory superior colliculus (SC) neurons. These SC neurons are believed to play important roles in attentive and orientation behaviors. In the present experiments we sought to determine the effect of reversibly deactivating these cortices on such behavior. The testing apparatus consisted of a semi-circular array of paired LEDs and speakers at 15° intervals. Two cats were trained to approach a near-threshold visual stimulus, regardless of location, for a food reward. The auditory stimulus was presented at the same site as the visual stimulus, or 45° lateral or medial to that site. In normative testing, animals showed a multiplicative enhancement of their responses to visual stimuli when an auditory stimulus was presented spatially coincident with the visual stimulus. Conversely, they showed dramatic inhibition of responses to the visual stimulus when the auditory stimulus was 45° disparate to it. During testing, a cortical area (either AES, LS, AI/AII, or striate cortex) was deactivated with lidocaine through indwelling cannulae. Deactivation of AES disrupted multisensory enhancement and multisensory depression at all eccentricities tested. However, deactivation of the other cortical areas tested, and/or saline controls in AES, had no influence on these multisensory processes.
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432.4

RESPONSE PROPERTIES OF CORTICOTECTAL NEURONS IN THE LATERAL SUPRASYLVIAN CORTEX OF CAT.

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Because the projections from lateral suprasylvian cortex (LS) to the superior colliculus (SC) and striatum (ST) originate primarily from non-overlapping populations of corticofugal neurons (Norita *et al. Neurosci. Res.* **10**:149-155, 1991), it has been suggested that the information they process is also quite different. The present experiment represents a first step in examining this possibility. Forty-three neurons that could be antidromically-activated from the SC, but not from the ST, have been studied thus far in halothane anesthetized, paralyzed cats. Twenty-four of these were located in the medial bank of LS and 19 in the lateral bank. Most corticotectal neurons were binocular (95%) and dominated by the contralateral eye (93%). They generally exhibited spatial summation (77%) and surround inhibition (79%), responded well to flow field vectors (71%), had wide variations in velocity selectivity, and showed obvious direction selectivity (84%), with the majority (81%) preferring centrifugal movements. Comparatively few (5%) exhibited orientation selectivity. These data are consistent with the disruption of many of these same characteristics in SC neurons during deactivation of LS (Ogasawara *et al. J. Neurophysiol.* **52**:1226-1255, 1984) and underscores their corticotectal origin. Supported by NEI grant EY05554.

432.5

CORTICOSTRIATAL AND CORTICOTECTAL PROJECTIONS FROM THE FRONTAL EYE FIELDS OF THE CAT.

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The relationships between the frontal eye fields (FEF) and the striatum (ST) of the cat are poorly understood. The present experiments were an attempt to determine how regions surrounding the presylvian and cruciate sulci, known to send projections to the superior colliculus (SC), project to the ST. Injections of WGA-HRP made into both cortical regions resulted in dense label in the ST and the SC. In the ST, anterograde labeling was found bilaterally (with a ipsilateral predominance) in the caudal part of the head of the caudate nucleus (AP +17.5 to +14.0); only presylvian injections resulted in the labeling in the putamen, where it was restricted to the caudal aspect. Presylvian injections resulted in label distributed dorsally whereas cruciate injections produced label ventromedially. These same cortical injections also resulted in label restricted to the deep laminae of the ipsilateral SC. After injections of WGA-HRP into ST, numerous labeled corticostriatal neurons were observed. In the presylvian, they were found in lamina III and the upper aspects of lamina V while in the cruciate, they were located in lamina III, upper aspects of lamina V, and lamina VI. In both regions, corticotectal neurons were found only in lamina V. These data are consistent with the idea that, in addition to a direct influence on deep laminae SC neurons, the FEF may modulate tectospinal neurons indirectly via the ST and substantia nigra. Supported by NEI grant EY05554.

432.6

DEACTIVATION OF UNIMODAL CORTICES ALTERS THE PROPERTIES OF MULTISENSORY NEURONS IN CAT SUPERIOR COLLICULUS. M.T. Wallace[1]*, L.K. Wilkinson[2], M.A. Meredith[3], and B.E. Stein[1]. Depts of Physiol[1], Psychol[2] & Anatomy[3]. Medical College of Virginia, Richmond, VA, 23298

Visual, auditory and somatosensory inputs from anterior ectosylvian (AES) and lateral suprasylvian (LS) cortices converge onto multisensory superior colliculus (SC) neurons (Wallace et al., 1991). In this study we sought to determine how these corticotectal inputs influence the response properties of SC neurons. Reversible deactivation (cooling) of a single modality-specific cortical region (i.e., SIV-somatosensory, Field AES-auditory, AEV and LS-visual) was found to depress the corresponding unimodal response of a multisensory SC neuron (e.g., cooling SIV depressed somatosensory responses of a visual-somatosensory neuron). In many cases this deactivation also resulted in a proportional decrease in the integrative product produced by presenting two different sensory stimuli simultaneously. However, in certain instances deactivating a single cortical region altered multisensory integration in SC neurons in ways not readily predictable from its influences on unimodal responses. For example, in some neurons deactivation of SIV decreased visual-somatosensory integration to a far greater extent than that expected based on the slight depression of the unimodal somatosensory response. In at least one case a seemingly paradoxical effect was observed when multiple cortices (i.e., LS, AEV and SIV) were deactivated simultaneously: unimodal visual and somatosensory responses were depressed, but the magnitude of the multisensory interaction was dramatically increased (from 84 to 495%). This is likely due to the principle of 'inverse effectiveness', wherein the combination of weakly effective unimodal stimuli produce disproportionally high enhancements of one another's effect on SC activity. These data demonstrate the importance of the different unimodal cortices in maintaining the integrity of multisensory integration in the SC. Supported by NIH grants NS 08902 and NS 22543

432.7

"Extrastriate" Visual Pathways in the Pigeon: Descending Projections upon the Optic Tectum. Harvey J. Karten*, Kevin Cox and Toru Shimizu. Dept. Neurosciences, University of California San Diego, La Jolla, CA 92093 and Dept. Psychology, University of South Florida, Tampa, FL 33620

Two major visual pathways to the forebrain have been identified in birds: 1) A tectofugal pathway, the retino-tecto-rotundo-ecto system similar to the extrastriate pathways through inferior pulvinar of mammals; and 2) A retino-thalamo-wulst pathway, similar to the geniculostriate system of mammals.

Two major descending pathways from telencephalon upon the optic tectum in pigeon have been identified. These arise from: a) The wulst, (Karten et al., 1973, Reiner and Karten, 1982), and b) the archistriatum (Zeier and Karten, 1971), a purported target of the rotundo-ectostriatal pathway. Cholera toxin B (CTb) was used to identify the locus of origin within the archistriatum of projections upon the tectum, and to then characterize the pattern of termination of these projections within the tectum.

Telencephalic Origins of Tectal Afferents: Following injections of CTb limited to the optic tectum, without spread to underlying ventricle or subtectal nuclei, retrogradely labeled cells in the telencephalon were confined to two regions: a) A sharply restricted zone within the ventrolateral portion of the archistriatum intermedium (Aivl); and, b) The hyperstriatum accessorium of the wulst, and adjacent lateral corticoid region.

Projections of the Archistriatum upon the Optic Tectum: Injection of CTb in the Aivl resulted in axonal labeling in the tractus occipito-mesencephalicus with terminations in Layers 10-13 of the ipsilateral optic tectum. Projections of the wulst terminate in more superficial laminae of the tectum.

The pattern of differential distribution of wulst vs. archistriatal projections resembles the differential pattern of projections of corticocollicular connections in mammals arising from the striate vs. extrastriate cortices, respectively.

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432.8

WHOLE-FIELD MOTION SENSITIVITY OF SINGLE UNITS IN THE LARGE-CELLED PRETECTAL NUCLEUS LENTIFORMIS MESENCEPHALI (nLM) IN RANA PIPIENS Z. Li* and K. V. Fite, Neuroscience and Behavior Program, University of Massachusetts, Amherst MA 01003

The large-celled pretectal nucleus lentiformis mesencephali (nLM) is considered to be essential for horizontal optokinetic nystagmus in frogs. Although the relationship between directionally specific pretectal neurons and horizontal optokinetic nystagmus has been demonstrated in various vertebrates, the significance of velocity sensitive neurons in nLM needs clarification. The present study consists of a single-unit analysis of nLM using a large-field (100 x 100°), random dot patterned stimulus. This stimulus was presented for 4 directions and 4 velocities (0.4-40°/s) of movement centered on the receptive field of the unit.

All units localized in nLM were spontaneously active, and sensitive to "off" changes in ambient illumination. A majority (more than 2/3's) of the recorded units showed velocity sensitivity. These response profiles could be categorized into three types: (1) Units responding in the lower range (0.4-10°/s) of stimulus velocity, which increase in response when the stimulus velocity increases. (2) units responding in the lower range (0.4-10°/s) of stimulus velocity, which decrease in response when the stimulus velocity increases. (3) Units showing a nearly linear increase in response when stimulus velocity increased from 0.4 to 40° /s. Approximately one-third of the units could be classified as directional, and no response bias for horizontal or temporal-to-nasal motion was observed. (supported by NSF grant BNS 8819870 to K.V.F.)

432.9

LINEARITY AND DYNAMICS OF THE RETINAL SLIP CODE IN TURTLE ACCESSORY OPTIC SYSTEM NEURONS. A.F. Rosenberg* and M. Ariel, Dept. of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260, USA.

The basal optic nucleus (BON), the major retinal recipient area of the reptilian accessory optic system, contains direction-sensitive (DS) cells that respond to full-field, textured visual stimuli. It is thought that these neurons provide a retinal slip signal that drives the optokinetic reflex. The purpose of this study was to quantitatively investigate how retinal slip is encoded in these neurons using linear system theory.

Single unit responses of BON neurons were characterized using whole-field visual stimuli projected directly onto retinas of isolated turtle brains. Direction-tuning curves were obtained either by using constant velocity stimulation (linear sweeps) along several polar axes or by presenting a constant speed stimulus that moved along a circular path (circular sweeps). The hypothesis that stimulus velocity is encoded along a single linear axis was first tested by fitting direction-tuning curves to cosines. Second, the direction-tuning derived from circular sweeps was compared to the sum of responses of two orthogonally oriented stimuli, each of which followed a linear path with sinusoidally modulated velocity (sinusoidal sweeps). Finally, linear sweeps were applied along the axis with maximal DS modulation at different speeds to directly test the cosine rule. The tuning of these cells obeys a cosine rule to a first approximation, with the addition of a non-DS component.

To assess the dynamic responses of BON neurons, sinusoidal sweeps were applied along the axis with maximal DS modulation at several frequencies. The resulting discharge rates were fit to rectified sinusoids to determine gain and phase. Strong sinusoidal modulation persisted at frequencies to 1 Hz with peak speeds below 15°/s.

BON neurons exhibit DS responses at a broad range of stimulus velocities, yet the range of movement for which these cells exhibit somewhat linear responses seems to be restricted to slow velocities.

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432.10

VISUAL INPUTS TO THE TURTLE CEREBELLAR CORTEX STUDIED *IN VITRO*. M. Ariel* and T. X. Fan, Department of Behavioral Neuroscience. University of Pittsburgh, Pittsburgh, PA 15260.

Single units were recorded extracellularly in the cerebellar cortex (Cb) from an isolated turtle brain preparation during visual stimuli and current pulses to the basal optic nucleus (BON). Only a small fraction of isolated units responded to visual stimuli. They were all direction-sensitive (DS), driven only by the contralateral eye and found below the Purkinje cell layer. The most effective stimulus was a moving check pattern imaged onto the retina from a computer-generated video monitor. A unit's spike frequency in the preferred direction increased as a function of stimulus velocity until about 10 °/s, but was DS up to 100 °/sec.

The cellular identity of the visual units was analyzed based on their location in Cb, their spike waveform and by their responses to single current pulses (30-150 μA) to the BON. Of the 15 units driven by BON stimulation, 6 units followed high frequency stimulation at a fixed short latencies. Such responses were also recorded following a transection in the frontal plane just caudal to the Cb peduncles. Although this lesion presumably removes climbing fiber input from the inferior olivary nuclei, the visual and electrical response properties in Cb were unaffected. Thus, it appears that at least some of the visually responsive units in the granule layer are recordings from mossy fibers originating in the BON.

The properties of these units to visual and BON stimulation indicate that they relay retinal slip information directly from BON to Cb. As the BON receives direct input from DS retinal ganglion cells, these results thus provide electrophysiological evidence of the bisynaptic retinocerebellar pathway described anatomically by Reiner and Karten in 1978. The possible role of these units in oculomotor control is discussed.

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432.11

CENTRIFUGAL PROJECTIONS TO THE RETINA IN THE TURTLE *Pseudemys scripta elegans*. D. Zhang and W.D. Eldred*. Dept. of Biology, Boston University, Boston, MA 02215

The existence of centrifugal or efferent fibers to the retina from the brain has been demonstrated in many vertebrate species, including the turtle. However, little detailed information is known regarding the number of cells involved and the location of the cells in the brain which give rise to these centrifugal fibers. To investigate this question, we used intraocular injections of cholera toxin B to retrogradely label the cell bodies in the brain which project to the eye. The labeled cell bodies in the brain were visualized using an antibody directed against cholera toxin B. Approximately 40 centrifugal cell bodies were found on each side of the brain. The locations of these neurons were not confined to specific nuclei within the brain. The majority of cell bodies were concentrated in the nucleus of isthmi parvocellularis. This nucleus is homologous to the isthmo-optic nucleus in birds, which also gives rise to centrifugal fibers to the retina. Additional cells were observed in the nucleus reticularis isthmi, the lateral lemniscus, and in the lateral wing of the dorsal raphe nucleus in the reticular formation of the mesencephalon. The localization of these cell bodies within the brain will provide the basis for future studies of their role in visual processing. This research supported by EY04785 to WDE.

432.12

THE MORPHOLOGY OF PRESUMPTIVE CENTRIFUGAL TERMINALS IN RETINAE OF PIGEON (*Columba livia*) AND CHICK (*Gallus gallus*) . W. Woodson*, A. Brzozowska-Prechtl, K. Cox, and H.J. Karten, Dept. of Neurosciences, University of California, San Diego, 92093.

Woodson et. al. (Soc. Neurosci Abstr.('90) 16: 1313)reported three distinct morphological types of presumptive centrifugal terminals (pTs) in the pigeon retina. Subsequent investigations revealed that: (i) the isthmo-optic nucleus (ION) proper and the ectopic cells each give rise to different types of centrifugal pT endings; (ii) high density areas were observed in the inferior and mid-temporal to nasal retina and on both sides of the pecten; (iii) overlap of different types of centrifugal pTs was observed in all portions of the retina.

The distribution and morphology of centrifugal endings of 21 day old chicks were examined following injections of 1% cholera toxin B into ION and the surrounding tegmentum. As in pigeons, centrifugals are found mainly in the inferior retina. One type has a thin axon with small varicosities (~ 1 to 4 μm in diameter) arborizing over a wide area of the retina. The main differences between chicks and pigeons included: (i) the presence of a centrifugal fiber having a thick axon arborizing with one large and compact bulbous ending (~ 10 μm in diameter) consisting of 5-10 smaller varicosities (~ 1 μm in diameter); (ii) the presence of two types of arborizations in the contralateral retina, and only the thin widely arborizing type in the ipsilateral retina. The results suggest a transient developmental stage or a species difference in the morphology of centrifugal pT arborizations. Moreover, we suggest that the thin and widely arborizing ipsilateral centrifugal projections in the chick retina arise from the ectopic cell region.

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432.13

TECTAL VISUAL RESPONSES ARE MODULATED BY ISTHMIC NEURONS IN PIGEONS. Y.-C. Wang*, S.-R. Wang and B.J. Frost. Departments of Physiology and Psychology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Anatomical studies in pigeons have shown that both nucleus isthmi parvocellularis (Ipc) and nucleus isthmi magnocellularis (Imc) receive input from, and feedback to, the optic tectum. However, the functional effects of these neural circuits on the tectal cells are still unknown. The present study evaluates the roles of Ipc and Imc in modulating the visual response properties of tectal neurons. In Ketamine/Rompun anaesthetized pigeons, Ipc or Imc was reversibly blocked by lidocaine (2%, 5–9 nl) or excited by NMDA (50 mM, 5–9 nl) while stimulating tectal neurons (n=165) with moving visual stimuli. Single cell recordings showed that 80% of the tectal neurons were almost totally inhibited after injection of lidocaine into the region of Imc having receptive fields (RFs) overlapping the tectal RFs. However, 68% of the tectal neurons were unchanged when the same drug was applied to Ipc under the same conditions. In contrast, adminstration of NMDA into Ipc dramatically reduced the response of 71% of tectal cells, while NMDA applied to Imc had no effect. These feedback effects are tightly related to the topographic relation between RFs in nucleus isthmi (Ipc and Imc) and optic tectum. Removal of Imc–tectal feedback loop (lidocaine) or excitation of Ipc–tectal pathway (NMDA) significantly reduced the excitability of tectal neurons whose RFs were located within the isthmic RFs, otherwise, there was no effect. These results suggest that the subdivisions of nucleus isthmi (Ipc and Imc) play a differential feedback role in modulation of visual processing in the avian optic tectum. Supported by NSERC OGP0000353 and ISE0117027.

433.1

LOCAL MECHANISMS OF CORTICAL DIRECTION SELECTIVITY: GABA-INACTIVATION COMBINED WITH INJECTIONS OF [^{3}H]-NIPECOTIC ACID.
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Iontophoresis of GABA was used to inactivate iso-orientation sites or cross-orientation sites at a horizontal distance of 400–700μm from single cells recorded in cat area 18, and the effects on direction selectivity studied. During iso-orientation inactivation, 71% of 51 cells showed a significant (>0.2) change in the directionality index [DI: (response to preferred direction − response to nonpreferred direction)/(response to preferred direction − spontaneous activity)], with a mean change of 0.58. When the inactivation site showed strong direction bias (DI>0.5), the effects were predictable in the sense that remote inactivation caused a change in a cell's response to the direction of motion that was closest to the preferred direction at the inactivation site: *viz.* an increase or a decrease in response to the cell's preferred direction (due presumably to the loss of iso-orientation inhibition or excitation, respectively), or an increase in response to the nonpreferred direction. In the latter case, pressure injections of [^{3}H]-nipecotic acid were sometimes made at the recording site in order to retrogradely label inhibitory interneurones. The histological reconstructions revealed the presence of labelled cells in the vicinity of the inactivation site, thus demonstrating an anatomical substrate for the effect on direction selectivity in terms of the loss of inhibition between cells with opposite preferred directions. During cross-orientation inactivation, a significant change in DI was seen in only 18% of 68 cells, and was always accompanied by an increase in response to non-optimal orientations. These effects were probably due to the loss of a cross-orientation inhibitory input which contributes mainly to orientation tuning. Overall, iso-orientation inactivation had a significantly greater effect on direction selectivity than did cross-orientation inactivation (mean change in DI 0.43 and 0.14; P <0.001). The results thus emphasize the importance of local iso-orientation interactions in the generation of cortical direction selectivity, with cross-orientation inhibition playing a relatively small but significant role.

433.2

NEURAL CORRELATES OF THE MOTION AFTEREFFECT: THE TIME COURSE OF DIRECTION-SELECTIVE ADAPTATION.
D.E. Giaschi*, S.G. Marlin, R.M. Douglas and M.S. Cynader. Depart. of Ophthalmology, University of British Columbia, Vancouver, BC, Canada.

Following prolonged viewing of a moving pattern, a stationary pattern appears to drift in the opposite direction. This is the well-known motion aftereffect, and it probably results from adaptation of direction-selective neurons in the visual cortex. We used single-unit recording techniques to investigate the time course of direction-selective adaptation in simple and complex cells in the striate cortex of anesthetized cat. Adaptation to prolonged unidirectional visual stimulation and subsequent recovery of responsiveness were compared in the adapted and non-adapted directions for each neuron. Following adaptation in either the preferred or the non-preferred direction, all neurons were less responsive to motion in both the adapted and the non-adapted direction. For complex cells adapted in their preferred direction, the time course of adaptation was exponential with a fast time constant of 3s in both directions. The time course of recovery was also exponential with time constants of 6s and 9s in the adapted and non-adapted directions respectively. An overshoot of the response was observed in the non-adapted direction. Simple cells showed a similar adaptation and recovery in the non-adapted direction, but the change in responsiveness in the adapted direction was characterized by a fast (3s) initial drop followed by a slow (23s) gradual decline. Similarly, the recovery was characterized by a fast (6s) initial rise followed by a slow (200s) gradual increase in responsiveness. For adaptation in the non-preferred direction, there was less adaptation overall, and no overshoot of the response in the non-adapted direction during recovery. The time constants of adaptation and recovery were 5s and 3s respectively for both simple and complex cells and for adapted and non-adapted directions. Thus, there appears to be a fast general process underlying adaptation in cortical neurons. An additional slow direction-selective process is revealed when simple cells are adapted in the preferred direction. We suggest that this latter type of adaptation is a key feature underlying the perceptual motion aftereffect.

433.3

ADAPTATION OF POST-SYNAPTIC POTENTIALS (PSPs) IN RAT VISUAL CORTEX,
P. G. Finlayson*, S. Marlin and M. Cynader, Dept. Ophthal., Univ. of British Columbia, Vancouver, B.C. V5Z-3N9.

Adaptation to repeated patterned electrical stimulation was recorded intracellularly in cortical tissue slices. PSPs, often monosynaptic, were evoked by short trains (3-5 stimuli at 100 Hz) of electrical stimuli to the slice. Control and recovery responses were to stimulus trains separated by 10s intervals. All neurons recorded exhibited an exponential decrease (tau: 6 - 30s) in PSP amplitude and area with stimulus trains presented at short (0.3s) intervals for 30 to 120 s. The recovery of adapted responses was exponential (tau:10s - 8 min). Cortical cells recorded extracellularly *in vivo* show similar time constants of adaptation with moving stripes. Cells initially depolarized 5-10 mV at the start of adaptation, and repolarized often to or below resting potentials. Input resistance initially decreased with the membrane depolarization and increased as the size of the PSPs decreased and the membrane repolarized. Input resistance often continued to increase during the recovery period. These effects were also observed with KCl recording electrodes. Adaptation of PSPs was observed whether stimuli evoked spikes or only PSPs. Adaptation was not affected by hyperpolarizing or depolarizing the cell. Repetitively activating action potentials in the cell with intracellular current pulses did not result in adaptation of PSPs. Adaptation in cortical cells is not dependent on cell firing or due to $GABA_A$ (Cl-dependent) inhibition, or spike adaptation. These results show that the most likely mechanisms of adaptation in cortical cells are presynaptic, involving changes in transmitter release. Post-synaptic post-adaptation changes may also occur.

433.4

RESPONSES OF SIMPLE CELLS IN CAT'S AREA 17 TO MOVING TEXTURE PATTERNS: COMPARISON WITH COMPLEX CELLS.
C. Casanova*. Dept. of Surgery-Ophthalmology and Dept. of Physiology and Biophysics, Fac. of Medicine, University of Sherbrooke, Canada, J1H 5N4.

There is still some controversy surrounding the responsiveness of simple cells in the cat's striate cortex to moving random dot patterns. Initial reports of Hammond and colleagues (e.g Hammond and McKay, Exp. Brain Res. 1977) indicated that all complex cells respond vigorously to visual noise while simple cells are virtually unresponsive to the same stimulus. This clear distinction between cells has been challenged in subsequent studies (e.g. Skottun et al., J. Neurophysiol. 1988) which reported that the noise could also drive simple cells. The present study re-examined this issue.

Experiments were performed with anesthetized and paralyzed normal adult cats. Receptive fields in area 17 were first characterized with drifting sine-wave gratings. Under computer control, the direction of a random texture field (Julesz's type, 256x256 of density) was varied over 360° in 12 steps. Each condition was randomly interleaved. The 2D Fourier power spectrum showed that all frequencies have the same expected amount of power.

We recorded from 55 complex cells. 32 units responded vigorously to moving visual noise with sustained discharges (mean direction bandwidth of 39 deg). A few cells (11) could simply not be activated by the pattern. Out of the 38 simple cells recorded, a total of 18 responded well to the motion of the texture. Most responses were not as sustained as those of complex cells, nor as robust. Rather, they consisted of multiple peaks while responses of a few cells showed some periodicity. These results support the notion that the two types of cells in area 17 respond to moving texture patterns.

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433.5

RELATION OF LINEAR SPATIAL AND TEMPORAL PROPERTIES TO DIRECTION SELECTIVITY IN VISUAL CORTEX NEURONS.
C.L.Baker (Jr.)* McGill Vision Research Centre, Montreal, PQ Canada H3A 1A1.

The "reverse correlation" method was used to analyze responses of simple cells in cat visual cortex (A17 and A18) to ternary white noise stimuli (Emerson *et al*, J.Neurophysiol. 58: 33, 1987). To insure adequate spatial resolution, stimuli were scaled in relation to the period of a given neuron's optimal spatial frequency.

In contour plots of first-order spatiotemporal correlograms, positive and negative "islands" were clearly resolved, corresponding to classical ON and OFF-responding zones spatially and to biphasic responses temporally. Typically there were prominent asymmetries in the degree to which adjacent zones were biphasic or monophasic (ie, transient or sustained). In some cases these regions were weakly spatiotemporally oriented, especially at reduced spatial resolution.

Power spectra derived from these correlograms were narrowband for spatial and temporal frequency, and directionally biased. The magnitude and direction of this bias were in general poor predictors of measured direction selectivity to drifting sinewave gratings, suggesting an important role of nonlinear mechanisms.

Supported by Canadian MRC grant (MA-9685).

433.6

ILLUSORY SHIFTS OF THE FOCUS OF EXPANSION IN OPTIC FLOW FIELDS.
C. J. Duffy* and R. H. Wurtz. Lab. of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

While moving through the visual environment, we see an optic flow field having a radial pattern of motion emanating from a focus of expansion (FOE). When we make a smooth pursuit eye movement during self-movement, reafferent planar motion from eye rotation is combined with the radial motion to displace our retinal image of the FOE. This complicates the task of interpreting optic flow field information about our direction of self-movement.

We studied human perception of optic flow fields by asking 14 observers to indicate the location of FOE in computer generated simulations of optic flow fields projected onto a tangent screen covering 100° X 100° of their central visual field. All of our subjects accurately judged the position of FOE displaced along the horizontal meridian. When we used an artificial optic flow stimulus in which we overlapped radial motion having a centered FOE with horizontal planar motion, our observers experienced an illusory shift of the FOE. The illusory shift of the FOE was always in the direction of the overlapping planar motion; the magnitude of the shift increased with increasing planar motion speed, and decreased with increasing radial motion speed. The illusory shift of the FOE persisted when the observer was free to make eye movements, required to fixate a stationary target, or required to pursue a moving target.

We suggest that the illusory shift of the FOE occurs because the planar motion is interpreted as a reafferent signal indicating a smooth pursuit eye movement. This signal might trigger an attempt to compensate for the expected effects of eye movements on the retinal image of the optic flow field by shifting the perceived FOE. This is consistent with a two stage, parallel processing model of optic flow field analysis, and with the response properties of neurons found in the medial superior temporal area (MST) of monkey cerebral cortex.

433.7

OCULOMOTOR RESPONSES TO PERCEPTUALLY COHERENT AND NON-COHERENT PLAIDS. KR Dobkins*, GR Stoner and TD Albright, The Salk Institute, La Jolla, CA 92037.

When two moving gratings are superimposed, they can be perceived as either a single coherently moving "plaid pattern" or as two component gratings sliding non-coherently across one another. Since visual motion signals are known to exert a strong influence over oculomotor activity, we sought to determine whether there is correspondence between involuntary eye movements elicited by moving plaids and judgments of motion coherency. Specifically, we asked whether direction and speed of eye motion in human subjects were independent of perceptual coherence or, alternatively, approximated component motion when non-coherence was reported and pattern motion when coherence was reported.

Plaid patterns were constructed using two heterochromatic (red/green) sinusoidal gratings oriented 67.5° apart. Motion of the plaid was produced by moving only *one* of the two gratings. Unlike conventional plaids constructed from two moving components, coherent and non-coherent percepts elicited by these novel plaids are each associated with a single direction of motion. Non-coherence is characterized by perceived motion in the direction of the single moving component. By contrast, coherence is associated with perceived motion along the axis of the stationary component. Plaid stimuli were 22° diameter and positioned at the center of gaze.

We used color asymmetries to manipulate perceptual coherence of moving plaid patterns and we recorded accompanying oculomotor activity. Individual gratings within the plaid contained either + (red brighter) or - (green brighter) luminance contrast. When the two heterochromatic gratings contained "symmetric" (+/+ or -/-) luminance contrasts, coherent motion was perceived. When the gratings contained "asymmetric" (+/-) luminance contrasts, non-coherent motion was perceived.

When the plaids were perceived as moving coherently, eye movements were relatively fast and closer to the coherent direction. Conversely, when non-coherent motion was perceived, eye movements were relatively slow and closer to the component direction. These results suggest that the neural mechanisms underlying perceptual motion coherency also participate in the generation of eye movements.

433.8

AREA MST REPRESENTS ABSTRACT FEATURES OF MOTION FOR A BROAD RANGE OF STIMULI. B. J. Geesaman and R. A. Andersen*. Department of Brain & Cognitive Sciences, MIT, MA 02139.

It has been suggested that area MST of the macaque monkey has a role in processing optical flow information relevant to determining direction of observer heading. It may, as well, be important in the perception of object motion. We are interested in determining the diversity of stimulus types over which these cells can extract information about motion; e.g. expansion, contraction, rotation, etc.

To test the response of MST cells to different motion patterns, we recorded from 50 cells in the awake behaving monkey. We probed the receptive fields of these cells with different classes of stimuli and constructed tuning curves for each class based on the responses. Each class contained either 20 degree or 40 degree stimuli that covered 8 equally distributed directions in "spiral space". Spiral space is defined such that 0 degrees in this space represents a stimulus with a velocity vector field consistent with pure expansion, 180 degrees is pure contraction, 90 degrees is counter-clockwise rotation, and 270 degrees is clockwise rotation. This is a continuous space; therefore, a stimulus lying between these cardinal orientations would have elements of two types of motion and perceptually appear as a spiral. The different classes of stimuli included moving random dot patterns (44 out of 50 cells responded), a single solid square (46/50 cells), an outline of a square (8/9 cells), and a square aperture moving relative to an underlying stationary pattern of equally spaced diagonal lines (6/8 cells). Interestingly, although the magnitude of response often varied between classes, for those cells that responded strongly to different classes of stimuli, the orientation and shape of the tuning curves were similar for a given cell. Thus, many cells ignored the differences in spatial structure between the stimuli and were selective for the type of motion. In conclusion, MST is able to extract information about motion type for a range of stimuli, making it an appropriate candidate for analysis of object motion, as well as motion introduced by observer translation.

433.9

LESIONS OF LATERAL SUPRASYLVIAN CORTEX IN THE CAT REVEAL DEFICITS IN THE PERCEPTION OF GLOBAL MOTION. K. K. Rudolph* and T. Pasternak, Center for Visual Science and Department of Neurobiology and Anatomy, University of Rochester, Rochester, NY 14627.

High incidence of directional selectivity and large receptive fields make neurons in the lateral suprasylvian (LS) cortex of the cat well-suited for the analysis of visual motion. We examined the role of LS cortex in the integration of local motion signals into a global percept. We placed bilateral ibotenic acid lesions in LS cortex and used dynamic random-dot targets to test the discrimination of motion direction. The targets consisted of dots that were displaced with a constant step size in directions chosen at random from a specified distribution. When the range of the distribution of such displays is limited to about 300 deg, the entire display appears to move in the direction of the mean.

LS lesions produced deficits in the discrimination of opposite directions for large (1 deg) but not small (0.15 deg) step sizes. The spatial limit for the task (Dmax) was also substantially decreased. These results suggest that there is a change in spatial scale of residual motion mechanisms in the absence of LS cortex. Postoperative motion thresholds were also affected by the addition of dots moving in random directions (directional noise), even at small step sizes. Thus, LS lesions affected the integration of local motion signals in the presence of directional noise. Finally, cats with LS lesions showed a two-fold loss in accuracy in discrimination of small direction differences.

These results provide further evidence for the role of LS cortex in processing visual motion. However, observed deficits were relatively limited: cats with LS lesions were still able to perform tasks requiring integration of local motion signals. Thus, it is likely that the mechanisms underlying these discriminations are not limited to LS neurons. Rather, LS cortex may be only one component of a larger motion network. It is of interest that the effects of LS lesions bear striking similarity to those recently reported after MT/MST lesions in the monkey (Pasternak et al., Neurosci. Abstr. 17, 1991).

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433.10

A MATHEMATICAL MODEL FOR THE SELF-ORGANIZATION OF DIRECTION COLUMNS IN AREA MT. S. Tanaka,* H. Shinbata and M. Miyashita. Fundamental Res. Labs. NEC, Tsukuba, Ibaraki 305, Japan.

Computer simulations were conducted to study which neural connections give rise to modular structures in the middle temporal area (MT) of monkeys. These simulations were based on our thermodynamic model for the activity-dependent self-organization of neural networks. All presynaptic cells regarded as V1 and/or V2 cells were assumed to be direction-selective. Direction selectivity of MT cells in response to moving visual stimuli and the related modular structures in MT were successfully reproduced. The simulated results showed that singular lines with respect to preferred directions of afferent fiber terminals emerge in the postsynaptic layer. The topological arrangement of preferred directions of afferent inputs was found to determine the direction selectivity of postsynaptic cells. That is, pan-directional cells appeared at the ends of the singular lines, while bi-directional cells were along the length of the lines, although all presynaptic cells were assumed to be direction-selective. Both the frequency distribution of changes in the preferred direction between adjacent cells and the distribution according to the value of the directionality index closely agreed with those observed in physiological experiments. In the absence of intracortical inhibition, all postsynaptic cells tended to be pan-directional cells. This suggests that intracortical inhibition in area MT as well as inputs from directional cells in area V1 and/or V2 are necessary for the generation of the direction selectivity characteristic of MT.

433.11

MOTION DETECTION AND IDENTIFICATION FOR LUMINANCE AND CHROMATIC STIMULI

Karl R. Gegenfurtner* (1), Michael J. Hawken and Daniel C. Kiper(1)
Howard Hughes Medical Institute (1), Center for Neural Science, New York University, New York 10003.

We have measured thresholds for detection and identification of a vertically oriented sinewave grating vignetted by a two-dimensional spatial Gaussian window. The stimulus appeared as a contrast-reversing grating to the left or to the right of fixation on the horizontal meridian and then moved either towards or away from the fovea. The temporal frequency of the contrast reversing and drifting gratings were identical. The color of the grating was modulated along different directions in color space around an equal energy white, thus leading to different input contrasts for the L- and M- cones. This allowed us to measure complete detection and identification contours in cone contrast space (Stromeyer, Eskew, Ryu & Kronauer, ARVO 1991).

We find that contrast sensitivity is particularly impaired for stimuli having no L-cone contrast. When considering the mechanisms that mediate motion detection and identification, we find that under certain spatio-temporal conditions contrast sensitivity is determined by a mechanism primarily stimulated by the L-cones.

These results are compared to behavioral and physiological data in macaque monkeys.

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433.12

SPATIAL SUMMATION OF MOVEMENT AFTEREFFECTS AND ITS MODIFICATION BY EYE PURSUIT MOVEMENTS

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Dept. Comp. Physiology, Rijksuniversiteit Utrecht, The Netherlands, and Dept. Physiology, Freie Universität, Berlin 33, Germany.

A random dot pattern (RDP), generated on a computer screen and moving across the foveal and parafoveal retina (stimulation field 6-8 degrees, 30 s stimulation period), evokes a movement aftereffect (MAE) in the direction opposite to the RDP movement direction for both viewing condition: (a) fixation and (b) eye pursuit movements (head fixed, 0.25 - 8.0 deg/s).

When simultaneously two RDPs ("transparencies") are moved perpendicularly to each other across the stimulation field, two moving patterns are seen, but only one rather uniform MAE is evoked (Verstraaten et al. ,1990). Its direction D is in between the MAE directions expected for each of the two moving RDPs. D depends on the angular velocities of the two RDPs and on the stimulus conditions (a) or (b). A sensitivity-weighted vector summation predicts the outcome for stimulus condition (a). When, however, with stimulus condition (b) one of the moving RDPs is pursued, D is determined by "retinal" stimulation and by efference copy mechanisms.

It is concluded that the "weighted vector summation" of the MAE may be attributed to cortical areas (MST, FST ?), where gaze movement signals interact with retinal movement signals. Thus MAE depends on the "perceived visual movement". Only with the restricted stimulus condition (a) MAE depends merely on the activation of the retinotopically organized parts of the visual system.(O.-J.G. was supported by a F.C.Donders-professorship, University of Utrecht).

434.1

ORGANIZATION OF GABAERGIC NEURONS AND AXON TERMINALS IN CAT PRIMARY AUDITORY CORTEX (AI). J. J. Prieto* and J. A. Winer. Division of Neurobiology, Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-2097.

We (i) identified the GABAergic cell types, (ii) counted the proportion of GABAergic neurons in AI, and (iii) analyzed the density of GABAergic terminals in the neuropil. The neurons were studied in adult animals in 25 µm-thick sections. The number and proportion of GABAergic cells were determined in 1 µm-thick plastic sections, counterstained with toluidine blue, in 500 µm-wide samples at systematic intervals.

The proportion of GABAergic neurons (24.6%) resembled the values in other neocortical areas; the laminar differences were also similar. However, GABAergic neurons were not homogeneously distributed, and changed along the dorsoventral axis: more lay in the central part of AI, where physiological studies find the most sharply tuned neurons.

The types of GABAergic cells include: horizontal cells (layers I and VI), extraverted multipolar cells (layer II), bitufted cells (layers II-IV), small (layers I-VI), medium-sized (layers I-VI), and large (layers II-VI) multipolar neurons, bipolar cells (layers II-VI), inverted pyramidal cells (layers V-VI), and large ovoid neurons (layer VI).

There were more puncta in the neuropil in the supragranular than in the infragranular layers, especially in layer Ia. They were variable in size, with the largest in layers III and IV, and the finest in layer I. Many of the biggest GABAergic puncta end near the somata and primary dendrites of immunopositive large multipolar neurons in layers II to VI; in layers II, V and VI these terminals are rare in the neuropil. Thus, a specific circuit for the axosomatic inhibition of these neurons may be independent of the pattern in the neuropil.

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434.2

EXPRESSION OF AMPA-SELECTIVE GLUTAMATE RECEPTOR SUBUNIT mRNAs IS RELATED TO SYNAPTIC POPULATIONS IN THE RAT COCHLEAR NUCLEUS. C. Hunter*, T. Yu, and R. J. Wenthold. Lab. of Neurochemistry, NIDCD, NIH, Bethesda, MD 20892.

We have previously used *in situ* hybridization histochemistry to localize AMPA-selective glutamate receptor (GluR) subunit mRNAs in morphologically defined cell types in the rat cochlear nucleus. Selective antisense oligonucleotides to GluR1-4, 45 nucleotides in length (see Keinanen et al., Science 249:556-560, 1990) and their sense controls were hybridized to tissue sections and the sections processed for autoradiography. Three main patterns of GluR subunit expression were identified with antisense probes: All four GluR subunits were expressed in cartwheel/stellate neurons of the dorsal cochlear nucleus, which receive excitatory input from cochlear nucleus granule cells; GluR2, 3, and 4 subunits were expressed in globular, spherical, fusiform and round cell types, which receive excitatory input from the ipsilateral auditory nerve; GluR2 and 4 subunits were expressed in granule cells, with, as yet, unidentified excitatory inputs. Quantitative analyses in addition, showed that the level of GluR3 expression, as measured by grain density, is heterogeneous within one or two cell types. Thus GluR1-4 expression is likely related to the presynaptic input to these cells.

We now further examine the role of presynaptic input on GluR1-4 subunit expression in neurons: 1) following 7-day and 21-day lesions of the auditory nerve in the adult rat, eliminating ipsilateral, excitatory input to the cochlear nucleus and 2) during postnatal development during the period of synaptogenesis in the cochlear nucleus.

434.3

SALICYLATE INDUCED SHIFTS IN INTENSITY THRESHOLDS AT VARYING FREQUENCIES IN ADOLESCENT RATS. J.F. Brennan[1]*, C.A. Brown[1] and P.J. Jastreboff[2]. [1]Dept Psychology, Univ of Massachusetts/Boston, MA 02125 and [2]Dept Surgery, Univ of Maryland Sch Med., Baltimore, MD 21201.

25 day old pigmented rats were trained in a 2-way shuttle box to avoid a footshock signalled by 5 s tonal presentations of 10 kHz, 62 dBC. Reliable avoidance levels were obtained by the end of the 2nd training day of 150 trials. Changes in auditory intensity thresholds were tested at 23 different frequencies (1-16 kHz) that were presented in counterbalanced order across testing days and subjects. 2 hrs prior to each of 5 successive extinction sessions, groups of 6 pups received either saline injections or salicylate injections of either 200- or 300-mg/kg. For each frequency series initial probes began at 62 dB and decreased in 5 dB units until an error (i.e., response latency greater than 10 s) was recorded, at which time the series increased in 2.5 dB units until a correct response (i.e., latency less than 10 s), followed by a decrease in units of 1 dB. Salicylate injections induced threshold increases at a broad range of frequencies, and were not as selective as induced threshold deficits reported in adults. (NIH DC00299).

434.4

A DUAL SYNAPTIC SYSTEM REVEALED IN RAT AUDITORY THALAMUS IN VITRO. B. HU* *Loeb Research Institute, Ottawa Civic Hospital, Ottawa, Ontario, Canada. K1Y 4E9*

The ventral (MGv) and dorsal (MGd) divisions of the rat medial geniculate body (MGB) represent the "principal" and "association" thalamic auditory systems respectively. They transmit the auditory signals arising from the inferior colliculus (IC) to the cortex in a parallel manner. The present study examined the synaptic properties of the two systems in response to IC input. Thalamic explants retaining the entire lateral surface of the MGB and IC brachium (ICB) were continuously superfused via artificial CSF at 32–34°C. Single current pulse was used to stimulate ICB and the evoked responses were recorded from MGv (n=75) and MGd (n=98) regions. Extracellularly, most MGv cells responded with a single spike at a short, consistent latency (9.4±4.3 ms). In contrast, MGd neurons responded primarily with a brief high-frequency burst (10–27 ms; 300–420 Hz), whose latency shifts from 8 to 94 ms depending upon the intensity of input. The MGd bursting response was reversibly blocked by bath application of nickel (100–250 µM) or APV (50 µM) but was unaffected by bicuculline (100 µM) or phaclofen (60 µM). The evoked responses in both regions were totally and reversibly blocked by TTX and by co-application of DNQX (30 µM) and APV (50 µM). Intracellular recordings showed that the ICB-evoked bursting responses in MGd, but not in MGv neurons were mediated by a low-threshold spike (LTS), whose activation latency shifts widely (12 to 100 ms). Failure of LTS revealed (up to 100 ms) an underneath slow EPSP, which could be antagonized by APV and ketamine in a dose-dependent manner. These data suggest that distinct synaptic signalling processes occur in MGv and MGd systems. Supported by OMHF and MRC.

434.5

HOMOGENEOUS DISTRIBUTION OF GABA NEURONS ACROSS CAT PRIMARY AUDITORY CORTEX. S. H. C. Hendry* Dept. of Anatomy & Neurobiology, University of California, Irvine, CA 92717.

The primary auditory area (AI) of cat cerebral cortex contains a map of the cochlea, with each sound frequency represented as a band of dorsoventrally oriented cells (isofrequency bands). In cat AI, callosal afferents are unevenly distributed and terminate in broad anteroposteriorly oriented zones that intersect isofrequency bands. These callosally innervated zones are physiologically distinct from acallosal zones in ways suggestive of inhibitory influences that are greater within the latter. To determine if the physiological differences are correlated with differences in elements intrinsic to AI, GABA immunoreactive neurons were examined for evidence of an uneven distribution of inhibitory interneurons across AI. Stereological methods were applied to determine the numerical density and proportion of GABA immunoreactive somata in 1 µm-thick sections through 5 hemispheres of 3 normal cats. Mean values of 21,400-22,800 GABA neurons underlying 1 mm^2 of cortex, which make up 20.4-21.8% of the total neuronal population was calculated for AI. Values for individual layers varied with the greatest density of GABA neurons in layers III and IV and greatest proportion in layer I. Those values for AI, in general, and for individual layers did not vary significantly either dorsoventrally across AI (parallel to isofrequency bands) or anteroposteriorly (perpendicular to the bands). These data suggest that regional variations in the total population of GABA neurons do not exist across AI and, thus, cannot account for physiological differences in this area. Evidence for variations in GABA neuronal subpopulations is currently under investigation. Supported by DC 00450.

434.6

UNILATERAL KAINIC ACID LESIONS OF THE LATERAL LEMNISCUS: EFFECTS ON INTERAURAL INTENSITY DIFFERENCE FUNCTIONS RECORDED FROM SINGLE NEURONS IN INFERIOR COLLICULUS. L. Li* and J.B. Kelly. Laboratory of Sensory Neuroscience, Psychology Department, Carleton University, Ottawa, Ontario K1S 5B6.

Recent anatomical and physiological data suggest that the dorsal nucleus of the lateral lemniscus (DNLL) plays a role in binaural processing in the auditory system. The neurons in DNLL are largely GABAergic and give rise to substantial projections to the contralateral DNLL and inferior colliculus (Shneiderman, Oliver and Henkel, 1988). Kainic acid lesions of DNLL alter evoked responses recorded from the rat's auditory cortex by reducing the extent of binaural suppression in the hemisphere contralateral to the lesion site (Glenn and Kelly, 1991). The present study was undertaken to determine the effects of kainic acid lesions of the lateral lemniscus on single neuron responses recorded from the central nucleus of the inferior colliculus of the rat. Lesions were made by local injection of kainic acid into the lateral lemniscus through a glass pipette. After a recovery period of at least two weeks, recordings were made from the inferior colliculus with glass microelectrodes. Tone pulses were delivered separately to the two ears through a sealed sound system. Results show that unilateral destruction of the intermediate nucleus of the lateral lemniscus has little or no effect on interaural intensity difference functions recorded from ipsilateral or contralateral inferior colliculus. After unilateral DNLL lesions, binaural responses were still present in both left and right inferior colliculus, but there was a tendency toward a reduced slope of interaural intensity difference functions contralateral to the lesion. (Supported by NSERC.)

434.7

LONG-TERM EFFECTS OF COCHLEAR AND MIDDLE EAR LESIONS ON SYNAPTIC TRANSMITTER RELEASE IN THE COCHLEAR NUCLEUS. C.G. Benson* and S.J. Potashner. Department of Anatomy, University of Connecticut Health Center, Farmington, CT, 06030.

To determine if cochlear activity affects the regulation of synaptic function in the cochlear nucleus (CN), we measured the effects of unilateral cochlear ablation and unilateral ossicular disarticulation on the activity of glutamatergic or aspartatergic and glycinergic synaptic endings in the CN of adult guinea pigs. Guinea pigs were anesthetized and either the left cochlea was destroyed mechanically or the left middle ear ossicles were disarticulated. Two days, 8 weeks or 16 weeks after surgery, the uptake and release of ^{3}H-D-aspartate (^{3}H-D-ASP) and ^{14}C-glycine were measured bilaterally in dissected segments of the CN, in vitro. Two days and 8 weeks after cochlear ablation, ^{3}H-D-ASP uptake and release were reduced ipsilaterally in all segments. However, by 16 weeks after the lesion these measures had recovered and were no longer significantly different from controls. There were no significant changes in the uptake and release of ^{14}C-glycine in the CN after cochlear ablation. Sixteen weeks after unilateral ossicular disarticulation there were significant decreases in the uptake and release of ^{3}H-D-ASP bilaterally in the CN. These findings suggest that chronic changes in cochlear activity can lead to changes in synaptic release in the CN. (Supported by DC00199 from NIH NIDCD).

434.8

RELEASE OF D-ASPARTATE, GABA, AND GLYCINE FROM GUINEA PIG BRAIN STEM AUDITORY NUCLEI. S.K. Suneja, J. Gross, C.G. Benson and S.J. Potashner*. Dept. of Anatomy, University of Connecticut Health Center, Farmington, CT, 06030.

Evidence suggests that glutamate, aspartate, GABA, and glycine may be transmitters in the cochlear nucleus. In this study, we measured the release of ^{3}H-D-aspartate, ^{14}C-GABA and ^{14}C-glycine from several other brain stem auditory nuclei to determine if they too might contain synaptic endings that use these amino acids as transmitters. After excision, the brain stem was cut transversely into 500 μm sections from which auditory nuclei were punched. The accuracy of punching was confirmed by histological analysis. Punches were incubated with ^{3}H-D-aspartate and ^{14}C-GABA or ^{14}C-glycine before the release of radioactivity was assessed in a superfusion system. Electrical stimulation evoked the Ca^{2+}-dependent release of D-aspartate from each punched nucleus, namely the LSO, MSO, MNTB, VNLL, and the central nucleus of the IC (ICc). Ca^{2+}-dependent release of GABA was observed from the MNTB, VNLL, and ICc while release of glycine was observed from the LSO and MSO. These findings are consistent with the hypothesis that these nuclei contain glutamatergic or aspartatergic synaptic endings. They also suggest that some of these nuclei may contain GABAergic and glycinergic synaptic endings. (Supported by DC00199 from NIH-NIDCD)

434.9

PLASTICITY OF CALBINDIN IMMUNOREACTIVITY IN THE MATURE GERBIL SUPERIOR OLIVARY COMPLEX WITH ALTERED AUDITORY EXPERIENCE M.D. McGinn[1]*, I.R. Schwartz[2], and P.R. Eager[2] [1]Dept. of Otolaryngology, UC-Davis, Davis, CA 95616 and [2]Sect. of Otolaryngology, Yale University School of Medicine, New Haven, CT 06510 U.S.A.

Calbindin D-28k immunoreactivity (CaBP+) showed consistent and distinctly different patterns in the MNTB and LSO which varied with auditory experience. Animals were colony reared(COL), exposed to 3 wks of intermittent (30 min on/off) high (HF)(80dB) or low frequency (LF)(74dB) noise, or to low sound (LIG)(ear canal ligation).

The MNTB showed the most intense CaBP+ cells in all conditions. HF animals showed the largest number and most intense CaBP+ cells and the fewest light CaBP+ cells. LF animals had a greater proportion of light CaBP+ cells; COL animals were similar. LIG animals showed the highest proportion of light CaBP+ cells.

The LSO showed the greatest variation with the different treatment conditions. HF animals showed a densely stained neuropil in the medial limb. Moderately stained cells were found throughout, but some cells in the medial limb were darker. LF animals showed many moderately stained cells in the lateral limb, but fewer in the medial limb. Medial limb CaBP+ cells stained more darkly, but less intensely than in HF animals. The medial limb neuropil stained more densely than the lateral limb, but less darkly than in HF animals. LIG animals showed many lightly stained cells throughout the LSO in a uniformly stained neuropil comparable in intensity to that in the medial limb of LF animals.

(Supported by NIH/NIDCD grants DC00132 and DC00057).

434.10

PLASTICITY OF CALCIUM BINDING PROTEIN AND GABA IMMUNOREACTIVITY IN THE ADULT GERBIL COCHLEAR NUCLEUS WITH ALTERED AUDITORY EXPERIENCE I.R. Schwartz[1]*, P.R. Eager[1] and M.D. McGinn[2], [1]Sect. of Otolaryngology, Yale University School of Medicine, New Haven, CT 06510 and [2]Dept. of Otolaryngology, UC-Davis, Davis, CA 95616 U.S.A.

Calbindin D-28k (CaBP+), parvalbumin (PV+) and GABA (GABA+) immunoreactivity showed patterns in the cochlear nucleus (CN) which varied with auditory experience. Animals were colony reared (COL), exposed to 3 weeks of intermittent (30 min on/off) high (HF)(80dB) or low frequency (LF)(74dB) noise, or to low sound (LIG)(ear canal ligation). LF animals showed large numbers of intensely stained GABA+ puncta in the DCN and PVCN which were not seen in HF, LIG or COL animals. The intensity and number of CaBP+ dendrites in the DCN molecular layer decreased from LIG to HF to LF animals. COL & LF animals were similar. In octopus cell regions the darkest CaBP+ somata were in HF. The greatest extent of dendritic staining was in LIG animals which also showed the greatest number and size of CaBP+ puncta. PV+ somata in the DCN molecular layer decreased in intensity from HF, to LF to LIG. Changes in CaBP+ and PV+ in the CN were less marked than the changes observed elsewhere in the auditory brainstem. In the superior colliculus LF animals showed an almost complete absence of PV+ stained cells and dendrites compared to HF animals.

The observations are consistent with the hypothesis that a strongly driven auditory system protects neurons from overstimulation by an upregulation of inhibitory inputs.

(Supported by NIH/NIDCD grants DC00132 and DC00057).

434.11

QUANTITATIVE EVIDENCE THAT COCHLEAR ROOT NEURONS ARE NOT CHOLINERGIC. W.Yao and D.A.Godfrey*. Dept. of Otolaryngology, Med. Col. of Ohio, Toledo, OH 43699.

Cochlear root neurons (CRN) are distinct morphologically from globular bushy cells in rodent auditory nerve root (Merchan et al, 1988). CRN do not immunoreact for glycine or GABA (Osen et al, 1991). Our preparations have shown immunoreactivity of CRN for choline aceytltransferase (ChAT) which, despite their very weak histochemical reaction for acetylcholinesterase, suggests that they might be cholinergic. To provide evidence concerning this, we used a radiometric assay to measure ChAT activities (μmol/kg/min) both of samples containing CRN and of adjacent samples not containing CRN from 5 rats. Mean ($\pm$SD)(# samples) ChAT activity of CRN-containing samples was $35 \pm 20(9)$, while that of the non-CRN samples was $31 \pm 18(13)$. By measuring proportions of the samples occupied by CRN cytoplasm, we estimated the ChAT activity of CRN to be $229 \pm 406(9)$. By contrast, the ChAT activity for samples of the facial nucleus, which contains cholinergic motoneurons, was $4823 \pm 1184(5)$, and that for samples of the facial motor root was $6074 \pm 1397(4)$. Thus, CRN are not likely to be cholinergic. However, the presence of ChAT activity in some samples suggests that they might receive some cholinergic innervation. (Supported by NIH grant DC00172)

434.12

EFFECTS OF CHOLINERGIC AGONISTS AND ANTAGONISTS ON SPONTANEOUS ACTIVITY OF RAT DORSAL COCHLEAR NUCLEUS NEURONS: IN VITRO STUDIES. K. Chen[1], H. J. Waller[2]* and D. A. Godfrey[1], Depts. of Otolaryngology[1] and Neurological Surgery[2], Medical College of Ohio, Toledo, OH 43699.

Extracellular recordings from rat brain stem slices tested the effects of bath application of cholinergic agonists and antagonists on cochlear nucleus neuronal activity. Recordings were made from 54 neurons in 30 slices from 28 rats. Of neurons tested, 89% showed increased firing rates in response to 10 μM carbachol (mean increase = 249%, n=18), 63% showed increased firing to 10 μM muscarine (mean increase = 529%, n=35); and 50% showed increased firing to 200 μM nicotine (mean increase =103%, n=12). Only 3% and 25% of the neurons showed decreased firing to muscarine and nicotine, respectively. The excitatory effect of carbachol was eliminated by atropine (1 μM, n=3), but not by d-tubocurarine (2 μM, n=2). Excitatory effects of muscarine were blocked by 1 μM atropine (n=6), but not by selective antagonists of muscarinic receptor subtypes: pirenzepine (1-5 μM, M_1 receptor, n=3), gallamine (10-20 μM, M_2 receptor, n=3) or p-fluorohexahydro-siladifenidol (1-2 μM, M_3 recepter, n=2). We also found that 40% of the neurons tested showed an excitatory response to 50-100 μM serotonin (mean increase = 190%, n=20); 60% were unaffected. (Supported by NIH grant DC00172).

435.1

CHARACTERIZATION OF PRINCIPAL NEURONS IN THE RAT MEDIAL NUCLEUS OF THE TRAPEZOID BODY BY INTRACELLULAR RECORDING AND LABELING. I. Sommer, K. Lingenhöhl*, and E. Friauf. Dept. Animal Physiology, Univ. Tübingen, 7400 Tübingen, Germany.

Neurons of the medial nucleus of the trapezoid body (MNTB) are classically thought to function as a relay station in the ascending auditory system, transmitting information from the contralateral cochlear nucleus to neurons in the lateral superior olive and thereby inhibiting those. We have analyzed the physiological and morphological characteristics of MNTB neurons by means of intracellular recordings *in vivo* and subsequent intracellular injection of HRP. MNTB neurons responded in a primary-like fashion to tone pulses presented to the contralateral ear. Their spontaneous activity was high (>100 Hz), indicating that they may massively inhibit their target neurons. HRP-injection identified that we recorded from principal MNTB neurons; those with higher best frequency were located more medially than those with lower best frequency, thus demonstrating the tonotopic organization of the MNTB (lateral-to-medial from low-to-high). The morphological analysis revealed a complex system of axon collaterals and projections to several ipsilateral brainstem nuclei. Each MNTB principal neuron projected to the LSO and the superior paraolivary nucleus (SPN). Axon collaterals and terminals in the lateral nucleus of the trapezoid body, the medial olivary nucleus, and the ventral and dorsal nucleus of the lateral lemniscus were also present, although not always. Axon terminals could be located perisomatically or in the neuropil. The location of these terminals within the LSO and the SPN correlated with the MNTB neurons' best frequency and indicates that the LSO and the SPN are likely to be tonotopically organized (both lateral-to-medial from low-to-high). The divergent projection patterns of MNTB principal neurons suggest that these neurons are likely to play a complex role both in the ascending and descending auditory system. Supported by the DFG (Fr 772/1-2).

435.2

AUDITORY CORTICOGENICULATE PROJECTIONS IN THE CAT: LAMINAR ORIGINS AND AREAL ORGANIZATION. D.T. Larue*, B.A. Peterson, and J.A. Winer. Division of Neurobiology, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-2097.

The corticothalamic system comprises a massive descending projection whose organization and functional role in sensory processing is obscure. We studied the auditory corticogeniculate cells of origin using retrograde tracing methods. Our goal was to define which cortical areas and which cell layers within them project to particular subdivisions of the auditory thalamus. Wheat germ agglutinin-horseradish peroxidase was injected stereotaxically into different subdivisions of the medial geniculate body in adult cats and processed with tetramethylbenzidine.

At least ten different cortical fields project onto the medial geniculate body. They include primary (AI) and secondary (AII) auditory cortex, anterior auditory field (AAF), suprasylvian fringe (SF), insular (Ins), temporal (Te), and perirhinal (Pr) cortex, and posterior ectosylvian (EP), posterior (P) and ventroposterior (VP) auditory fields. Each geniculate subdivision receives a projection from a unique constellation of these cortical areas. Injections in the ventral division labeled cells in AI, AAF, P, and VP, and since the injections also involved the ventrolateral part, which is considered part of the non-lemniscal pathway, labeling was also seen in SF, EP and AII. Injections of the dorsal and caudal dorsal divisions revealed heavy projections from AII, Te, ventral Ep (including area VP) and a lighter input from Ins. In contrast, a smaller injection in the suprageniculate nucleus labeled many neurons in SF, Ins, dorsal EP, and a smaller number in AII, distinguishing it from other parts of the dorsal division. Injections of the medial division produced robust cortical labeling in AAF,Te, and Pr, and lighter labeling in VP, AI and AII, while involvement of suprageniculate in these injections produced labeling in SF and Ins.

The cortical layers of origin typically had a single pattern: a continuous band of cells was labeled in layers VI and Vb, with a discontinuous line of large layer Va cells separated from the deeper Vb cells by a gap without labeling. An exception to this pattern occured in the perirhinal cortex, which was labeled in every case. Injections involving the medial division produced the three-layered pattern described above (layer VI, Vb, and Va). The projection to all other thalamic divisions involved layer VI exclusively.

These findings suggest that, like the thalamocortical projection system, the descending corticothalamic projection is segregated into parallel pathways involving both tonotopic and non-tonotopically organized regions, and that multiple cortical inputs converge onto individual thalamic nuclei. Supported by U.S.P.H.S. grant RO1 NS16832-13.

435.3

PARALLEL PATHWAYS IN THE AUDITORY FOREBRAIN: AFFERENT AND EFFERENT CONNECTIONS OF THE MEDIAL GENICULATE BODY. B.A. Peterson* and J.A. Winer. Division of Neurobiology, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-2097.

Studies of the auditory thalamocortical system suggest that more than one pathway ascends to the cortex. To study this question, we injected wheat germ agglutin-horseradish peroxidase into various parts of the cat auditory thalamus to determine how many patterns exist.

A different pattern of connections was defined for each subdivision of the auditory thalamus with the midbrain, cerebral cortex, and subcortical forebrain. Some findings are common to each case. Thus, (i) the thalamic reticular nucleus projects to all parts of the medial geniculate body; (ii) each thalamic injection labels terminals in a different set of cortical fields; (iii) the origins of corticothalamic projections are more widespread than are the thalamocortical terminals; and (iv) corticothalamic projections have multiple laminar origins.

Each division of the auditory thalamus has a unique set of connections. Thus, the ventral division projects to layers III and IV in primary auditory cortex (AI), and to the anterior, posterior and ventroposterior auditory fields. It receives input from the thalamic reticular nucleus and from one or more subregions of the inferior colliculus.

The medial division has a different pattern of connectivity. It projects to layer I in every auditory cortical field, to the perirhinal cortex, and upon the amygdala and striatum as well. It receives projections from the superior colliculus, the thalamic reticular nucleus, and parts of the inferior colliculus outside the central nucleus.

The suprageniculate nucleus projects to layers I, III, and IV of non-primary auditory fields, including the insular, suprasylvian fringe, and dorsal posterior ectosylvian areas. It projects also to layer I in perirhinal cortex and to the amygdala and striatum. Its afferents arise from the superior colliculus mainly, with only modest input from the inferior colliculus.

A different pattern of thalamic connections involves the caudal nucleus of the dorsal division, which projects heavily to AII and temporal field, and less so to posterior ectosylvian and insular fields. Thalamic axons terminate predominantly in layers III and IV, with light input to layer I in perirhinal cortex, and moderate input to the infragranular layers of the temporal cortex. The major brain stem inputs arise from the dorsal cortex and lateral nucleus of the inferior colliculus. Surprisingly, it did not project to the amygdala or striatum.

These findings suggest that each part of the medial geniculate body has a specific constellation of midbrain and forebrain connections that must be related to their functional organization.

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435.4

INTRINSIC CONNECTIONS OF NEURONS IN THE COCHLEAR NUCLEAR COMPLEX OF MICE IN SLICES. S. Zhang*, D. Oertel. Dept. of Neurophysiology, Univ. of Wisconsin, Madison, WI 53706.

Synaptic potentials, spontaneous and evoked by electrical or chemical stimuli at various sites of the cochlear nuclei, were recorded intracellularly from 41 biocytin-labeled neurons: 6 tuberculoventral, 5 giant, 8 cartwheel, 1 superficial stellate, and 21 fusiform cells. Neuronal connections were deduced from temporal patterns, maps of synaptic inputs and the morphology of neurons.

Cartwheel, superficial stellate, and fusiform cells received spontaneous EPSPs. These same cells responded to electrical and chemical stimulation of the surface of the ventral cochlear nucleus (VCN) with EPSPs. Shocks evoked slowly rising EPSPs after 7 msec. The EPSPs probably reflected granule cell input.

Tuberculoventral neurons were excited by shocks to the auditory nerve with early and later EPSPs that were often suprathreshold. Mapping of excitatory afferents suggested that T stellate cells of the VCN were the source of polysynaptic EPSPs. IPSPs were occasionally recorded; these could have arisen from D stellate or other tuberculoventral neurons.

Giant cells responded to shocks of the auditory nerve with a monosynaptic EPSP that was usually cut short by two sequential IPSPs. Chemical stimulation of the VCN evoked EPSPs and IPSPs from widespread regions. The IPSPs could have arisen from tuberculoventral cells; EPSPs from auditory nerve fibers and T stellate or granule cells.

Fusiform cells receive spontaneous, bursty, IPSPs that are blocked by strychnine. Spontaneous IPSPs are often preceded by slowly rising EPSPs suggesting that a common source, probably granule cells, drive fusiform cells and inhibitory interneurons. The latencies and bursting patterns of spontaneous and late driven IPSPs match firing patterns of cartwheel cells. Fusiform cells also have early IPSPs that may arise from tuberculoventral cells.

435.5

REPRESENTATION OF PINNA-PRODUCED SPECTRAL NOTCHES IN DORSAL COCHLEAR NUCLEUS (DCN). E.D. Young*, G.A. Spirou, and J.J. Rice, Center for Hearing Sciences, Johns Hopkins School of Medicine, Baltimore, MD 21205.

The pinnae produce sound localization cues by modifying the spectra of acoustic stimuli in a way that depends on sound-source direction. In cats, the pinnae produce prominent notches (spectral minima) in the sound at the eardrum at frequencies >8 kHz; these notches provide information about both stimulus azimuth and elevation. Here, we describe responses of DCN neurons to stimuli containing such notches. Extracellular single-unit recordings were made in decerebrate cats; the stimuli were broadband noise electronically filtered with previously-measured pinna transfer functions, so as to simulate free-field spectra at the eardrum. The stimuli were scaled in frequency so as to move the center frequency of notches relative to a unit's best frequency (BF). In some DCN units, the only effect of changing notch frequency is to elevate threshold when the notch is centered on BF. This response probably reflects loss of effective stimulus energy when the notch is centered over the unit's tuning curve plus some effect of lateral inhibition; it is the type of response seen in all ventral cochlear nucleus neurons. In some DCN type III and type IV units, a sharp excitation-inhibition-excitation sequence is seen as the notch is swept past the unit's BF. Such responses have not been seen to date in units with BFs<8 kHz; at these frequencies, pinna transfer functions do not contain notches. These results suggest a role for the DCN in processing of complex, biologically relevant spectral features of stimuli, including features related to sound localization. Supported by grants from NIDCD.

435.6

FUNCTIONAL ORGANIZATION OF ACOUSTIC AND VOCALIZATION AREAS OF THE BAT CENTRAL NERVOUS SYSTEM REVEALED BY HIGH RESOLUTION AUTORADIOGRAPHIC IMAGING OF ^{3}H-2-DEOXYGLUCOSE UPTAKE. G.E. Duncan*, W.E. Stumpf, and O.W. Henson. Dept. of Cell Biology and Anatomy, Univ. North Carolina, Chapel Hill, NC 27599

Brain activity patterns associated with the generation and processing of echolocation signals in the bat Pteronotus p. parnellii were studied by high resolution autoradiographic imaging of ^{3}H-2-deoxyglucose (2-DG) uptake. Bats were injected i.p. with 2-DG and restrained in a foam holder or allowed to fly in the laboratory. Bats were killed 20 min after the injection of 2-DG. In the flying bats, marked alterations in patterns of 2-DG uptake occurred in brainstem and forebrain regions. Regions activated during flight included the nucleus ambiguous, inferior colliculus, superior colliculus, medial geniculate, auditory cortex, stratum lacunosum molecular of the hippocampus, anterior cingulate cortex, claustrum, and frontal cortex. The results help to define the functional organization of brain regions involved in vocalization and processing of biosonar signals.

435.7

CYTOARCHITECTURE AND HISTOCHEMICAL ORGANIZATION OF ODONTOCETE WHALE AUDITORY BRAINSTEM. W.E. O'Neill[1], M.L. Zettel[1], and S.N. Haber[2]. Depts. of Physiology[1] and Neurobiology and Anatomy[2], Univ. of Rochester School of Medicine, Rochester, NY 14642.

Relatively little is known about the anatomy of the central auditory systems of echolocating whales. We studied the brainstem of the beluga whale and bottlenose dolphin. Sections stained with cresylviolet, acetylcholinesterase (AchE), and antibodies to Calbindin D-28k (cabp) and parvalbumin (pv) were examined to localize and characterize the structures of the auditory brainstem. Comparisons were made to material from bats, rodents, human and non-human primates.

In the beluga whale, an extremely large (5 mm diameter) auditory nerve leads into a large cochlear nucleus (CN). Many cabp(+) and pv(+) cells were scattered throughout the anteroventral CN. Cells in the prominent lateral superior olive were cabp(-) and pv(+), as in other mammals. The medial nucleus of the trapezoid body (MNTB) was both cabp and pv positive. Medial to and apparently associated with the MNTB, there is an unusual dorsoventrally oriented, sickle-shaped sheet of cells, embedded in a band of AchE(+) fibers. These cells may belong to the medial olivocochlear bundle system.

Although the general features are consistent with those in other mammals, certain aspects appear unique to the whale, and may reflect adaptations to echolocation in an aquatic environment.

(Supported by NIH NS-22511 and DC-0267)

435.8

DORSAL NUCLEUS OF THE LATERAL LEMNISCUS: ORGANIZATION OF SOME LEMNISCAL AFFERENTS IN THE INFERIOR COLLICULUS OF THE FERRET. C. K. Henkel.*Dept. of Neurobiology and Anatomy, Bowman Gray Sch. of Med., Winston-Salem, NC 27157.

The dorsal nucleus of the lateral lemniscus (DNLL) projects bilaterally to the inferior colliculus. The crossed projection was studied in adult ferret using PHA-L as an axonally transported marker. Most axons crossed to the contralateral side under the periaqueductal gray in the dorsal tegmental commissure of Probst. In cases with injections that involved either the nucleus sagulum or rostral tegmental areas, labeled fibers were seen crossing to the contralateral side in the commissure of the inferior colliculus. The latter fibers typically ended in the rostral pole of the inferior colliculus, superficial layers of the dorsal cortex or ascended into the superior colliculus and were not further studied. Fibers that crossed in the dorsal tegmental commissure approached the contralateral DNLL where they frequently gave off collateral branches. What appeared to be primary axonal branches in the subcollicular tegmentum gave rise to multiple, secondary branches that ascended into the central nucleus of the inferior colliculus with each secondary branch being reduced in size and giving rise to a larger number of tertiary varicose branches. Short side branches ended in small clusters of boutons. Small injections labeled a thin lamina of fibers on the contralateral side that extended from ventrolateral to dorsomedial through the central nucleus and into the deeper layers of the dorsal cortex. The laminae were 90-160 μm in thickness. Axonal branches diverged and distributed branches and endings over 1000 μm rostral-caudally in the laminae. Some axons entered the laminae from a rostral perspective and coursed caudally while others entered caudally and coursed rostrally. Injection sites of only 100-200 μm segment of DNLL labeled axons that diverged over the full expanse of the laminae. Single axons that were reconstructed could be traced over much of the laminae as well.

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435.9

PARVALBUMIN IMMUNOCYTOCHEMISTRY DELINEATES PRIMARY AUDITORY NEOCORTEX. C.B. Smelser and N.T. McMullen*. Department of Anatomy, University of Arizona College of Medicine, Tucson, AZ 85724.

Electrophysiological and anatomical studies of rabbit auditory neocortex have revealed a large primary field (AI) characterized by a distinct cytoarchitecture: a cell-dense lamina III/IV and a broad cell-sparse lamina V. We report that monoclonal antibodies to parvalbumin (PV), a calcium-binding protein present in GABA-ergic local circuit neurons, precisely delineate the cytoarchitectonically defined AI. PV immunocytochemistry (PVi; SWANT, 1:10K) was performed on 100 um thick sections obtained from young rabbits. Serial coronal sections through the entire temporal pole were processed and every third section was Nissl stained with methylene blue. PVi yields an extraordinary Golgi-like dendritic (and, in some cases, axonal) labeling of nonpyramidal cells in all cortical layers. The dorsal boundary of AI is demarcated by an abrupt increase in the number of PV-positive nonpyramidal cells and dense terminal labeling within lamina III/IV. Lamina II and upper parts of III are populated by nonpyramidal cells with small somata and less often, large cells of the bitufted, bipolar and stellate variety. Lamina III/IV is characterized by a dense population of large spine-free nonpyramidal cells with bitufted dendritic domains and tangentially-oriented local axonal plexi. Basket-type axonal terminals outline pyramidal cell somata and contribute to the dense terminal labeling within III/IV. Cell-sparse lamina V contains large nonpyramidal cells remarkable for their tangentially oriented dendritic fields. Lamina VI can be partitioned into sublamina VIa and VIb. Lightly labeled pyramidal neurons compose VIa while a diverse population of nonpyramidal cells occupy VIb. The ventral border of AI stands in stark contrast to the near absence of PV labeling in perirhinal cortex (Supported by NIH, Deafness Research and Whitehall Foundations).

435.10

DIFFERENTIAL CORTICAL PROJECTIONS FROM SUBNUCLEI OF MONKEY MEDIAL GENICULATE COMPLEX REVEALED BY ANTEROGRADE TRANSPORT OF PHA-L. T. Hashikawa*, H. Ojima and E. G. Jones. Laboratory for Neural Systems, F.R.P., RIKEN, Wako 351-01, Japan, and Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Cortical auditory fields have been shown to receive their thalamic afferents from specific subdivisions of the medial geniculate body (MGB). In the present study, in order to examine how fibers originating from each MGB subdivision contribute to these projections, *Phaseolus vulgaris* leucoagglutinin (PHA-L) was iontophoretically injected under physiological control in limited regions of the MGB in Japanese monkeys, *Macaca fuscata*.

Fibers from the *ventral nucleus* formed several terminal plexuses in a patch-like fashion in the primary auditory cortex (AI), mainly in middle cortical layers. When measured in tangential sections, each patch was 500 - 1000 μm in width. A main patch was surrounded by "satellite" patches and the distance between the patches from center to center was about 1000 μm. Fibers from the *dorsal nuclei* also formed terminal patches in the same layers in cortical areas surrounding AI. Their principal projection was not to AI. Size of the main patches was twice as wide as those in AI, even with smaller injections. Fibers from the *magnocellular nucleus* had rather few terminal branches in middle cortical layers as well as in layer I, but they innervated extremely wide areas, both in AI and surrounding auditory areas by means of collaterals of single axons. Formation of discontinuous terminal patches suggests the presence of unitary modular structures in the auditory cortices based on thalamocortical connections.

435.11

TERMINAL MORPHOLOGY AND LAMINAR ORIGINS OF DUAL CORTICOTHALAMIC SYSTEMS IN CAT PRIMARY AUDITORY CORTEX. H. OJIMA* and E.G. JONES. Neural Systems Laboratory, Frontier Research Program, RIKEN, 351-01 JAPAN.

Dual terminal patterns of auditory corticothalamic fibers and their differential laminar origins were investigated with the anterograde tracers, PHA-L and biocytin, in the cat. Cats (n=8) anesthetized with Nembutal (40 mg/kg) were used. A tracer-filled electrode was introduced into the cortex while monitoring field potentials in response to single square waves applied to an earphone.

Injections of PHA-L into nearly the entire depth of AI resulted in staining of fibers with large or small boutons in the medial geniculate body (MGB). Fibers ending in large boutons were found preferentially in the dorsal and medial divisions, while most with small boutons were in the ventral division, with a smaller number in the dorsal and medial divisions. Large boutons formed clusters resembling bunches of grapes.

Injections of biocytin involving layer V without layer VI always stained fibers with large boutons which were localized in areas corresponding to those revealed by PHA-L. In contrast, localized injections solely into layer VI stained no large boutons but always demonstrated a significant number of small boutons in the ventral division and occasionally in other divisions of the MGB.

The present findings accord with previous findings that neuronal populations projecting to the MGB are separated in superficial layer V and layer VI and provide evidence that their terminal endings and patterns are also different in the MGB.

435.12

RELATIONSHIP BETWEEN NEURONAL BIRTHDATES AND THE LATERALITY OF THE PROJECTIONS IN THE RAT LATERAL SUPERIOR OLIVE. M.Kudo*, Y.Kitao, S.Okoyama and T.Moriizumi. Dept. of Anatomy, Sch. of Medicine, Kanazawa University, Kanazawa 920, JAPAN.

The crossed and the uncrossed projections from the lateral superior olive (LSO) to the inferior colliculus (IC) are distinct in all respects. The correlation between birthdates of the LSO neurons and the laterality of their projections was studied in the rat by double-labeling techniques using 5-bromodeoxyuridine (BrdU), the thymidine analogue, and Fluoro-Gold (FG), a retrograde fluorescent tracer. BrdU was given to a pregnant rat on each day throughout the LSO generation period (E12-E16). In the progeny rats as adults, FG was injected into the IC unilaterally to differentiate the crossed and the uncrossed projection neurons. The results indicate that the crossed projection neurons were mainly produced on day E13, whereas the uncrossed projection neurons generated between days E14 and E16 with peak production on day E16. Thus, one of the factors initiating the target laterality of the projections is the temporal order of neurogenesis.

435.13

INFERIOR COLLICULUS PROJECTIONS TO THE MEDIAL GENICULATE BODY: A STUDY OF THE ANATOMICAL BASIS OF COMBINATION-SENSITIVE NEURONS IN THE MUSTACHED BAT Jeffrey J. Wenstrup*. Dept. of Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

This study used dual anterograde tracers to examine projections to the medial geniculate body (MGB) from regions of the central nucleus of the inferior colliculus (ICC) analyzing different harmonic elements of the mustached bat's biosonar signal. The goal was to investigate whether direct converging input from ICC can provide the anatomical basis for combination-sensitive neurons known to exist in parts of the MGB. Multiple deposits of WGA-HRP were placed at physiologically-identified sites in ICC tuned to 26-30 kHz, while multiple deposits of biocytin were placed at ICC sites tuned either to 51-57 kHz or 60-62 kHz.

ICC regions tuned to 26-30 kHz projected to a set of targets nearer the margins of MGB. These included the suprageniculate nucleus, medial division, lateral part of the ventral division, and dorsal and superficial dorsal nuclei. Small patches of label were also found occasionally within the central part of MGB, but these were inconsistent and of low intensity.

ICC regions tuned to 60-62 kHz or 51-57 kHz each projected strongly to parts of MGB believed to contain combination-sensitive neurons. Thus, 60-62 kHz regions projected to the medial part of the ventral division and to the adjacent dorsal nucleus, while 51-57 kHz regions projected more rostrally to the dorsal nucleus and to the medial division. These major target zones did not overlap significantly with 26-30 kHz projections.

A working hypothesis is that direct projections from 26-30 kHz representations in ICC are insufficient to account for combination-sensitive neurons in MGB. (Supported by USPHS grant DC00937.)

435.14

PROJECTIONS TO THE COCHLEAR NUCLEUS FROM PRINCIPAL CELLS IN THE MEDIAL NUCLEUS OF THE TRAPEZOID BODY. B. R. Schofield* and N. B. Cant. Dept. of Neurobiology, Duke Univ. Medical Center., Durham, NC 27710

The superior olivary complex is a major source of descending projections to the cochlear nuclei (CN). In guinea pigs, a large percentage of the olivary cells that project to the CN are located in the medial nucleus of the trapezoid body (MNTB). We have used fluorescent tracers (Fluoro-Gold, Fluoro-Ruby [tetramethyl rhodamine-dextran] and Fast Blue) to determine whether MNTB principal cells, which are post-synaptic to the large synaptic terminals known as calyces of Held, contribute to this projection.

Injection of one of the tracers into the CN labels cells in the ipsilateral MNTB and calyces of Held in the contralateral MNTB. By injecting different tracers into the CN on each side, we could examine each MNTB for retrograde transport from the ipsilateral side and anterograde transport from the contralateral side. In every case, some of the labelled cells were enveloped by a labelled calyx of Held, identifying them as principal cells. It is unclear whether the remaining labelled cells were non-principal cells or were principal cells whose afferent calyx was unlabelled.

Our study demonstrates a projection from one CN to the contralateral CN via the calyces of Held and MNTB principal cells.

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435.15

LEFT HEMISPHERE SPECIALIZATION FOR AUDITORY DISCRIMINATION IN MALE AND FEMALE RATS R.H. Fitch*, C. Brown, and P. Tallal. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102

Left-hemisphere lateralization for language processing, as well as for the discrimination of species-typical calls in monkeys and mice, may derive from underlying specialization of the left hemisphere for processing rapidly changing acoustic information. In support of this hypothesis we now report findings of a right-ear advantage for discriminating 2-tone sequences in rats. Adult rats were trained in a modified operant conditioning paradigm, culminating in a testing phase where one of four 2-tone sequences was presented to the right or left ear (alternating in blocks). White noise was simultaneously presented to the contra-lateral ear. Subjects were required to identify their target via a Go-No Go response paradigm. Results from 2 studies showed that male rats were significantly better at identifying their target with the right as compared to the left ear (Study 1, $p<.01$;$df=1,8$; Study 2, $p<.001$;$df=1,8$). Since sex differences in lateralization have been reported for a variety of functions including language, adult female rats were also tested in Study 2. There were no sex differences in overall discrimination. Females, like males, showed a pattern of better discrimination with the right ear, but this effect was much weaker as evidenced by a near-significant interaction between response type, ear, and sex ($p<.09$; $df=1,13$). Implications of these findings for the role of early hormonal exposure in the development of language lateralization, and sex differences in the incidence of language disorders, will be addressed.

Supported by an award from the Rita Rudel Foundation, and by NIDCD grant #RO3-DC01038.

435.16

SYNAPTIC CONNECTIONS MADE BY THE SUPERIOR OLIVARY COMPLEX (SOC) IN THE INFERIOR COLLICULUS (IC). AN EM AUTORADIOGRAPHIC STUDY. Gretchen E. Beckius and Douglas L. Oliver*, Dept. of Anatomy, Univ. of Connecticut Health Center, Farmington, Ct 06030

We made injections of 3H-leucine and wheat germ-HRP into either the lateral (LSO) or medial superior olives (MSO) of the adult cat. The nuclei were located by stereotaxic coordinates and by their responses to binaural tones. EM autoradiographs from the IC were prepared with Ilford L4 emulsion and exposed 4-66 wk. ***Axonal endings from the contralateral LSO*** usually contain round synaptic vesicles and make asymmetric synapses (RA endings) in the IC. However, one third have pleomorphic synaptic vesicles and make symmetrical contacts (PS endings). ***Endings from the ipsilateral LSO*** are more numerous and varied. About half of this projection to the IC is PS endings; 45% are RA endings; and 5% are unusual with pleomorphic vesicles and asymmetrical contacts (PA endings). ***Endings from ipsilateral MSO*** are invariably RA endings in the IC.

Overall, endings from the SOC represent the largest source of RA endings for the IC. The MSO and LSO projections make up 33% and 26% of all the presumed excitatory inputs to the ipsilateral IC, but the projection from the contralateral LSO represents only 17%. The bilateral LSO projections account for 38% of the presumed inhibitory PS endings.

These data support the concept that synaptic domains are distinct functional zones where different types of inputs are segregated in the IC. The sum of all RA endings from cochlear nucleus and superior olive exceeds 130% of the RA endings in IC. In addition, PS endings from dorsal nucleus of the lateral lemniscus and LSO total 76% of all PS endings in IC. The surplus of RA endings suggests that some excitatory inputs to the central nucleus are mutually exclusive and do not overlap on the same postsynaptic neurons.

Supported by N.I.H. grant R01-DC00189.

CHEMICAL SENSES: PATHWAYS—GUSTATION

436.1

EXCITOTOXIC PARABRACHIAL NUCLEUS LESIONS DISRUPT CONDITIONED TASTE AVERSION, CONDITIONED ODOR AVERSION, AND SODIUM APPETITE IN RATS. G. Scalera*, P.S. Grigson, T. Shimura, S. Reilly, and R. Norgren. College of Medicine, Penn State Univ., Hershey, PA 17033

Previous studies have demonstrated that electrolytic lesions in the parabrachial nuclei (PBN) disrupt both conditioned taste aversions and salt appetite in rats. In order to assess the contributions of neurons and fibers of passage to these effects, we made electrophysiologically guided bilateral injections of ibotenic acid into the PBN of 10 rats (IBO, 0.2 ul; 20 ug/ul). Six other rats received the same volume of vehicle in the PBN (PBS, pH=7.4) and 8 animals served as non-surgical controls. Following overnight fluid deprivation, rats were given 15 min access to 0.3 M alanine and immediately injected ip with LiCl (1.5 mEq/kg, 0.15 M). Three alanine-LiCl pairings were spaced 3 days apart. All non-lesioned rats rejected alanine following a single pairing with LiCl. Even after 3 pairings, however, the IBO rats failed to reject alanine. Subsequently, these rats were acutely sodium depleted with furosemide (7.0 mg, sc) and given simultaneous access to 0.5 M NaCl and water. The IBO rats failed to increase intake of NaCl, while the other groups consumed at least 3 times control levels. In a second set of rats, using a higher concentration of LiCl (0.3 M), IBO lesions of the PBN prevented acquisition of both an alanine taste aversion and an almond odor aversion. Finally, the lesioned rats exhibited an apparent lack of neophobia in 2 situations -- when first presented with alanine and when put on a novel, Na-free diet.

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436.2

CODING OF TASTE INTENSITY IN THE HAMSTER SOLITARY NUCLEUS. H. J. Duncan* and D. V. Smith. Dept. Otolaryngology-Head and Neck Surgery, Univ. Cincinnati Coll. Med., Cincinnati OH 45267-0528.

In order to examine the contributions of Na^+, H^+ and Cl^- receptor mechanisms to the responsiveness of cells in the nucleus of the solitary tract (NST), we have determined concentration-response functions for a wide array of chemical stimuli. This array includes 8 sodium salts, 2 lithium salts, 5 non-sodium salts, 5 acids, 5 sweet-tasting stimuli, and 5 bitter-tasting stimuli. We recorded multiunit activity from the NST evoked by anterior tongue stimulation with five or more concentrations of each tastant. The peak of the integrated multiunit response to a 10 sec test stimulus was measured; these values were expressed as proportions of the peak response to a 0.032M NaCl standard. The slopes of the concentration-response functions derived in this way varied with respect to taste quality and chemical composition. In particular, the slopes for some salts were more steep than for NaCl, some were less steep, yet many stimuli were similarly effective at 0.032M. The data for salts are well described by power functions and the slopes for these functions are similar to those from human psychophysical studies on the anterior tongue. Equally effective concentrations are chosen from these functions for single unit studies; the matching concentrations (M) for stimuli determined to date are: NaCl=0.032, $NaNO_3$=0.032, Na_2SO_4=0.023, Na-acetate=0.044, LiCl=0.028, KCl=0.11, NH_4Cl=0.032, citric acid=0.0026, tartaric acid=0.0018, Na-saccharin=0.0014, dextrose=0.73, sucrose=0.028, quinine-HCl=0.032 and urea=1.73.

Supported in part by NIDCD grant DC00353-07.

436.3

A PATCH-CLAMP ANALYSIS OF NEUROKININ RECEPTOR ACTIVATION IN THE GUSTATORY PORTION OF THE SOLITARY NUCLEUS. H. Liu, M. Behbehani, S. Chandler* and D. V. Smith. Depts. Physiology and Otolaryngology, Univ. Cincinnati Coll. Med., Cincinnati, OH 45267

In previous experiments, we have shown that substance P (SP) can excite many neurons in the gustatory portion of the nucleus of the solitary tract (NST) of the hamster. Using whole cell patch-clamp techniques in an *in vitro* slice preparation, we have examined the effects of SP on the membrane properties of cells in the rostral hamster NST. Recordings were made from 21 cells with stable resting membrane potentials (mean = 55 ± 5 mv sd). Most of the cells fired repetitive action potentials with a mean spike overshoot of 64.7 ± 7 mv. Short application of the neurokinin receptor agonist SP depolarized 13 neurons with a mean depolarization of 4.5 mv that lasted for 1 - 2 min; in 10 cells this depolarization was accompanied by a decrease in conductance and an increase in spontaneous activity. Another 3 cells were hyperpolarized 4 - 6 mv with an increase in membrane conductance and a decrease in spontaneous firing. The remaining 5 cells were not affected by SP. In the presence of TTX, the depolarizing effect of SP was not abolished by perfusion of the slices with high Mg^{++}/low Ca^{++} PSS, indicating a direct postsynaptic action of SP. The effect of SP was blocked by its antagonist ([D-Pro^2, D-$Trp^{7,9}$] substance P) in 6 cells. Current/voltage curves indicated a conductance decrease during the depolarization with a reversal potential of the SP-dependent current close to the K^+ equilibrium potential. We conclude that activation of neurokinin receptors decreases K^+ conductance, leading to membrane depolarization, which may interact with gustatory afferent processing.

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436.4

RAT TASTERS AND NON-TASTERS REVEALED IN NEURAL ACTIVITY. B.K.Giza*, T.R.Scott & L.Zhang, U.Delaware, Newark DE 19716

Preferences for the bittersweet taste of NaSaccharin (Sac) in rats may be related to the distribution of PROP sensitivity, as it is in humans. Do differences in NTS taste-evoked activity mediate these preferences? We offered rats water and 0.03M Sac, then selected those who consumed >75% Sac (S) or >75% water (W). We recorded the activity of 56 cells from S- and 43 from W-rats in response to 13 stimuli. Activity in the two groups was similar for sugars but greater for all other stimuli in W-rats. **Thus water-preferring animals were not insensitive to sweet stimuli, but rather hypersensitive to non-sweets.** We divided the cells of each group into three clusters: sugar-, sodium- and acid-oriented. Sugar and sodium cells showed few differences between S- and W-rats. Rather the greater responsiveness of W-rats was most evident among acid cells. Therefore, **hypersensitivity to non-sweet tastes in W-rats was carried primarily by cells whose activity is associated with aversive chemicals.** The activity profile evoked by Sac was similar to those of sugars in S-rats, but more like those of non-sweet stimuli in W-rats. In humans, the sweetness of Sac does not differ between tasters and non-tasters of PROP, but bitter sensitivity is greater in the former group. It is the exaggeration of Sac's bitterness that leads to its rejection by PROP tasters. A corresponding situation would seem to obtain in rats.

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436.5

PARABRACHIAL GUSTATORY LESIONS IMPAIR TASTE-GUIDED QUININE AVOIDANCE IN RATS. A.C. Spector*. Dept. of Psychology, University of Florida, Gainesville, FL 32611.

Rats with lesions centered in the gustatory zone of the parabrachial nuclei (PBN) are able to respond to sucrose in a concentration-dependent manner, but this responsiveness is blunted. This study examined the extent to which PBN lesions alter taste-guided avoidance of an aversive taste stimulus, quinine. Water-deprived rats were tested, in a specially-designed gustometer, for their licking responses to water and 7 concentrations of quinine hydrochloride (0.003 mM - 3.0 mM) during repeated 10 sec trials. Water rinses preceded each stimulus trial and testing occurred over three 40 min sessions. Next, six deeply anesthetized rats received electrophysiologically-guided bilateral electrolytic lesions of the gustatory zone of the PBN. Two lesions were placed on each side, one at the ventral border of the taste area (60 uA, 20 s) and one 200-300 um more dorsal (40 uA, 20 s). A second group (n = 13) received similar lesions, but the current and time were doubled. Eight rats served as surgical controls. After recovery rats were retested for quinine responsiveness. In all groups, licking to quinine significantly decreased as a function of concentration both before and after surgery. There was a slight rightward shift in the concentration-response curve of about 0.10 $\log_{10}$ units after surgery in the control group. The lower current-induced lesions shifted the curve about 0.5 $\log_{10}$ units to the right and the higher current-induced lesions caused a more substantial shift of about 1.0 $\log_{10}$ unit. Histological analysis is in progress to determine the relationship between the degree of behavioral impairment and the cytoarchitectural lesion parameters. These results suggest that rats with PBN lesions are not completely ageusic to quinine. These lesions, however, markedly attenuate taste-guided avoidance responses. Supported by PHS grant DC-00161.

436.6

THE PARABRACHIAL NUCLEUS AND INCENTIVE CONTRAST IN RATS. P.S. Grigson, S. Reilly*, A.C. Spector^, and R. Norgren. College of Medicine, Penn State Univ., Hershey, PA 17033 and Dept. Psychology^, Univ. Florida, Gainesville, FL 32611

Rats with bilateral electrophysiologically guided lesions of the parabrachial nuclei (PBN) and surgical controls were food deprived and divided into two groups. One group received 5-min daily access to 0.1 M sucrose for 14 days. The other received similar access to 1.0 M sucrose for the first 10 days and then was shifted to 0.1 M sucrose for the remaining 4 days. Prior to the shift, the lesioned rats consumed less of both solutions than the sham animals. Nevertheless, both groups licked the stronger solution with shorter latencies, at higher rates, and with shorter interburst intervals than they did the weaker stimulus. Following the concentration shift, the sham rats demonstrated contrast on three measures, i.e., they made fewer licks and initiated more bursts that were of shorter duration than did the unshifted controls. The lesioned rats failed to demonstrate a contrast effect on any measure; their licking simply dropped to the level of the lesioned, but unshifted rats. Although motor impairments might account for the differences in preshift licking rates between the lesioned and the sham groups, the data indicated that the incentive value of the stimuli was not eliminated, because the rats with PBN damage did respond more for the stronger than for the weaker sucrose. The absence of a contrast effect in the lesioned animals, however, indicates that the PBN damage disrupted the comparison process that typically occurs when rats are shifted from a more preferred to a less preferred solution. Supported by PHS grants DC-00240, DC-00047, MH-00653.

436.7

GUSTATORY FUNCTION AFTER LESIONS OF THE ROSTRAL NUCLEUS OF THE SOLITARY TRACT IN RATS. T. Shimura, P.S. Grigson*, and R. Norgren. Dept. Behavioral Science, College of Medicine, Penn State Univ., Hershey, PA 17033

In an automated gustometer, 12 rats generated concentration-response functions for 8 sapid chemicals and capsaicin. When water deprived, the rats progressively decreased their licking of citric acid, $MgCl_2$, NH_4Cl, quinine HCl, and capsaicin. When replete, they increased their licking of sucrose and polycose and decreased intake of NaCl and monosodium glutamate. Six of these animals and 3 additional, inexperienced rats had electrophysiologically guided lesions centered on taste neurons in the nucleus of the solitary tract (NST); 4 others (3 exp., 1 inexp.) served as controls. Subsequently, all animals were retested using the same stimuli under the same conditions. Control animals were unchanged, but the lesioned rats exhibited consistent deficits in responding to sapid stimuli. Licking of 1.0 M sucrose did not differ from water. The normally aversive substances reduced intake only about half as much as before surgery. Intake of capsaicin was not influenced by the NST lesions. In a second experiment, 3 presentations of 0.3 M alanine were paired with injections of LiCl (1.5 mEq/kg, 0.15 M). Both the lesioned and the control rats learned to avoid the alanine. Thus, rats with NST lesions show a marked impairment of gustatory preference and aversion, but still can use taste cues for learned aversions. With lesions in the parabrachial nuclei, the second central taste relay, rats exhibit only moderate changes in gustatory preference, but cannot learn a taste aversion. Supported by DC-00240, DC-00047, MH-00653.

436.8

SALT DEPRIVATION ALTERS TASTE RESPONSES IN THE NUCLEUS OF THE SOLITARY TRACT OF BEHAVING RATS. K. Nakamura and R. Norgren*. Dept. Electronics and Informatics, Toyama Prefectural Univ., Toyama, Japan 939-03 and Dept. Beh. Sci., Col. of Med., Penn State Univ., Hershey, PA 17033

Single neurons were isolated from the nucleus of the solitary tract of awake, behaving rats before (n=41), during (n=58), and after (n=12) they were placed on a sodium-free diet. Fluid stimuli (50 ul) were delivered via intraoral cannulae. During sodium deprivation, taste responses generally were reduced. Spontaneous activity increased by an average of 42%, while responses to water dropped by 28%. Mean response to NaCl decreased 47%; to sucrose, 59%; to citric acid, 32%; and to quinine HCl, 16%. Nevertheless, the response profiles of the neurons to 4 standard stimuli were not changed by the dietary conditions. In the replete condition, 61% of the activity elicited by NaCl occurred in Na-best cells; 33% in sucrose-best neurons. In the depleted state, the figures were 60% and 26%, respectively. At higher stimulus concentrations, however, the relative responsiveness was altered by sodium deprivation. When the animals were sodium replete, in sucrose-best neurons, 1.0 M NaCl elicited only 60% as much activity as that produced by 0.3 M sucrose. When depleted, the response to strong salt was 101% that of sucrose. Similarly, for Na-best neurons, the response to 1.0 M sucrose was only 38% of that to the 0.1 M NaCl standard in the replete condition, but rose to 71% when the rats were sodium deprived. Although the sample was small, these ratios appeared to revert toward previous values when sodium was added back to the diet. Supported by PHS grants DC-00240, MH-43787, MH-00653.

436.9

CYCLIC SPONTANEOUS ACTIVITY IN TASTE-RESPONSIVE UNITS IN THE NUCLEUS OF THE SOLITARY TRACT IN THE RAT. Scott Monroe* and Patricia M. Di Lorenzo. Dept. of Psychology, P.O. Box 6000, SUNY at Binghamton, Binghamton, NY 13902-6000.

Recent studies have shown that some taste-responsive units in the parabrachial pons (PbN), the second relay in the central taste pathway in the rat, show non-random, cyclic variations in spontaneous firing rate. The present study examined spontaneous activity in taste-responsive units in the nucleus of the solitary tract (NTS), the first relay in the central taste pathway, in the urethane-anesthetized rat. Initially, responses to representatives of the four basic taste stimuli were recorded followed by 15 min of spontaneous activity. Spontaneous activity (5 sec bins) was examined for cyclic characteristics using a Fourier analysis. Linear trends were removed prior to spectral analysis. Spontaneous activity was classified as cyclic if a peak in the activity spectrum exceeded the 95% confidence limits of the spectral estimates of random data. Preliminary results show that 4 of 12 taste-responsive units showed significant cyclic spontaneous activity. These units showed single peaks in the spectral analysis ranging from .11 - 4.43 cycles/min, with a median of .18 cycles/min. Ten of the NTS units were recorded simultaneously with taste-responsive PbN units and in five of those pairs one unit showed cyclicity where the other did not. This suggests that the occurrence of cyclicity of spontaneous rate may be independent in the NTS and PbN. In contrast to the PbN units, spontaneous firing rates in NTS units were not suppressed following taste stimulation.

Supported by a grant form the Whitehall Foundation to P.Di Lorenzo.

436.10

THE EFFECTS OF A CTA ON GUSTATORY EVOKED ACTIVITY IN THE RAT NTS REMAIN AFTER BEHAVIORAL EXTINCTION. L.J. Nolan* and T.R. Scott, Dept. of Psychology, University of Delaware, Newark, DE 19716.

Chang and Scott (1984) reported modification of gustatory evoked activity in the rat NTS as a consequence of a CTA. We investigated whether this modification would be lost with the complete behavioral extinction of the aversion. Rats were conditioned to avoid .0025M sodium saccharin (CS) by pairing its taste three times with i.p. LiCl. The aversion was then extinguished over a period of weeks by daily presentations of the CS when the rats were 23-hour fluid deprived. A control group received saline injections. After rats consumed the CS at pre-illness levels, responses of single units in the NTS were recorded to the CS plus an array of 12 stimuli. The stimulus space from the control group presented a typical distribution of chemicals, with the CS among the sugars. In the CTA-extinguished group, the CS shifted decisively away from the sugars towards salt and quinine. This change in location of the CS was similar to that reported in rats with intact CTAs. Therefore, the effect of an intense aversion to the taste system remains even after the rat has regained full acceptance of the CS. This vestige of the initial conditioning may account for the ease with which relearning occurs following extinction.

436.11

LOCATION OF POSTERIOR TONGUE-RESPONSIVE GUSTATORY ACTIVITY WITHIN THE NUCLEUS OF THE SOLITARY TRACT. C.B. Halsell*, J.B. Travers and S.P. Travers. Dept. of Oral Biology, The Ohio State University, Columbus, OH 43210.

The posterior lingual taste buds located within the foliate (FOL) and circumvallate (CV) papillae account for the majority of tongue taste buds in the rat (46% and 35%, respectively). However, there is a paucity of data on central processing of taste-responses arising from the posterior tongue, especially the CV. In the present study, in rat, the location of neurons responsive to specific CV and FOL stimulation were mapped within the nucleus of the solitary tract (NST) using standard extracellular recording techniques. Both posterior taste responses extended from the anterior tongue taste area to about the level where the NST meets the 4th ventricle, with a trend for CV responses to extend caudal to FOL responses. In most cases, CV and FOL taste responses were recorded in the same electrode track, with FOL responses located dorsal to CV responses. Tactile responses from these two fields were co-extensive with taste responsive sites, but were more widespread. All multiunit sites and four single units responsive to specific CV stimulation had similar response profiles: 0.01M HCl > quaternary mixture > 0.003M quinine•HCl, with no response to 0.3M NaCl and 0.01M Na saccharin. This central response profile is more specific than glossopharyngeal nerve response profiles previously described, but is in agreement with the robust responsiveness of this nerve to acids and alkaloids. Supported by NIH grants DC00416 and DC00417

436.12

DISTRIBUTION OF TASTE-RESPONSIVE NEURONS IN THE HAMSTER SOLITARY NUCLEUS. A.P. Knox*, L.D. Savoy, T.P. Hettinger and M.E. Frank. Department of BioStructure and Function, Univ. of Connecticut Health Center, Farmington, CT 06030.

The distribution of taste-responsive neurons in the rostral solitary nucleus (SN) was studied in anesthetized hamsters (*Mesocricetus auratus*). The anterior tongue was isolated in a flow chamber and seven taste stimuli (0.03M NH_4Cl, 0.01M citric acid, 0.1M glycine, 0.1M KCl, 0.03M Na acetate, 0.03M NaCl, 0.1M sucrose) applied. Extracellular recordings from SN neurons were made with glass microelectrodes (1-8 MΩ) filled with 4% HRP in 0.5 M KCl and 0.05 M Tris buffer. Units were recorded on a VCR and played back through a window discriminator for data analysis. Successful recording sites were marked by ionophoresis of HRP, which filled stellate and elongate cells. Perfused brainstems were cut in 40μm frozen sections in the transverse, parasagittal or horizontal plane. The SN is prominent and can be reconstructed from sections in any plane. Sections were processed for HRP, counterstained and the taste-responsive area containing small, densely packed cells was mapped. A high percentage of cells in this area responded to one or more stimulus. Rates as high as 100 spikes/s were obtained to sucrose. Responses in the rostral SN may be stimulus selective at any one multiunit site. Single units, however, could display disparate responses at contiguous sites. These data argue that taste responsiveness of neurons may have a "modular" organization that selects for basic taste stimuli. This work was supported by NIH grant DC 00853.

436.13

TRANSGANGLIONIC DEGENERATION IN THE GUSTATORY SYSTEM. S.T. McGlathery and M.C. Whitehead*. Department of Surgery, Division of Anatomy, UCSD, La Jolla, CA 92093

Peripheral taste nerves are prone to damage in humans and have been experimentally altered in animal studies of the deafferented gustatory system. It is not known whether peripheral nerve damage in the mammalian taste system results in degeneration of central axonal endings as in other sensory systems (e.g. trigeminal, vestibular, auditory). In this study, using 11 Golden hamsters, the right chorda tympani was severed in the middle ear. In 8 cases a toxin, Formocresol, was applied to the nerve stump. The animals survived for 2-15 days. The brains were sectioned and stained for degenerating axons and endings using the Fink-Heimer method. In all cases there was evidence of degenerating axons in the rostral nucleus of the solitary tract. Degeneration was concentrated in the chorda tympani terminal field. In animals treated with both the nerve-cut and Formocresol the degeneration was dense, especially at 11-13 days. In cases where the chorda tympani had merely been severed, degeneration was less robust but relatively heavy at 11 days and observable as early as two days after nerve-cut. The degeneration observed after Formocresol treatment could result from the death of geniculate ganglion cells. However, degeneration after simple nerve cut is best explained as transganglionic, not necessarily requiring cell death. The present findings suggest that damaging peripheral taste nerves can profoundly disrupt the synaptology of the NST. Altered synaptology could relate to the dysgeusia or other taste phenomena of central origin reported for some patients after taste nerve damage (e.g. in middle ear or oral surgery). (Supported by NIH Grant DC00452).

436.14

CONSUMPTION AND LICK ANALYSIS OF SACCHARIN-ETHANOL COCKTAILS IN RATS LACKING GUSTATORY CORTEX. C.C. Horn*, S.A. Bailey, J.C. Mitchell, and S.W. Kiefer. Department of Psychology, Kansas State University, Manhattan, KS 66506.

Research on alcohol ingestion has customarily employed water-alcohol mixtures, but in the real world alcohol is often flavored with another pleasant tastant, often something sweet. Alcohol-saccharin mixtures were used in the present study to achieve a degree of external validity. Gustatory cortex lesion (n=6), control lesion (n=4), and normal rats (n=5) were given 30 min lick analysis tests; volume consumed was measured at the end of each session. Solutions of saccharin (.006 M) and alcohol-saccharin mixtures (3%, 6%, 9%, and 12%, v/v) were presented in ascending and descending orders, one solution each day. Contrary to previous findings with water-alcohol mixtures [Kiefer et al., Alcohol, 4, 1987] rats lacking gustatory cortex did not consume more alcohol-saccharin at any concentration compared to controls. Preliminary analysis of lick rate also revealed no significant differences between groups. It is suggested that the synergy of saccharin and alcohol contributes to the lack of differences. There were significant differences across concentrations for all groups, e. g., rats consumed more saccharin and 3% alcohol-saccharin than other concentrations.

436.15

INCREASED SODIUM CHLORIDE PALATABILITY IN RATS LACKING GUSTATORY CORTEX. S.A. Bailey*, S.W. Kiefer, and S.L. Rock. Department of Psychology, Kansas State University, Manhattan, KS 66506-5302.

It has been reported that rats lacking gustatory cortex (GC) consumed significantly more sodium chloride (NaCl) solution over a range of concentrations than did control rats during simple preference tests (Braun et al., 1982). Whether this pattern of consumption could be attributed to palatability, postingestive effects, or some other mechanism could not be delineated using intake as a measure. The present study, which employed taste reactivity as a dependent variable, compared GC rats (n=7) to control rats (n=7) across four NaCl concentrations (0.1M, 0.3M, 0.6M, 1.0M). Each of four daily fluid presentations (given randomly) consisted of videotaping orofacial reactivity to the intraoral delivery of NaCl solution (1 ml/min) for 1 min. The tapes were individually scored for ingestive and aversive reactivity. The data indicated that GC rats produced significantly more ingestive reactivity than control rats. There were no significant group differences on aversive reactivity. The increase in ingestive responding by GC rats may be the basis for the increased consumption of NaCl shown previously.

BASAL GANGLIA AND THALAMUS VI

437.1

LOCALIZATION OF STRIATAL EXCITATORY AMINO ACID BINDING SITE SUBTYPES TO STRIATONIGRAL PROJECTION NEURONS . S. J. Tallaksen-Greene[1]*, R. G. Wiley[2] and R. L. Albin[1]. [1]Dept. of Neurology, U. of MI., Ann Arbor, MI 48109. and [2]Depts. of Neurology and Pharmacology, DVAMC and Vanderbilt Univ., Nashville, TN 37212.

Autoradiographic studies have identified high levels of excitatory amino acid (EAA) binding site subtypes in striatum. However, the localization of EAA receptor subtypes to specific populations of striatal projection neurons has not been determined. We used quantitative autoradiography to examine the cellular localization of NMDA, AMPA, metabotropic and kainate EAA binding sites in the striatum following selective lesion of striatonigral projection neurons. Degeneration of striatonigral neurons was induced unilaterally by injection of the suicide transport toxin, volkensin (0.5-0.8 ng in PBS), into the left substantia nigra. Following a survival period of 12 days, we observed a reduction of all EAA binding site subtypes in the striatum ipsilateral to the injected nigra. Striatal NMDA binding sites were reduced approximately 50%. The other EAA binding site subtypes exhibited more modest reductions; up to 18% for AMPA, 27% for metabotropic and 15% for kainate.

These results indicate that there are NMDA, AMPA, metabotropic and kainate binding sites on striatonigral projection neurons and suggest that the NMDA subtype may be selectively enriched on striatonigral neurons. Supported by NS01300, NS19613, NS07222 and the Hereditary Disease Foundation.

437.2

HALOPERIDOL-INDUCED MORPHOLOGICAL CHANGES IN STRIATUM ARE MEDIATED VIA GLUTAMATE SYNAPSES. C. K. Meshul*, R.K. Stallbaumer, B. Taylor and A. Janowsky. V.A. Medical Center and The Oregon Health Sciences Univ., Portland, OR 97201.

We have shown that 14d treatment with haloperidol (0.5 mg/kg/d, s.c.) causes a signficant increase in the percentage of synapses associated with a perforated postsynaptic density (i.e. perforated synapses)(Meshul *et al*, 1992). Based on the size of the presynaptic terminal, we hypothesized that the perforated synapse was not dopaminergic but could be glutamatergic. Co-administration of haloperidol and MK-801 (0.3 mg/kg/d, s.c.), an NMDA non-competitive antagonist, for 14d prevented the haloperidol-induced increase in striatal perforated synapses, but had no effect on the increase in density of dopamine D-2 receptors. Since this glutamate antagonist could be having its effect outside the striatum, MK-801 (10 nmole/0.5 ul/d) was injected directly into the caudate, followed 30 min later by systemic haloperidol administration or saline for 14d. The haloperidol-induced increase in striatal perforated synapses was reduced following pre-treatment with intrastriatal MK-801. Surprisingly, intracaudate injection of MK-801 alone caused a significant increase in the percentage of perforated synapses compared to the saline control. This suggests that blockade of the NMDA channel within the striatum could allow activation of other glutamate receptors, which may then play a role in the appearance of perforated synapses. In order to demonstrate whether the perforated synapse contains glutamate, post-embedding immuno-gold electron microscopy was carried out. Glutamate antibody (Arnel, Brooklyn, NY, diluted 1:400,000 + 1 mM aspartate) was localized within presynaptic terminals associated with perforated postsynaptic densities. Supported by the Dept. of Veterans Affairs and NIAAA.

437.3

TENASCIN AND ASTROCYTES IN THE STRIATUM OF PROGRESSIVE GRADES OF HUNTINGTON'S DISEASE. D. Steindler, T. O'Brien*, M. Gates, K. Harrington, E. Laywell, A. Reiner, and A. Faissner. Dept. of Anatomy & Neurobiol., Univ. of TN, Memphis; Dept. of Neurobiol., Univ. of Heidelberg.

Various neuron types may be at risk during the course of Huntington's Disease (HD). Glial cells have not been extensively studied during the course of neurodegeneration within the structure most involved in the disease process, the caudate-putamen (or neostriatum). Since the astrocyte could be a cell at risk, or even associated with the primary gene defect, we examined two markers that have been shown to be specific for astrocyte "reactivity," (glial fibrillary acidic protein, GFAP), and development, (tenascin, a constituent of the extracellular matrix that is developmentally regulated in the neostriatum and other structures, and also expressed early in CNS injury).

Immunocytochemistry for GFAP and tenascin, using well-characterized mono- and polyclonal antibodies, was carried out on presymptomatic, and Grades 2& 3 HD striatum (using the grading scale of Vonsattel). Our studies have revealed that astrocytes show changes in the presymptomatic HD tissue, and that their reactivity, distribution, and expression of tenascin and GFAP mirrors the degenerative process through the caudate and putamen as the disease progresses. In particular, we have found that GFAP and tenascin are upregulated in the HD striatum, and distributed in a patchy manner that may either represent striosomes or a matrix subcompartment. In presymptomatic tissue, GFAP and tenascin patchy staining is light, as compared to Grades 2 & 3. By Grade 3, the tenascin and GFAP immunostaining in the caudate and putamen is dense and pervasive. In Grades 2 & 3, GFAP staining is associated with classical reactive astrocytes, but in the presymptomatic tissue the GFAP labels what resemble immature astrocytes. Thus, astrocytes exhibit morphological and biochemical changes early in HD that may be associated with the onset of disease. Supported by the Hereditary Disease Foundation.

437.4

COMPARISON OF THE DOPAMINE EFFECTS ON GABA NEURONS IN THE MEDIAL FRONTAL CORTEX AND THE STRIATUM OF THE RAT. S. Rétaux, N. Kayadjanian, P. Vernier, J. Caboche, M. Mavridis, J. P.Bourgeois*, M.J. Besson and J. Penit-Soria. Lab. Neurochimie-Anatomie, IDN-UPMC, CNRS URA 1488, 75005 Paris; Lab. Neurobiol. Cell. Mol. CNRS UPR 23, 91198 Gif-sur-Yvette, Lab. Biol. Mol., Inst. Pasteur, CNRS URA 1284, 75005 Paris

Previous *in vitro* electrophysiological studies have shown that DA increased the spontaneous firing of GABA interneurons in the medial frontal cortex (MFC). This activation could be involved in the inhibition of the firing rate of pyramidal cells observed *in vivo* following DA neuron stimulation of the ventral tegmental area (VTA). The DA control of MFC GABA interneurons was further investigated by studying the spontaneous 3H-GABA release on MFC slices. Three D2 agonists (quinpirole, RU 24926 and lisuride) dose dependently enhanced 3H-GABA release. These effects were antagonized by the classical D2 antagonist : sulpiride. Endogenous DA released by amphetamine also increased the release of 3H-GABA through a D2 receptor activation. A permissive effect of D1 agonist was observed on the D2-mediated increase of 3H-GABA release. The D2 activatory effect observed on GABA interneurons of the MFC contrasted with the D2 inhibitory effect observed on the spontaneous 3H-GABA release in the striatum.

Finally, DA regulated the levels of mRNA encoding one of the isoforms of glutamic acid decarboxylase (GAD67). In situ hybridization studies showed that the electrolytic lesion of VTA neurons decreased GAD67-mRNA expression in the MFC. At the opposite, a 6-OHDA lesion of DA nigral neurons enhanced GAD67-mRNA levels in the striatum, an effect which can be reproduced by a chronic treatment with sulpiride and haloperidol. Supported by INSERM grant n° 90.0601

437.5

SCOPOLAMINE ATTENUATES HALOPERIDOL-INDUCED C-FOS EXPRESSION IN THE BRAIN. N. Guo,* G.S. Robertson and H.C. Fibiger, Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, 2255 Wesbrook Mall, Vancouver, B.C. Canada, V6T 1Z3.

Haloperidol increases the the expression of Fos in some parts of the central nervous system. Haloperidol also induces catalepsy in rodents and extrapyramidal side effects in humans, both of which are reduced by muscarinic receptor antagonists. In order to gain insight into the neurochemical and neuroanatomical substrates of haloperidol-induced catalepsy we examined the effects of the muscarinic receptor antagonist scopolamine on haloperidol-induced c-fos expression in the striatum, nucleus accumbens, and lateral septal nucleus. Male Wistar rats received subcutaneous injections of scopolamine (2.5mg/kg), or vehicle (1mg/kg) 30 minutes before haloperidol (2mg/kg). The rats were perfused 2 hours after the injection. Immunohistochemical staining was performed on brain sections (30μm) to visualize Fos protein. At a dose that reduced the cataleptic effect of haloperidol, scopolamine decreased the neuroleptic-induced Fos expression in the striatum. Striatal cholinergic neurons are known to be interneurons that express D2 receptors and innervate striatal medium spiny neurons. The finding that scopolamine attenuates haloperidol-induced increases in striatal c-fos expression raises the possibility that these increases are mediated indirectly by the ability of this neuroleptic to increase ACh release which in turn acts on muscarinic receptors located on striato-pallidal neurons.

437.6

METABOLIC EFFECTS OF CONTINUOUS L-DOPA INFUSION IN RATS WITH UNILATERAL SUBSTANTIA NIGRA LESIONS. C.A. Hubbard, J.P. Bennett, and J.M. Trugman* Dept. of Neurology, Univ. of Virginia, Charlottesville, VA 22908

We used the 2-deoxyglucose (2-DG) method of measuring regional cerebral glucose utilization (RCGU) to study the effects of continuous L-dopa infusion in rats with unilateral 6-hydroxydopamine nigral lesions. Rats were implanted with osmotic minipumps (Alzet) delivering 100, 800, or 1200 mg/kg/day L-dopa and 25 mg/kg/d benserazide and were studied 20-22 h later. Rats receiving L-dopa, 100 mg/kg/d, rotated 0-1 turns/min; rats receiving 800-1200 mg/kg/d rotated 3-7 turns/min. Compared to lesioned controls, administration of L-dopa, 100 mg/kg/d, increased 2-DG uptake in the subthalamic nucleus (up 40%) and mildly increased RCGU in the entopeduncular nucleus (EP) and substantia nigra pars reticulata (SNr; up 30%). At this dose, an asymmetrical RCGU response on the denervated and nondenervated sides of the brain was not seen. These metabolic effects are comparable to those elicited by acute administration of a selective D2 agonist. Infusion of L-dopa, 800-1200 mg/kg/d, decreased 2-DG uptake in the lateral habenula (down 30%) and markedly increased 2-DG uptake in the EP and SNr (up 70-100%) ipsilateral to the lesion, resulting in prominent metabolic asymmetry. Increased RCGU in the EP and SNr of this magnitude has been shown previously to be mediated by D1 receptor stimulation. We conclude that continuous infusion of L-dopa at 100 mg/kg/d produces metabolic effects consistent with selective D2 stimulation whereas infusion at 800-1200 mg/kg/d elicits metabolic effects consistent with both D1 and D2 receptor stimulation.

437.7

PROGRESSION OF CEREBRAL METABOLIC CHANGES IN MPTP-INDUCED HEMIPARKINSONISM IN MONKEYS. E. Palombo[1], KS Banckiewicz[2], IJ Kopin[2], L. Sokoloff[2,*] and LJ Porrino[3], [1]NIMH, Bethesda, MD 20892; [2]NINDS, Bethesda, MD 20892; [3]Bowman Gray School of Medicine, Winston-Salem, NC 27157.

Local rates of glucose utilization (LCGU) were determined in 6 hemiparkinsonian monkeys, either 6-10 weeks (short-term, n=3) or 16 to 26 weeks (long-term, n=3) after a unilateral intracarotid infusion of MPTP. Four additional monkeys served as normal controls. MPTP-treament resulted in similar clinical signs of hemiparkinsonism, e.g. unilateral bradykinesia and rigidity that were apparent in all 6 treated monkeys.

In the short-term group, significant side-to-side asymmetries were restricted to the substantia nigra compacta (SNpc) medialis and to the zona incerta (ZI). Additional smaller differences were seen in the external globus pallidus (GPe) and subthalamic nucleus (STN).

In the long-term group, LCGU decreases in the SNpc medialis were similar (-31 short-term vs -34% long-term). Changes in the GPe (+30 vs +55%), STN (-14 vs -20%), and ZI (+9 vs +18%) were far more pronounced. In addition, LCGU was increased in the dorsolateral caudate (+18%), all parts of the putamen (+ 22%), and pedunculopontine nucleus (+19%) ipsilateral to the lesion. Furthermore, bilateral reductions were present in the ventral tegmental tegmental area as compared to controls; bilateral increases were also present in the pars oralis of ventrolateral thalamus and in the supplementary motor area.

Within the time frame of this study, the pattern of LCGU changes in the basal ganglia and associated structures becomes more complex as the length of time after SNpc denervation increases. This suggests that pathophysiological events in the brain progress in spite of a relatively stable clinical syndrome.

437.8

THE GLUTAMATE AGONIST QUINOLINIC ACID REGULATES THE EXPRESSION OF *NGFI-A*-LIKE IMMUNOREACTIVITY IN STRIATAL NEURONS. S. Berretta[1]*, J. Mildbrandt[2], G. Evan[3] and A.M. Graybiel[1]. [1]MIT E25-618, Cambridge, MA 02139 USA; [2]Washington University School of Medicine, St Louis, MO 63110 USA; [3]Imperial Cancer Res. Fund, London UK

NGFI-A (*egr-1, zif/268, krox-24*), an immediate-early gene of the zinc finger family, is constitutively expressed at low levels in neurons of the caudoputamen (CP) and therefore in principal could be either up- or down-regulated by neurotransmitters in the striatum. Evidence for such regulation has already been obtained for monoamines: dopamine indirect agonists induce marked increases in expression of *NGFI-A* mRNA in striatal neurons. We investigated the effects of glutamate, the major neurotransmitter in cortical afferents to the CP. We injected the glutamate NMDA receptor agonist quinolinic acid, or vehicle (CSF), intrastriatally in adult male rats and tested for the expression of NGFI-A by immunohistochemistry with two polyclonal antisera. On the side of the injection of quinolinic acid, NGFI-A immunostaining in the CP was markedly increased compared to that on the uninjected (or vehicle injected) side, except around the injection site, where staining was sharply decreased. These effects were blocked by pretreatment with the glutamate NMDA receptor antagonist MK801 (3 mg/kg). In a CSF control mild NGFI-A-induction occurred. In both treated and non-treated CP, we characterized NGFI-A-positive striatal neurons by double immunostaining. Most if not all of the neurons expressing NGFI-A were DARPP-32-positive. This suggests that striatal neurons expressing constitutive or induced NGFI-A bear D1-like dopamine receptors. Many neurons positive for NGFI-A also expressed immunodetectable enkephalin, which is contained in GABAergic medium spiny striatal neurons projecting to the globus pallidus. Striatal interneurons expressing parvalbumin, putative GABAergic striatal interneurons, were not NGFI-A positive. Supported by The National Parkinson Foundation and NIH Javits RO1 NS25529.

437.9

EFFECTS OF CHRONIC CLOZAPINE TREATMENT ON INDUCTION OF FOS-LIKE PROTEINS IN THE STRIATUM. N. Hiroi* and A. M. Graybiel, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

We have previously reported that acute injections of the atypical antipsychotic drug clozapine (CLZ) induced Fos-like immunoreactivity (FLI) in neurons of the nucleus accumbens and caudoputamen acutely (by 2 hrs) in a dose-dependent manner (Hiroi et al. Soc. Neurosci. Abstr., 1991). We have now compared the effects of acute CLZ treatment with the effects of chronic (8 days) CLZ treatment. Groups of rats received one of the following treatments: chronic CLZ followed by acute CLZ (CC), chronic CLZ followed by acute vehicle(CV), chronic vehicle followed by acute CLZ (VC), and chronic vehicle followed by acute vehicle (VV). Rats received 8 days of injections of CLZ (20 mg/kg, i.p.) or vehicle, and on the 9th day they received acute injections of CLZ (20 mg/kg, i.p.) or vehicle 2 hours before perfusion. Brain sections were immunostained with polyclonal Fos antiserum (Oncogene Science, Inc.) or with a monoclonal Fos antibody (Dr. F. R. Sharp). With the polyclonal Fos immunostaining, the CC and VC groups both showed abundant FLI in the nucleus accumbens and caudoputamen. When assessed with the monoclonal Fos antibody, the VC group showed FLI mostly in the dorsomedial nucleus accumbens, but the CC group showed no FLI in either the nucleus accumbens or the caudoputamen. These results suggest that 1) chronic treatment with CLZ changes the genomic responses of nucleus accumbens neurons to acute doses of CLZ and 2) these changes may reflect regulation of Fos-like antigens recognized by the monoclonal Fos antibody but not other Fos-related antigens recognized by the polyclonal Fos antiserum. (Supported by the Human Frontier Science Program and Javits Award NIH. We thank Dr. F. R. Sharp for a Fos monoclonal antibody and Sandoz Pharmaceutical for a gift of clozapine).

437.10

LOCAL INJECTION OF DOPAMINERGIC AND MUSCARINIC AGONISTS MODULATE STRIATAL PEPTIDE GENE EXPRESSION. L.K. Nisenbaum*, S.T. Kitai, and C.R. Gerfen, Dept. of Anatomy and Neurobiology, University of Tennessee, Memphis, Memphis, TN, and Lab of Cell Biology, NIMH, Bethesda, MD.

The antagonistic balance between acetylcholine (ACh) and dopamine (DA) within the striatum has long been recognized to be important in the functional activity of the basal ganglia. In order to investigate how ACh and DA influence the output of striatal projection neurons, we have examined the regulation of neuropeptide gene expression by dopaminergic and muscarinic agonists. Lesions of the nigrostriatal dopaminergic pathway have previously been shown to result in an increase in the expression of enkephalin mRNA in striatopallidal projection neurons, while the level of dynorphin mRNA remains unchanged in striatonigral cells. Systemic application of D-1 and D-2 agonists further modifies the expression of dynorphin and enkephalin mRNA in the striatum of lesioned rats. In order to test whether the action of these drugs are mediated locally within the striatum, SKF-38393, a D-1 agonist, was injected directly into the striatum or lateral ventricle (icv) of 6-OHDA lesioned rats. *In situ* hybridization histochemistry was used to measure the expression of dynorphin and enkephalin mRNA. Injection of 5 μg SKF-38393 directly into the striatum produced a local increase in dynorphin mRNA surrounding the injection site. Likewise, 5-10 μg SKF-38393 administered icv produced a 100% increase in dynorphin mRNA within the dorsal medial striatum adjacent to the ventricle. Next, the effect of a muscarinic agonist on striatal gene expression in 6-OHDA lesioned rats was examined. Injection of pilocarpine (120 μg) icv resulted in a further 25% increase in enkephalin mRNA above the lesion-induced level in striatal neurons within the dorsal medial region adjacent to the ventricle. Together, these data suggest that the effects of both D-1 and muscarinic agonists on peptide expression are mediated locally within the striatum. Further, this raises the possibility that DA and ACh may interact locally to modulate peptide gene expression of striatal projection neurons. *Supported by USPHS grant NS20702 and the Human Frontiers Program.*

437.11

TIME COURSE OF ALTERATIONS IN SUBSTANCE P, DYNORPHIN, ENKEPHALIN AND *c-FOS* GENE EXPRESSION IN STRIATAL NEURONS DURING CHRONIC COCAINE TREATMENT.

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Projection neurons of the striatum differ in the expression of neuropeptides: striatonigral neurons mainly express substance P and dynorphin, whereas striatopallidal neurons express enkephalin. Dopamine modulates the levels of these peptides. We examined with *in situ* hybridization histochemistry short-term and long-term changes in gene expression of these peptides, as well as of the immediate-early gene *c-fos*, during chronic treatment with the indirect dopaminergic agonist cocaine (30 mg/kg, i.p., twice daily). Thirty minutes after acute application of cocaine, mRNA levels of substance P and *c-fos* are significantly increased, mostly in dorsal striatal regions. By day 3 of the chronic treatment, the cocaine-induced increase in mRNA levels is significantly reduced (as compared to the levels at the beginning of the treatment), and remains reduced thereafter, in most regions. Conversely, mRNA levels of dynorphin and enkephalin are unchanged 30 min after the acute cocaine application, but are significantly increased on treatment day 2, also predominantly in dorsal regions, and remain elevated in some regions after a longer treatment. These results show that chronic cocaine treatment produces different temporal and regional patterns of alterations in gene expression in striatonigral and striatopallidal neurons.

437.12

MORPHOLOGICAL SEQUELAE OF SUICIDE TRANSPORT LESIONS OF THE SUBSTANTIA NIGRA OR GLOBUS PALLIDUS ON SUBSETS OF NEOSTRIATAL NEURONS.

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Suicide transport agents produce retrograde degeneration of neurons projecting to an injection site. In the basal ganglia these toxins have been shown to produce selective lesions of the striatopallidal and striatonigral pathways based on receptor binding and neuropeptide mRNA studies. In the present immunohistochemical study, the neostriata of adult rats were examined 10 days after an injection of volkensin into the substantia nigra (SN) (n=5) or an injection of OX-7 saporin into the globus pallidus (GP) (n=5). Adjacent sections were processed for 1) Nissl stain to study the density of all neurons and large interneurons, 2) NADPH-diaphorase (-d) histochemistry, to mark medium-sized interneurons, 3) enkephalin (ENK) immunocytochemistry (ICC), to label striatopallidal neurons, or 4) substance P (SUB P) ICC to label striatonigral neurons. Analyses of Nissl stained sections revealed that the striata ipsilateral to the lesions appeared healthy and did not exhibit shrinkage or gliosis; however, a moderate decrease in cell density was detected by quantitation (12-14% loss). The densities of large neurons and NADPH-d labeled neurons in the ipsilateral striata were unchanged after GP lesions and showed a slight decrease (7-8%) after SN lesions. After GP lesions the density of ENK labeled cells (21% loss) decreased more than that of SUB P labeled cells (16% loss). Conversly, after SN lesions, the density of SUB P labeled cells (26% loss) decreased more than that of ENK labeled cells (20% loss). These data suggest that interneurons are relatively spared and that projection neurons may be differentially affected after GP and SN lesions. Supported by DRIF, U. of MD (RCR) & NS01454 (MBH).

437.13

IMMUNOHISTOCHEMICAL LOCALIZATION OF 3-HYDROXYANTHRANILIC ACID OXYGENASE (3HAO) AND KYNURENINE AMINOTRANSFERASE IN ASTROCYTES IN THE RAT SUBSTANTIA NIGRA.

R. Schwarcz*, F. Du, K.E. McCarthy, E. Okuno and R.C. Roberts. Maryland Psychiatric Research Center, Baltimore, MD 21228.

Endogenous excitotoxins, such as quinolinic acid (QUIN), may be involved in the pathogenesis of several brain disorders. Kynurenic acid (KYNA), an endogenous antagonist of excitatory amino acid receptors, has neuroprotective and anticonvulsant properties. The immunocytochemical localization of 3-hydroxyanthranilic acid oxygenase (3HAO) and kynurenine aminotransferase (KAT), the biosynthetic enzymes of QUIN and KYNA, respectively, was examined in the adult rat substantia nigra, pars compacta (SNpc). At the light microscopic level, KAT- and 3HAO-immunoreactivity (-i) were present in glia in a robust and homogeneous pattern in both the nucleus and cytoplasm. At the ultrastructural level, both 3HAO-i and KAT-i were present in astrocytic processes surrounding capillaries. 3HAO-i was abundant throughout the neuropil in fine calibre glial processes which often encircled synaptic profiles, both asymmetric (excitatory) and symmetric (inhibitory). KAT-i was also present in astrocytic processes, but usually of larger calibre than those labeled by 3HAO antibodies. KAT-i glial processes abutted, rather than surrounded, both symmetric and asymmetric synapses. Thus, astrocytic QUIN and KYNA appear to be in a position to modulate excitatory amino acid receptor function in the SNpc. Supported by USPHS grants NS28236 and MH44211 and a HDSA fellowship (to F.D.).

437.14

Acute nicotine injections induce c-fos expression mostly in nondopaminergic neurons of the ventral tegmental area (VTA).

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Induction of c-fos gene is considered to be an immediate and early response in the cascade of molecular events that ultimately lead to long-term alterations in gene expression in neurons. The psychomotor stimulant and positive reinforcing effects of nicotine have been speculated to be mediated by the dopaminergic neurons of VTA. To identify the precise subsets of VTA neurons that mediate the nicotinergic effects, the pattern of expression of c-fos gene was mapped using immunocytochemical methods.

Acute nicotine injections (0.3-1.4 mg/kg;s.c) resulted in a prominent Fos-like immunoreactivity (Fos-Li) in the interpeduncular nucleus, medial terminal nucleus of the accessory optic tract and also prominently in the caudal linear subnucleus of VTA. The neurons of other VTA subnuclei, viz., the rostral linear, paranigralis, nucleus parabrachialis pigmentosus, and nucleus interfascicularis did not contain any cells with Fos-Li. Mecamylamine abolished Fos-Li in neurons of VTA, superficial layers of superior colliculus, periaqueductal gray areas and the interpeduncular nucleus.

These results suggest that acute nicotine injections induce c-fos expression mostly in nondopaminergic neurons of the ventral tegmental area.

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437.15

GABA-ergic interneurons of the striatum express D2 receptor mRNA: a double-labelling study with digoxigenin- and radiolabeled-RNA probes.

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We have previously hypothesized that those striatal neurons expressing very high levels of a 67 kD isoform of glutamic acid decarboxylase (GAD67) mRNA are interneurons (Chesselet and Robbins, 1989). To further test this hypothesis, striatal sections were hybridized simulataneously with a digoxigenin-labelled RNA probe for GAD67 and a ^{35}S-RNA probe for parvalbumin, a calcium binding protein present in striatal GABA-ergic interneurons (Cowan et al. '90; Kita et al. '90). The majority of neurons densely labelled for GAD mRNA also expressed parvalbumin mRNA, confirming that they are interneurons. Previous studies have shown that dopamine depletion selectively decreased GAD67 mRNA levels in the densely labelled cells (Soghomonian et al. Brain Res. 1992). Further double-labelling experiments revealed the presence of mRNA for dopamine D2, but not D1 receptors in these neurons, suggesting that the D2 receptor subytpe may be involved in the effects of dopamine on striatal GABA-ergic interneurons. Supported by PHS grant MH-448894. We thank A.J. Tobin (UCLA) and M. Berchtold (Zurich) for the cDNAs.

437.16

STRIATAL SUBREGIONS ARE DIFFERENTIALLY VULNERABLE TO THE NEUROTOXIC EFFECTS OF METHAMPHETAMINE.

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The processes underlying dopaminergic neurotoxicity produced by chronic administration of methamphetamine [m-AMPH] are incompletely understood. Insights into m-AMPH's cytotoxic mechanisms may be gained by assessing the effect a neurotoxic regimen of m-AMPH has on subpopulations of dopaminergic striatal projections. Using measures of dopamine content and [^{3}H]mazindol-labeled dopamine transport sites (visualized with quantitative autoradiography), we report differential vulnerability of striatal regions of rats given m-AMPH one week earlier (4 injections (s.c.), each 4 mg/kg, 2 hrs apart). Three rostrocaudal levels of the striatum were sampled (anterior, middle and posterior), and several regions were examined at each level (e.g. dorsal, lateral, ventral and medial regions; nucleus accumbens at the anterior level). Treatment with m-AMPH resulted in a significant decrease in [^{3}H]mazindol binding at both the anterior and middle levels, with the ventral and medial subregions exhibiting greater reduction than the dorsal striatum or the nucleus accumbens. Parallel to the heterogeneous reduction of striatal [^{3}H]mazindol binding by m-AMPH, striatal dopamine content depletion at the anterior level was most severe in the ventral region. The results encourage attempts to identify factors within the ventral-medial caudate nucleus that make it more susceptible to m-AMPH's neurotoxic effects as compared to the adjacent nucleus accumbens and the dorsal caudate nucleus.

438.1

PERCEPTION OF DURATIONS OF KINESTHETIC STIMULI IN CEREBELLAR PATIENTS. J.S. Lou, S.E. Grill,M. Hallett*, Human Motor Control Section, NINDS, NIH, Bethesda, MD 20892.

Coordinated movement depends upon precise temporal control of muscle activation. Persons with cerebellar disorders have difficulty with such movements as well as in discrimination of durations of auditory and visual stimuli. We have compared the ability to perceive durations of *kinesthetic* stimuli of seven patients with cerebellar disorders (without clinical evidence for peripheral neuropathy) to that of six normal controls.

Subjects were seated with their right forearm resting on a table midway between pronation and supination, the wrist rigidly fixed, and the index finger placed securely in a finger holder attached to a computer-controlled torque motor. This allowed imposition of controlled changes in joint angle about the metacarpal-phalangeal joint. A trial consisted of presentation of a pair of joint angle steps two seconds apart, of different durations but the same magnitude (about one degree). Each pair of stimuli, consisted of a standard 150 msec or 250 msec duration step and a variable duration step ranging from 50 msec to 250 msec when the standard was 150 ms long, and between 150 and 350 ms when the standard was 250 ms long. Stimuli were presented in random order. Subjects were instructed to report which stimulus was longer, by replying "one" or "two". The session continued until each pairing of stimuli was presented ten times.

There were striking differences in the ability to distinguish the durations of stimuli between normal subjects and cerebellar patients (Chi-square, p<.0001). For example, normal subjects were able to distinguish a 150 ms stimulus from one of 110 msec duration with only a 17% error rate, while cerebellar patients did not perform better than chance (making errors 48% of the time).

Impaired resolution of durations of kinesthetic stimuli by motor centers may contribute to deficits in coordinated movements in persons with cerebellar disorders.

438.2

PERFORMANCE OF RATS ON A PERCEPTUAL TIMING TASK FOLLOWING CEREBELLAR LESIONS. J.V. Baldo and R. Ivry*. University of California, Berkeley, CA 94720.

In previous research, we have found that patients with cerebellar lesions are impaired on motor and perceptual tasks that require precise timing. The present study used an animal model to investigate the role of the cerebellum in perceptual timing. Rats were trained on a two-choice discrimination task in which the duration of a visual stimulus was either 300 or 750ms. Probe stimuli of intermediate durations were also presented without reward. Animals received either sham lesions or bilateral electrolytic lesions targeted at the lateral cerebellar nuclei. Preliminary results indicate: 1)the lesions have minimal effect on overall response rate; 2)the experimental and control animals perform similarly on the endpoint stimuli; and 3)the experimental animals are less consistent in their judgements of probe stimuli (difficult discriminations). The observed deficit is not large and may be transient. Further experiments and analyses with this paradigm should help clairfy the role of the cerebellum in time perception.

438.3

CEREBELLECTOMY DOES NOT ELIMINATE THE BEHAVIORAL SUPERSENSITIVITY OF THE GENETICALLY DYSTONIC RAT TO SEROTONERGIC AGONISTS. V.L. Michela*, J.F. Lorden, and K. Vanwanderham. Dept. Psych., Univ. of Alabama at Birmingham, Birmingham, AL 35294.

In normal rats there is a decline in sensitivity to serotonergic (5-HT) agonists during the second postnatal week. During this period, the genetically dystonic *(dt)* rat, an animal model of inherited movement disorder, exhibits enhanced behavioral sensitivity to both quipazine and 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (Michela, et al., 1990; Wieland and Lucki, 1991). Since this is also the period during which the motor syndrome of the *dt* rat appears, the enhanced sensitivity may be a secondary consequence of the movement disorder per se. To test this hypothesis, the behavioral effects of 5-HT agonists were examined in intact and cerebellectomized *dt* and normal rats at 16-25 days of age. Earlier studies indicated that the output of the cerebellum is abnormal in *dt* rats, and it has recently been found that cerebellectomy (CBX) eliminates the expression of the motor syndrome. Despite the improvement in locomotor ability observed after CBX in the *dt* rats, 8-OH-DPAT produced a characteristic dose-dependent behavioral syndrome in all groups, and the enhanced sensitivity of the *dt* rats was maintained when compared to normal littermates. Thus, the behavioral sensitivity of the *dt* rats is not secondary to their movement disorder. Since experimentally-induced damage to the olivo-cerebellar system in normal rats also increases sensitivity to 5-HT agonists (Wieland, et al., 1990), the cerebellum may normally exert an inhibitory effect on the 5-HT syndrome that is absent in the *dt* rats. (Supported by the Dystonia Medical Research Foundation.)

438.4

EFFECT OF CEREBELLECTOMY ON THE MOTOR SYNDROME OF THE GENETICALLY DYSTONIC RAT. M.S. LeDoux,* J.F. Lorden and J.M. Ervin. Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

The dystonic(*dt*) rat is an autosomal recessive mutant with a motor syndrome that resembles generalized dystonia seen in humans. In the absence of intervention, motor function in the *dt* rat deteriorates, resulting in death by postnatal day 35. Glucose utilization studies and electrophysiological recordings have demonstrated abnormal neuronal activity in the deep cerebellar nuclei of the *dt* rat. To test the hypothesis that abnormal cerebellar output is responsible for the *dt* motor syndrome, total cerebellectomies, including ablation of the cerebellar nuclei, were performed on *dt* and normal rats at 15 and 20 days of age. As a control for non-specific lesion effects, a separate group of 15-day-old *dt* rats received bilateral kainic acid lesions of the entopeduncular nucleus.

Rats underwent behavioral testing just prior to surgery and again three days later. Separate groups of age-matched normal and *dt* rats served as unoperated controls. Behavioral testing included the determination of righting reflex times, locomotor and climbing ability. In addition, the severity of the motor syndrome was evaluated.

The *dt* rat demonstrated an immediate and permanent improvement in motor function and a marked decrease in abnormal motor signs after cerebellectomy. Cerebellectomy allows the *dt* rat to survive into adulthood and mate successfully. Interruption of basal ganglia output failed to improve the condition of the *dt* rats. These findings suggest that cerebellar output is critical to the expression of motor signs in the rat disease. (Supported by BNS 90-10187 and the Dystonia Med. Res. Fdn.)

438.5

THE EYE OPENING TIME, AS A FACTOR ACCELERATING MATURATION OF CEREBELLAR PURKINJE CELLS IN THE KITTENS. R.Grigorian* and E.Prigarina.I.M.Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Science , 194223, St.Petersburg, Russia.

In experiments performed on two age groups of anaesthetized kittens:(i)blind kittens (3-5 days after birth) and (ii) one week after eye opening (15-17days),the activity of identified cerebellar Purkinje cells (PC) was studied. In blind kittens the cerebellar PC discharges represented by low frequency complex(CS) and simple spikes (SS), namely 0.37 - 0.04 and 3.05-0.37 imp/s, respectively were recorded.A week after the eye opening (15 -17 days) the frequency of CS and SS in PC discharge was increased approximately on three fold, reaching 0.76 - 0.07 and 8.77 + 1,1 imp/s, respectively, i.e. the velocity of the increase of PC discharge frequency before or after eye opening is expressed as 1:3. In blind kittens the cerebellar PC in response to stimulation of n.ishiadici was ,as rule, silent dispite the superthreshold stimulus was applied, whereas PC of(ii)group kittens responded even on the threshold stimuli by CS discharge followed by inhibitory pause (IP). In this case the duration of IP was longer compared with that seen in background active Purkinje cells.

438.6

ALTERATIONS IN THE PHYSIOLOGY OF THE ROSTRAL DORSAL ACCESSORY OLIVE (rDAO) ACROSS HORMONE STATES. Sheryl S. Smith*. Dept. of Anatomy, Inst. of Neurosci., Hahnemann Univ., Phila., PA 19102-1192.

Ongoing studies in this laboratory have characterized the olivo-cerebellar circuitry across hormone (estrous) cycles. Limb response to changes in treadmill speed and negotiation of hurdles is most accurate and timely on estrus following endogenous increases in estradiol and progesterone, relative to diestrus (lower hormone levels). One mechanism by which limb coordination might be improved is via alterations in the error detection mechanism at the level of the rDAO (Gellman et al, 1985), i.e., via selective sensory gating of input to this structure during non-movement (unexpected stimulus) versus movement (expected stimulus). Additionally, synchronization of olivary activity is thought to facilitate rapid alternations in limb movement. Both parameters were tested in female rats chronically implanted with arrays of microwires (50 μ dia.) to record simultaneously from 3-8 individual neurons within the rDAO across the estrous cycle during intermittent treadmill locomotion. In some cases, cutaneous afferents to the rDAO were activated using 2 Hz stimulation of the forepaw. Increased amplitude (by 50%) of olivary response to forepaw stimulation during non-movement was observed on estrus vs. diestrus values. In contrast, estrus was associated with a greater decrease (by 30%) in olivary response during movement relative to diestrus values (n = 12, 3 rats). These results suggest that increased sensitivity of error detection mechanisms at the level of the rDAO accompanies estrous-enhanced limb coordination. In addition, use of cross-correlation analysis revealed synchronized discharge of olivary neurons recorded simultaneously on estrus, but not on diestrus (n = 6 in 2 rats, over 3 consecutive estrous cycles). In sum, estrous hormones may produce significant alterations in the physiology of the olivary network which correlate with improvements in both spatial and temporal aspects of trajectory of the distal limbs. (Supported by NS25809 and the Dept. of Anatomy)

438.7

TOPOGRAPHY OF SACCADIC EYE MOVEMENTS IN THE CEREBELLAR VERMIS OF THE RABBIT EVOKED BY MICROSTIMULATION. M. Godschalk*, J. van der Burg and B. van Duin. Dept. of Anatomy, Erasmus University Rotterdam, Rotterdam, The Netherlands.

In the oculomotor vermis in monkeys, consisting of parts of the cerebellar lobules VI and VII, saccades can be evoked with low intensity microstimulation (Noda and Fujikado, J. Neurophysiol, 1987). The direction of saccades is ipsilateral, with an upward component in the more rostral part of the oculomotor vermis, gradually changing to downward when going caudally. In rabbits, eye movements have been evoked with microstimulation in other parts of the cerebellum, but not the vermis.

Rabbits were prepared, under full anesthesia, for recording microstimulation-evoked eye movements. Scleral search coils were implanted under the conjunctiva in both eyes and a recording chamber was placed over the cerebellar vermis. After full recovery, penetrations were made in the vermis, searching for eye movements with a 200ms train of biphasic .2ms pulses at 330Hz and a maximum current of 60μA. When eye movements were observed, the current was lowered to assess the threshold. At the end of the experimental period, localization of the stimulation sites was verified by histological analysis.

In parts of lobules VI and VII, saccadic eye movements were evoked by currents ranging between 4 and 60μA. The movements were horizontal with no apparent vertical component. In a strip of approximately 4mm width on either side of the vermal midline, the cortex could be divided in two equal longitudinal zones. In the medial zone saccades were directed ipsilaterally, in the lateral zone contralaterally. Thus the topography of saccadic eye movements in the cerebellar vermis in of rabbits is unlike that of monkeys.

438.8

Neuronal activity in lateral cerebellar cortex (lobulus simplex) of cats, related to visual stimuli at rest and during locomotion. D.E. Marple-Horvat, J.M. Criado and D.M. Armstrong, SPON: Brain Research Association. Department of Physiology, School of Medical Sciences, University Walk, Bristol BS8 1TD

One of the major pathways from visual areas of cerebral cortex to motor cortex travels via the pontine nuclei to the cerebellar cortex. The great size of this projection suggests that it plays an important part in the visual guidance of movement, a conclusion that is supported by the devastating effect of cerebellar lesions on visually guided limb movements. We have therefore used full-field flash stimulation to probe for visual input to cerebellar Purkinje cells in cats stationary on a rest platform. Our recording chamber was placed over a region of cerebellar cortex (lobulus simplex, 5-7mm from midline) from which visual evoked potentials to flash stimulation have been recorded by others in anaesthetised cats. Of 16 cells tested, 12 responded to flash. Onset latencies were short, *circa* 25ms. Ten cells increased their simple spike discharge, peaking 40-50ms after flash. Since these neurones provide a highly convergent projection to the target cells in the deep cerebellar nuclei we have pooled these responses in a combined discharge histogram of all 12 cells, in which the visual event is signalled with exceptional clarity (peak z=15.8). Three of these cells were recorded whilst the cats walked on a horizontal ladder. Two rungs can be made to move up or down (6 cm) at two different times during the cat's approach. The 3 cells all gave early responses to rung movement with discharge peaking around 45-75ms. In one cell the response when the cat was close to the rung (2 steps, 40 cm away) was larger, 9.1 spikes per trial, than the response (6.2 spikes) when the cat was further away (3 steps, 60 cm). The magnitude of both responses exceeded the discharge evoked by flash (1.9 spikes), which was a more intense but behaviourally irrelevant stimulus. This cell appeared to have complex properties in which the behavioural significance of visual inputs is important (cf trained rhesus monkeys, Marple-Horvat and Stein 1990).

Marple-Horvat, D.E. and Stein, J.F. (1990) J. Physiol. 428, 595-614.

438.9

OPTICAL IMAGING OF RESPONSES TO ELECTRICAL AND NATURAL FACE STIMULATION IN THE RAT CEREBELLAR CORTEX. G. Chen, S.A. Elias and T.J. Ebner*. Departments of Neurosurgery and Physiology, University of Minnesota Medical School, Minneapolis, MN 55455.

Afferent responses to the cerebellar cortex have been characterized as a "fractured mosaic". In this study optical recordings with a voltage sensitive dye were used to evaluate the spatial patterns of activity evoked by afferent input. In anesthetized rats (ketamine/xylazine), the exposed cerebellar cortex was stained with RH795 for 2 hours. Using epifluorescence optics, Crus I and II were imaged with a Photometrics CCD system (14 bit A/D, 516 x 384 pixels). A saline filled chamber was used to control brain movement. Stimulation of the face consisted of either discrete bipolar electrical stimulation or "natural" stimulation by a Ling vibrator controlled probe. The basic experimental paradigm consisted of subtracting "background" images without stimulation from a corresponding "stimulus" image during which the face input was delivered. For both types of inputs optical signals could be obtained, characterized by patches of activity. Although small ($\sim$ 0.1% $\Delta F/F$) the responses were reproducible up to several hours. In several animals a "sagittal" organization to the patches was evident. In some animals the optical signal was distributed more widely across the entire folium. Field potential recordings revealed a close spatial correspondence with the optical signals. Consistent with a granule cell layer origin the optical signals were recorded at depths of 400-500μ. These results show that optical signals in the cerebellar cortex can be obtained with not only electrical but more physiological afferent inputs. (Supported by NIH grants NS-27210 and NS-18338).

438.10

ACTIVITY OF DENTATE NEURONS DURING SEQUENTIAL MOVEMENTS. H. Mushiake* and P.L. Strick. Research Service, VAMC and Depts. of Neurosurgery & Physiology, SUNY-HSC, Syracuse, NY 13210.

We recorded neurons in the dentate nucleus of the cerebellum while a monkey performed sequential pointing movements under two task conditions. The monkey faced a panel with 5 touch pads and placed his right hand on a hold key in front of him. In the Remembered Sequence Task (SEQ task), LEDs over 3 touch pads were illuminated in a pseudo-random sequence as an instruction to the monkey. After an instructed delay period, an auditory 'Go' signal told the monkey to release the hold key and press the touch pads according to the instructed sequence. In the Tracking Task (TRAC task), the monkey was required to press 3 touch pads immediately after the LED over each of them was illuminated. The two tasks were performed in blocks of 40 trials. We recorded from over 100 dentate neurons that displayed changes in activity during the reaction time period between the 'Go' signal and the release of the hold key. Approximately 40% of these neurons were classified *task dependent* because they displayed enhanced or exclusive changes in activity during the reaction time period for 1 of the 2 tasks. About 75% of these neurons displayed task dependence for the TRAC task and the remainder displayed task dependence for the SEQ task. In general, task dependent neurons were spatially separate from those that were task independent. Our observations suggest that the organization of the dentate is functionally heterogeneous. Supported by the VA Medical Research Service.

438.11

INFLUENCE OF ARTERIAL PRESSURE ON PURKINJE CELL ACTIVITY. L. T. Robertson*. Dept. Biological Structure and Function, School of Dentistry, Oreg. Health Sci. Univ., Portland, OR 97201-3097

We have shown that a significant proportion of Purkinje cells in lobule V have complex spikes elicited by stimulation of the vagal and renal afferent nerves. This study tested the hypothesis that the simple and complex spikes of Purkinje cells are modulated by changes in arterial pressure and by input from baroreceptors. We measured changes in spontaneous and peripherally-elicited simple and complex spike activity during pharmacologically-induced decreases in arterial pressure and during electrical stimulation of the buffer nerves in the decerebrate cat preparation (i.e., in absence of a hypothalamic-pituitary response to blood pressure changes). In about a third of the Purkinje cells tested, a nitroprusside-induced decrease in blood pressure resulted in a 75 to 90% reduction in simple spike activity. An arterial pressure decrease of 30 mmHg or more was required for the reduction in simple spike activity to occur. The reduction in activity involved both peripheral elicited and spontaneous simple spikes, but did not involve the complex spikes. The reduced simple activity often persisted for several minutes after the arterial pressure had returned to baseline. The prolonged reduction of simple spike activity may reflect a long-term response by the visceral receptors to the decrease in pressure. An increase in arterial pressure, induced by the administration of phenylephrine, affected the spontaneous activity in only a few cells, some of which had a 25% increase in simple spikes and some had a 40% reduction in simple spike, but did not affect the complex spikes. The relation between decreased blood pressure and reduced simple spike activity supports a cerebellar role in cardiovascular regulation.

438.12

IN VIVO INTRACELLULAR RECORDING OF PURKINJE CELL RESPONSES TO UPPER LIP STIMULATION IN CRUS IIA OF THE RAT CEREBELLUM. D. Jaeger* and J.M. Bower. Div. of Biology 216-76, California Institute of Technology, Pasadena, CA 91125

Earlier work in our group has shown that a brief tactile stimulus delivered to the lip of a rat can lead to prolonged increases in firing frequency of Purkinje cells in Crus IIA of the cerebellar cortex (Thompson and Bower, Soc. Neurosci. Abstr. 17, 552.8). Findings from intracellular recordings in the slice indicate that the ionic conductances of individual Purkinje cells are capable of generating prolonged depolarizations and increases of firing frequency in response to brief bursts of granule cell input (Jaeger and Bower, Soc. Neurosci. Abstr. 17, 552.10). In the present study we investigated the relevance of prolonged depolarizations in the generation of responses to sensory stimulation with the aid of intracellular recordings *in vivo*.

When inhibitory input to Purkinje cells was blocked locally with bicuculline, electrical stimulation of the upper lip lead to prolonged depolarizations in all recorded Purkinje cells located in the ipsilateral upper lip patch of crus IIA. Simultaneous extracellular recordings in the granule cell layer in close proximity to the recorded Purkinje cells showed a close correlation between discharge bursts in the granule cell layer and the rising phase of prolonged depolarizations in Purkinje cells. The depolarizations in Purkinje cells far outlasted granule cell bursts, however. These findings indicate that bursts of granule cell activity are sufficient to trigger prolonged depolarizations in Purkinje cells *in vivo* with concomitant increases of spike rate. Prolonged depolarizations may therefore provide an intracellular mechanism for lengthening the response to a brief sensory stimulus. Overlying inhibitory circuits are likely to create a sharpened spatial pattern of excitatory responses.

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438.13

CONTRIBUTION OF THE OLIVOCEREBELLAR SYSTEM TO THE INITIATION OF THE CONDITIONNED ARM MOVEMENT IN MONKEY. J-P. Pellerin*, M-T. Parent and Y. Lamarre. Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal (Qc), Canada, H3C 3J7.

Climbing fiber responses (CFR) were recorded in the lateral hemisphere of the cerebellum of a monkey trained to perform both flexion and extension of the elbow in response to conditionned stimuli randomly presented. Differences in the CFR frequency were found in relation to the initiation of the movement depending on whether the monkey moves in response to the presentation of the external stimuli (triggered movement) or performs a self-initiated movement. With triggered movements CFR increase about 100 ms before the onset of movement. With self-initiated movements, this response is very small or even absent in this period. In another experimental condition, the monkey is rewarded only when movements are initiated during a second cue presented at a fixed delay after the 'go' signal. In this 'time constraint' condition, CFR increase significativelv when the velocity exceed 220 degrees/second and when the corresponding reaction time (RT) is between 250 and 275 ms. These relationships are not shown when the monkey does not have to use a precise timing. The link between CFR, velocity and RT is stronger in time constraint task. We would like to suggest that CF are activated in relation to their own oscillatory rhythm. In a time contraint task, repetitive external stimuli may induce a change in the spontaneous rhythm of the CF and allow synchronization of CFR especially when the cue is delivered in phase with the imposed rhythm. These factors would have much less influence on self-initiated movement. Finally when a precise timing is required by the delivery of a second cue, CF are best activated and related to the kinematic parameters of the movement. These results suggest that olivocerebellar inputs induce synchronized activity in the cerebellum allowing a more efficient control of movement initiation.

Supported by MRC grant of Canada.

438.14

RESPONSES OF PURKINJE CELLS TO HINDLIMB JOINT ROTATION IN THE CAT. C. Gray, V. Perciavalle* and R. Poppele Dept of Physiology, Univ. of Minnesota, Minneapolis, MN 55455.

To determine the relationship between the response patterns of cerebellar cortical neurons and their locations in the cerebellum, we recorded from Purkinje cells in the anterior and posterior lobes during small amplitude passive rotations of the hind foot in barbiturate anesthetized cats. The 109 responsive cells were located in lobules I through IX between 1.4 and 4.2 mm lateral from the midline. 87% of the responsive cells were located in anterior lobules II and III and posterior lobules VII and VIII. 67% of the cells in the anterior and 79% of those in the posterior responded to both flexion and extension stimuli, the remainder responded to only one direction of rotation.

Response to a movement depended on a cell's location. Cells in the lateral parts of both lobes (3-4.2 mm) were excited at short latency (13-18 ms) by rotation in either direction. Medial cells generally exhibited longer latencies (25-28 ms) and longer response durations. In the posterior lobe they tended to respond identically to flexion and extension while in the anterior lobe those which were excited by movement in one direction were usually inhibited by the other.

The results suggest the possibility of a functional mapping of movement within the spinocerebellum.

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438.15

TRANSCRANIAL MAGNETIC STIMULATION OVER THE CEREBELLUM CAN DECREASE THE EXCITABILITY OF CONTRALATERAL MOTOR CORTEX IN MAN. K J Werhahn, B-U Meyer, JC Rothwell*, PD Thompson, BL Day and CD Marsden. MRC Human Movement & Balance Unit, Institute of Neurology, London WC1N 3BG, UK.

Ugawa et al (1991) reported that high voltage electric stimulation over the cerebellum could decrease the excitability of motor cortex at conditioning-test intervals of 5-12ms. The present experiments show that the same results can be obtained with much less discomfort to the subject using focal transcranial magnetic stimulation over the cerebellum.

With ethical committee approval, we recorded EMG responses from surface electrodes over the right hand muscles of five relaxed normal subjects following magnetic stimulation over the left motor cortex. These test stimuli were conditioned in random trials by a second magnetic stimulus given through a figure-of-eight coil (7cm loop diameter) placed over the right cerebellum and oriented so that the maximum current induced in the head flowed in the lateral to medial direction. With this arrangement, cortically-evoked test responses could be suppressed at conditioning-test intervals of 8-15ms. The effect was reduced if the conditioning coil was moved 2cm away from its optimal position, or if the direction of the stimulating current was reversed. We suggest that the cerebellar stimulus was producing suppression of motor cortical rather than spinal cord mechanisms since test responses evoked in active muscle by a small anodal electric stimulus over the motor cortex were not affected by a cerebellar conditioning shock. We conclude that both high voltage electric and transcranial magnetic conditioning stimuli may be capable of activating the cerebellum in man. The onset of the effect on cortical excitability is about 3ms longer with magnetic than with electrical conditioning stimuli. This may be due to activation of different neuronal elements in the cerebellum by the two forms of stimulation.

VESTIBULAR SYSTEM: BEHAVIORAL RESPONSES

439.1

NEURAL NETWORK MODEL FOR INTEGRATION IN THE VESTIBULO-OCULAR REFLEX. C.Y. Lee and J.H. Anderson*. Otolaryngology Dept., Univ. of Minn., Mpls., MN 55455.

A number of models for velocity storage and integration of eye velocity signals have been proposed which use positive feedback to integrate signals related to head velocity or retinal slip. Some of their limitations include a sensitivity to small changes in network or single unit parameters and a restricted number of units or a lack of variability related to the physiology of the vestibulo-ocular reflex (VOR). The aim of the present work was to study the properties of a bilateral network which included several different types of units representing brainstem neurons and could simulate the dynamic characteristics of the VOR over a large frequency range. For the initial work on the horizontal VOR twenty six units were used: sixteen representing the vestibular nuclei, eight representing brainstem and cerebellar structures, and two representing the horizontal ocular motoneurons. There were four inputs (representing two types of vestibular afferents) from the two horizontal semicircular canals. Each unit had a sigmoid input-output function and some constraints were put on the projection of the output from a given unit and on the sign of the connections between units. Since the intent was not to simulate physiological learning mechanisms, the method of Williams and Zipser and a non-linear optimization algorithm were used to train the network by adjusting the weights of the connections and the parameters of the sigmoid functions. After fewer than one thousand iterations whereby the weights were adjusted, the network learned to approximate the desired motoneuron output due to a sudden change in head position over 2 sec with an rms error of less than 1%. The dynamic range was extended by increasing the training time and adjusting the sigmoid function for individual units. The network was robust to perturbations in the connection weights and removal of a unit and predicted the response to other head movements. (Supported by NIH (DC00110) and Minn. Supercomputer Institute.)

439.2

VERTICAL AXIS PITCH VESTIBULOOCULAR REFLEX DURING LOW AND HIGH VELOCITY SINUSOIDAL ROTATION. S.A. Rude*, S.C. Brettler, J.F. Baker. Northwestern U, Chicago, IL 60611

Vertical vestibulo-ocular reflex (VOR) in cats tested in the 90° rolled position (vertical axis pitch) is less accurately compensatory than in the upright position (horizontal axis pitch). This inaccuracy could be related to a predominance of upward direction slow phase eye movements during low frequency rotations. Last year we reported a steady upward drift of the eyes in darkness in the absence of any rotation when the cat was in the 90° rolled position. Representing the drift as a velocity offset term allowed satisfactory sinusoidal fits to eye velocity during 2°/sec 0.01 Hz sinusoidal pitch rotation about the vertical axis. We are now testing vertical axis pitch VOR in darkness using electromagnetic search coils during 2-64°/s peak velocity oscillations at 0.01 Hz.

Sinusoidal functions with a positive offset term did not provide satisfactory fits to eye velocity during 0.01 Hz rotations above ~8°/sec. While higher head velocities resulted in greater downward slow phase velocities, the increase was not as great as would be predicted by the sum of drift and sinusoidal terms. Average peak upward slow phase eye velocity at 64°/sec head velocity was 43±14°/sec and average peak downward eye velocity was 9±6°/sec. Waveforms of slow phase eye velocities in response to high head velocities were markedly asymmetrical. Upward eye velocities rose steadily to the peak, while downward eye velocities appeared to saturate at a value near zero. These results suggest that in the 90° rolled position, upward but not downward slow phase vertical VOR is improved by a velocity storage mechanism as proposed by Matsuo and Cohen (Exp Brain Res 53:197, 1984). Supported by EY06485, EY07342, DC01559.

439.3

OPTOKINETIC-VESTIBULAR INTERACTION IN PATIENTS WITH INCREASED VESTIBULO-OCULAR REFLEX (VOR) GAIN. R.W. Baloh* and J. Demer Reed Neurological Research Center, UCLA Sch of Med, Los Angeles, CA 90024-1769

We studied optokinetic nystagmus (OKN) and visual-vestibular interaction in 5 patients with markedly elevated VOR gain (approximately 2 x normal over the frequency range of 0.01 - 1.6 Hz). Their lesions were localized to the cerebellum on clinical examination and on magnetic resonance imaging (MRI). All had impaired smooth pursuit and decreased initial slow phase velocity (SPV) of OKN. OKN SPV gradually built up over 25-35 sec reaching normal values for low stimulus velocities (≤ 30 deg/sec). The initial SPV of optokinetic after-nystagmus (OKAN) was increased but the rate of decay of OKAN was normal. Fixation-suppression of the VOR was equally impaired whether the patients fixated on a surrounding optokinetic drum, or a light-emitting diode (LED) in the dark, or imagined an LED in the dark. These findings can be explained by a model that includes a velocity storage element and a variable gain element shared by the vestibular and optokinetic systems. Cerebellar lesions do not affect velocity storage but change the output of the variable gain element.

439.4

VESTIBULAR HABITUATION AND HUMAN REHABILITATION. H. Cohen*, C. Hatfield, M. Kane, L. Miller. Dept. of Otorhinolaryngology, Baylor College of Medicine, Houston, TX 77030, Program in Occupational Therapy, Medical College of Ohio, Toledo, OH 43699, Detroit Receiving Hospital, Detroit, MI 48201

Repeated vestibular stimulation causes habituation of velocity storage in normal primates. Unilateral lesions reduce velocity storage but do not abolish it. Patients with unilateral peripheral vestibular lesions have vestibuloocular reflex phase leads when tested with sinusoids, suggesting that compensation may involve habituation of velocity storage.

We tested patients with unilateral peripheral vestibular lesions before and after receiving 12 biweekly therapy sessions involving repetitive vestibular stimuli. Subjects who had decreased vertigo and disequilibrium also had increased phase leads at low frequencies but not high frequencies. Therefore clinical compensation may involve low frequency habituation.

Supported by the Clayton Foundation for Research and the American Occupational Therapy Foundation.

439.5

CHORNOBYL VERTIGO: THE COMPARISON OF THE ACUTE AND CHRONIC FORMS. K.F.Trinus* BCA Foundation. Lukianivsky Str.27,Apt.47, Kiev UKRAINA 252071

197 Chornobyl clean-uppers were studied in 1986 during the works in the accident zone and 53 persons from the stuff of the Chornobyl station were studied in 1991. We used anamnesis, and traditional clinical tests: Uemura's, Fucuda's - stepping and wrighting, - indicating and tracking tests. In both groups the complaints were dominating over objective signs, provided by the test described. The most prominent features for both groups were complaints for vertigo attacks, which troubled several times per month, lasted minutes and hours, were provoked or strongly augmented by head movements and accompanied with nausea and vomiting. In chronic patients the headaches, tinnitus, phobias, cardiac pains and losses of consciousness were also added. If in the group of 1986 mostly the minor signs were recorded in the 1991 group there were medially expressed signs of the vestibular dysfunction. The most significant for both groups were the data of the Uemura's test. The data obtained with Fucuda's were well expressed in the 1986 group and almost insignificant in the 1991 group. The tacking and indicating tests data appeared to become significant in persons with distinctly expressed function changes. The comparison of the Chornobyl vertigo with the drop attacks, Meniere's disease, vestibular neuronitis, supratentorial lesion, - shown that it had some prominent features, which were not common to the pathologies compared.

439.6

PERCEIVED PASSIVE BODY VELOCITY AND ACTIVE MOTOR HAND VELOCITY INTERACT WITH TEMPORAL FREQUENCY

M. Aisen,[1,2] E. Katz,[1] C. Oman,[3] M. Gizzi[4]
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The motion profile is a plot of object location as a function of time. For an object moving back and forth at constant speed the motion profile has a triangular waveform, with slope and amplitude that represent object velocity and path length respectively. Increments in the period of this waveform, motion profile temporal frequency (MPTF), result in increases in perceived tactile (Katz et al., 1990) and visual (Katz et al. 1991) velocity, even when actual velocity is held constant. Here we studied MPTF in the vestibular and motor systems.

VESTIBULAR: Four velocities and four angular path lengths served to define 14 rotational stimuli. Subjects discriminated the faster of two stimuli in a 2AFC paradigm. While on average they discriminated velocity correctly, 68% of the times if stimuli were of identical paths (slower stimulus had lower MPTF), when stimuli-pair had identical velocities, the shorter (higher MPTF) was judged faster ($p<.05$).

MOTOR: Subjects were asked to move their right hand back and forth on an horizontal surface for a trial lasting about 30 sec. in darkness. At the beginning of each trial the subjects were instructed to move their hand faster than in the preceding trial. The data indicate that increases in velocity in successive trials was accompanied by reduction in path length and thus increasing MPTF.

These findings indicate MPTF interaction with perceived velocity in the vestibular and motor systems.

439.7

PHYSIOLOGICAL RESPONSES TO MOTION SICKNESS DURING SUDDEN-STOP VISUOVESTIBULAR STIMULATION. B. D. Lawson*, P. DiZio, N. S. Blaustein, J. Ventura, and J. R. Lackner. Ashton Graybiel Spatial Orientation Laboratory, Brandeis Univ., Waltham, MA. 02254. We studied how motion sickness (MS), anxiety (ANX), experience with the stimulus (EXP) and physical fitness (FIT) relate to heart rate (HR), blood pressure (BP), respiration rate (RESP) and electrogastrogram (EGG) during nauseogenic stimulation. Subjects (n=14) rotated at 270 deg/sec for 30 sec, then stopped suddenly and opened their eyes. During a 30 sec rest, they reported on MS and ANX. This procedure was repeated for 10 min while physiological responses were monitored. 10 min baseline and recovery periods were included. ANOVA's were performed on 4 time periods: baseline, early and late stimulation, and recovery. MS increased during stimulation (Friedman $X^2 \geq 22.5$, $p<.001$). HR and BP rose ($F \geq 8.0$, $p<.0001$), RESP fell ($F=3.9$, $p=.02$) and EGG did not change. Physiological measures did not distinguish high, medium and low MS groups. Subjects were ranked by MS, ANX, EXP, FIT and peak physiological response. MS correlated with RESP (.57). ANX correlated with HR (.50), BP (.60 dias.) and RESP (.54). EXP correlated with HR (-.47) and RESP (-.64), as did FIT with BP (-.68 sys and -.59 dias). (All 1-tailed $p \leq .05$.) Thus, the physiological responses elicited were not strongly related to MS severity, nor to MS per se, since ANX, EXP, and FIT also correlated to physiological response.

439.8

OBJECTIVE ASSESSMENT OF VESTIBULAR DYSFUNCTION B Segal*, G Fuoco, R Sweet[1] & A Zeitouni. Dept Otolaryngol, Jewish Gen. Hosp, [1]Montreal Gen Hosp & McGill U, Montreal, Canada H3T1E2

When assessing vestibular dysfunction, clinicians often rely more on subjective information (e.g., patient history) than on objective data (e.g., caloric test). As a result, vestibular dysfunction is usually characterized by gross descriptive classifications. We compared these broad classifications with objective measures of passive vestibular gaze stabilization, and of torso self-movement.

10 dizzy patients were examined in the clinic, and their overall vestibular dysfunction was classified (5-level rating: none, slight, moderate, etc). Then, passive gaze stabilization was characterized as the seated subject was turned, during brief darkness periods, while looking at a wall target. Next, active head-torso movement was evaluated while the standing subject made maximal-velocity, but "comfortable", ≈60° turns. 11 normals served as controls.

We found that only ≈60% of patients exhibited more gaze error, and slower torso movements, than normals. However, when gaze error was plotted against torso velocity, all abnormally-rated patients separated from the normals. Also, as a patient's rating became more abnormal, tendencies for gaze error to increase, and torso movements to slow, progressively widened this separation. Thus test results were closely related to the clinical ratings, perhaps because both vestibulo-ocular and -postural capabilities were assessed, the latter probably reflecting compensation to existing pathology. (Supported by Canadian MRC)

439.9

THE EFFECTS OF SPACEFLIGHT ON EYE-HEAD COORDINATION DURING LOCOMOTION. J. J. Bloomberg[1], W. P. Huebner*[2], M. F. Reschke[1], B. T. Peters[2]. [1]Space Biomedical Research Institute, NASA Johnson Space Center, Houston, TX, 77058 and [2]KRUG Life Sciences, Houston, TX. 77058

Exposure to the microgravity conditions of spaceflight are believed to induce adaptive sensory-motor modification that enable appropriate body movement in this unique environment. However, such adaptive changes in sensory-motor function may be inappropriate after return to a terrestrial 1-G environment leading to gait instability and oscillopsia during locomotion.

The aim of the present study is to characterize the effects of microgravity exposure on eye-head coordination during postflight terrestrial locomotion. Astronaut subjects walked (6.4 km/h) on a motorized treadmill while visually fixating on a centrally placed earth-fixed target positioned 30 cm from the head. Tests were conducted 10 days prior to launch and 2-4 hours after landing. A video-based motion analyzing system was used to measure head and body movement while standard DC-electrooculographic methods were used to record eye movements.

Preliminary results indicate that head stability during locomotion decreases immediately following spaceflight. In addition, during locomotion, the phase relationship between vertical head translations and the corresponding compensatory vertical eye movements is altered after prolonged exposure to microgravity. These results suggest that exposure to microgravity indeed causes adaptive modification of motor programs responsible for coordinated eye and head movement during locomotion.

439.10

VESTIBULAR COMPENSATION IN UNILATERAL AND BILATERAL LABYRINTHECTOMIZED RATS. J.D. Porter, S. Vrochopoulos and M.E. Meyer*. Dept. of Psychology, Univ. of Fla., Gainesville, FL 32611.

Unilateral loss of a labyrinth results in abnormalities involving the vestibulo-ocular and vestibulo-motor reflexes as well as postural asymmetry. Over time these symptoms diminish in a process known as vestibular compensation. It is known that neither the vestibular receptors nor Scarpa's ganglion regenerate following labyrinthectomy therefore, the mechanism behind this functional recovery is unknown. The reflexive abnormalities following bilateral labyrinthectomy are not well classified. This experiment focused on various locomotor and behavioral deficits observed at various time periods following unilateral and bilateral labyrinthectomy. On postoperative day 2,7,14,28,56,112 and 196, the animals were placed into a circular enclosure which allowed for unrestricted movement and videotaped for a period of 15 minutes. Their behaviors were scored from the tapes and placed into several categories: rearing, circling & pivoting, and horizontal activity. Both unilateral and bilateral rats displayed an initial inability to rear, circling & pivoting behavior and hyperactivity. However, there were differences in the magnitude and rate of vestibular compensation in the unilateral and bilateral labyrinthectomized rats. The unilateral rats recovered the ability to rear by day 56, circling & pivoting behavior was no longer present by day 28. The unilateral group remained hyperactive in relation to controls until day 112. The bilateral rats did not recover the ability to rear and remained hyperactive over the 196 days. Circling & pivoting behaviors in the bilateral group subsided by day 28.

SPINAL CORD AND BRAINSTEM V

440.1

MORPHOLOGY OF DEVELOPING HYPOGLOSSAL MOTONEURONS STUDIED IN SLICE PREPARATIONS OF THE RAT BRAINSTEM. F.He*, P.A.Núñez-Abades and W.E.Cameron. Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, PA 15260.

Between week 1 and 2 of postnatal life, there is a dramatic decrease in mean input resistance (R_{in}) of hypoglossal (presumptive genioglossal) motoneurons. One explanation for this decrease in R_{in} could be a doubling of total membrane surface area (A_n). We have quantitated the three-dimensional morphology of 35 genioglossal motoneurons at four different ages that have been labeled by intracellular injection of neurobiotin in rat brainstem slices.

Age (days)	A_n (um^2)	Area of Influence (um^2)	# terminals
1-2	15,707±3,086	126,705± 42,217	25± 7
5-6	14,529±2,023	176,537± 70,839	21± 5
13-15	14,468±2,361	147,647± 50,065	14± 4
19-30	26,669±4,374	302,982±112,623	24± 6

There was no increase in A_n between 5-6 and 13-15 days; therefore, we must propose another mechanisms to explain the decrease in R_{in}. However, the dendritic tree was not static during this period; there was a decrease in the mean number of terminals and the surface encompassing those terminals. This reduction resulted from a loss of 7th and 8th order branches while there was an increase in mean segment length at the 2nd and 3rd order branches.

This work was supported by NIH grant HD 22703.

440.2

POSTNATAL CHANGES IN SPECIFIC MEMBRANE RESISTANCE OF RAT BRAINSTEM MOTONEURONS. P.A.Núñez-Abades*, J.M.Spielmann and W.E.Cameron. Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, PA 15260.

The mean input resistance (R_{in}) of developing hypoglossal motoneurons undergoes a dramatic decrease between the first and second weeks of postnatal life. This alteration in membrane property could result from the growth of the cell membrane and/or a decrease in the specific membrane resistance (R_m). We have shown in these motoneurons that mean total area of membrane surface area (A_n) remains constant throughout this time period (see He et al). Thus, we set out to estimate the changes in R_m in these developing neurons. Intracellular recordings were made in hypoglossal (presumptive genioglossal) motoneurons from *in vitro* slices of the rat brainstem at various postnatal ages. R_{in} and membrane time constants were measured before the cells were labeled by intracellular injection of neurobiotin. R_m was estimated for each cell (n=21) using an equation derived by Rall (1975). No difference was found between the mean R_m measured at 1-2 days (7116 Ωcm^2) and 5-6 days (7489 Ωcm^2) while there was a 47% decrease in the mean between 5-6 and 13-15 days (3968 Ωcm^2). After the decrease at 2 weeks, the mean R_m rose to 6192 Ωcm^2 at 19-30 days as mean A_n expanded by 78% yielding only a modest decrease in R_{in}. We propose that the transient decrease in R_m results from i) an increase in number of leak channels in the postsynaptic cell and/or ii) an increase in the number of presynaptic inputs onto these neurons. These results suggest that there is a critical period during postnatal development when hypoglossal motoneurons are less excitable.

Supported by NIH grant HD 22703.

440.3

DYE-, TRACER- AND ELECTRICAL COUPLING IN DEVELOPING HYPOGLOSSAL MOTONEURONS OF THE RAT. W.E.Cameron*, E.Mazza, P.A.Núñez-Abades and J.M.Spielmann. Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, PA 15260.

Coupling among developing mammalian motoneurons was studied using *in vitro* brainstem slices from rats ages 1-30 days postnatal. The motoneurons were labeled by intramuscular injection of dextran-rhodamine into the posterior tongue at least one day prior to the experiment. Intracellular recording and/or labeling were obtained from a total of 194 hypoglossal motoneurons in three different age groups. In animals less than 9 days old, 6 of 41 cells (16.2%) injected with Lucifer Yellow showed evidence of *dye-coupling* while 18 of 51 cells (35%) injected with neurobiotin showed evidence of *tracer-coupling*. There was no difference in frequency of dye-coupling between animals at 1-2 days vs. 3-8 days while the frequency of tracer-coupling decreased from 40% (12/30) at 1-2 days to 29% (6/21) at 3-8 days. On average, dye-coupling occurred between two motoneurons whereas tracer-coupling was found among three motoneurons. In animals older than 10 days, there was no evidence of dye-coupling and only one instance of tracer-coupling in 52 motoneurons examined. Evidence of a short latency depolarization (SLD), indicative of *electrical coupling*, was found in 43% (3/7) at 1-2 days and in 42% (14/33) at 3-8 days. There were no SLDs found in animals older than 10 days. We conclude that among developing hypoglossal motoneurons, i) coupling is only transiently expressed and, ii) tracer-coupling provides a better estimate of the frequency of electrical coupling than does dye-coupling.

This work was supported by NIH grant HD 22703.

440.4

CHANGES IN MEMBRANE PROPERTIES OF DEVELOPING RAT PHRENIC MOTONEURONS STUDIED *IN VITRO*. J.M.Spielmann*, F.He and W.E.Cameron. Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, PA.

Previous *in vivo* studies (J Neurophysiol 65:671,1991) on developing phrenic motoneurons of the cat suggests a dramatic change in membrane during postnatal development. We developed an *in vitro* slice preparation of the rat cervical spinal cord to investigate in greater depth the time course and nature of these changes. Phrenic motoneurons of neonatal rats were retrogradely labeled following an injection of dextran-rhodamine into multiple sites in the sternocostal diaphragm. Horizontal slices (300 μm thick) were prepared from animals in one of four postnatal age groups: 1-3, 6-9, 14-15 and 19-28 days. Intracellular recording and labeling with neurobiotin were made in the phrenic motor nucleus as delineated by rhodamine fluorescence. Mean input resistance (R_{in}) of these neurons was significantly lower at 6-9 days (30.2 MΩ) than at 1-3 days (45.6 MΩ) or 19-28 days (43.4 MΩ) and not different than the mean at 14-15 days (39.4 MΩ). The decrease in R_{in} was not due to an increase in total membrane surface area. Mean surface area of these neurons actually decreased from 20,019 μm^2 (1-3 days) to 15,887 μm^2 (6-9 days) before expanding to 33,772 μm^2 (19-28 days). Estimates of specific membrane resistance (R_m) revealed a transient decline from 10,302 Ωcm^2 (1-3 days) to 2,873 Ωcm^2 (6-9 days); this value increased again in the older groups. In addition, we found a significant increase in the slope of the current-frequency plot from 22 Hz/nA (1-3 and 6-9 days) to 61 Hz/nA at 14-15 days that was followed by a subsequent decrease 24 Hz/nA in the oldest group. Similar to hypoglossal motoneurons (see Núñez-Abades et al), there is a transient decrease in R_m in developing phrenic motoneurons of the rat.

This work was supported by NIH grant HD 22703.

440.5

FAST MOTOR UNITS IN RAT GASTROCNEMIUS SHOWING EXTREMES IN FATIGUE RESISTANCE DO NOT DIFFER IN MOTONEURONE PROPERTIES: PAIRED OBSERVATIONS FROM SINGLE EXPERIMENTS. P. Gardiner.* Physical Activity Sciences, University of Montréal, Montréal, Québec, Canada H3C 3J7.

Differences in motoneurone (MN) membrane properties among motor unit types might be masked when grouped data from several experiments are analyzed. We consequently examined gastrocnemius motor units, paired from single experiments, that differed in type according to fatigue resistance and speed. Tibial MN of anesthetized Sprague-Dawley rats were impaled during in situ experiments using conventional microelectrode techniques, and gastrocnemius motor units were identified from their contractile response to MN current injection. Type slow (S) units had twitch half-relaxation times (Rt1/2) > 28 ms, were completely fatigue resistant, and demonstrated no force sag at 25 Hz stimulation. In fast fatigue-resistant (FR) and fast fatigue-sensitive (FF) motor unit pairs, twitch Rt1/2 were longer, twitches were weaker, and axon conduction velocities were faster, for FR. No differences in MN afterhyperpolarization (AHP) time-course or amplitude, in rheobase, or input resistance, were found between FF and FR. S units had lowest rheobase, highest input resistance, highest AHP amplitude and longest AHP 1/2 decay time, but not always the lowest axon conduction velocity, when compared to F units from the same experiments. Results show that MN properties, while showing distinct differences between fast and slow motor units, do not vary systematically among fast motor units which differ in fatigue resistance, in rat gastrocnemius.

Supported by NSERC Canada.

440.6

MUSCLE-SPECIFIC DISTRIBUTION OF SNB MOTONEURONS IN THE MONGOLIAN GERBIL. L. Abelson* and C. Ulibarri. School of Veterinary Medicine, University of California, Davis, CA 95616 & Dept of Veterinary & Comparative Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6520.

In the Mongolian gerbil the spinal nucleus of the bulbocavernosus (SNB) is a pool of about 200 androgen-sensitive motoneurons located above the central canal in lamina X. The gerbil SNB innervates three perineal muscles; the bulbocavernosus (BC), levator ani (LA), and anal sphincter (AS). We determined *1)* the percentage of motoneurons that innervates each muscle and *2)* the muscle-specific distribution of motoneurons within the SNB motor column.

Male gerbils (n=10) were castrated and implanted with Silastic capsules containing testosterone propionate (10 mm). One week later the BC, LA, and AS were injected with 10-20 μl of 4% dextran (Molecular Probes, Inc) conjugated to Rhodamine, FITC, or Cascade blue. Each muscle was injected with only one type of dextran. After ten days, gerbils were anesthetized and aldehyde perfused. The spinal cords and perineal muscles were removed, sectioned at 50 μm and then examined with fluorescence microscopy for visualization of labelled-dextran in both transverse (n=6) and longitudinal (n=4) sections. Only spinal cords from gerbils with injections discretely localized in each muscle were analyzed.

The majority of SNB motoneurons innervate the BC (around 75%). About 16% innervate the LA, and approximately 10% innervate the AS. No doubly-labelled motoneurons were identified. BC-projecting motoneurons were found equally distributed throughout the SNB motor column, whereas LA- and AS-projecting motoneurons were primarily found in the more lumbar portion of the SNB pool. These findings indicate that there is a reversed somatotopic representation within the SNB motor column. Supported by BNS-9112097 to CU.

440.7

MORPHOLOGY OF EXTERNAL URETHRAL SPHINCTER MOTONEURONS AND LOCALIZATION OF PUTATIVE SPHINCTER INTERNEURONS IN THE RAT SPINAL CORD. W.F. Collins, III*, D. Alspaugh, W. Hu, and J.T. Erichsen. Department of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

Spinal bladder/sphincter reflexes are: important for urinary continence, inhibited by supraspinal centers during micturition, and involved in spinal cord injury induced bladder/sphincter dyssynergia. However, little is known about the anatomical organization of these spinal reflexes.

In the present study, the dendritic morphology of external urethral sphincter (EUS) motoneurons and the distribution of putative EUS interneurons were examined using the retrograde tracer, cholera toxin B subunit (CTB), and the retrograde transneuronal tracer, wheat germ agglutinin (WGA), respectively. Adult male and female Sprague-Dawley rats were anesthetized, and either CTB (1%) or WGA (1%) was injected (0.5-3.0μl) into the EUS muscle. After a survival time of 2-5 days, spinal cords were removed and processed immunohistochemically to visualize labeled motoneurons and putative interneurons.

CTB-labeled EUS motoneuron somata were located in the ipsilateral dorsolateral (DL) nucleus. Three major dendritic projections of these EUS motoneurons were observed: (1) rostrocaudally oriented dendrites projecting throughout the DL nucleus, (2) dorsolaterally oriented dendrites projecting into the lateral funiculus, and (3) medially oriented dendrites projecting toward the dorsomedial nucleus. WGA-labeled putative interneurons were localized to the ipsilateral L5 and L6 ventral horn dorsomedial to the DL nucleus.

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440.8

THE ONTOGENESIS OF CORTICOBULBAR PROJECTIONS AND THE VIRTUAL ABSENCE OF CORTICOSPINAL CONNECTIONS IN THE OPOSSUM, *MONODELPHIS DOMESTICA*. T. Cabana*, C. Desrosiers and A. Nadeau. Sciences biologiques, Université de Montréal, C.P. 6128, Succ. "A", Montréal, QC, Canada, H3C 3J7.

In the *Didelphis* opossum, the corticospinal tract develops postnatally (Cabana & Martin, J. Comp. Neurol., 251:1986), and, as in other mammals, decussates at the medullary-spinal junction. We wished to use that model to study properties of the extracellular matrix at the decussation, but had to turn to the more amenable *Monodelphis* opossum. We had to repeat tracing the tract in order to determine the timetable of its growth and decussation. We herein report on those projections traced anterogradely with WGA-HRP. The sequence of corticobulbar innervation matches that reported for *Didelphis*, except for a few days of precedence in *Monodelphis*. A major difference between the two species is the virtual absence of spinal projections in *Monodelphis*. After crossing at the pyramidal decussation, few cortical axons grow into the white matter of the cord, and even less into the gray matter. *Monodelphis* demonstrates at least as good motor ability as *Didelphis*, whose corticospinal neurons are estimated to be 4 times more numerous, or as the rat, in whom they are 8 times more numerous (Nudo & Masterton, J. Comp. Neurol., 296:1990). *Monodelphis* has indeed the least developed corticospinal tract of all mammals studied. The quasi-absence of corticospinal connections in *Monodelphis* is not a total surprise, in the view of the absence of tactile placing in that species (Cassidy *et al.*, Soc. Neurosci. Abst. 146.1, 1990), and it raises questions about the function of the pyramidal tract in mammals. Supported by NSERC.

440.9

AGED-LIKE ELECTRICAL PROPERTIES INDUCED BY ACRYLAMIDE IN TRIGEMINAL MOTONEURONS OF THE AWAKE CAT. C. Weiss,* S.S. Chirwa, J.K. Engelhardt, F.R. Morales, and M.H. Chase. Dept. of Physiology, Dept. of Anatomy and Cell Biology, and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA. 90024

The effects of age on the electrical properties of motoneurons (MNs) include decreased conduction velocity (CV), increased input resistance (Rin), and an increase in the membrane time constant (Tm) (J. Neurophysiol. 58:180, 1987; 61:194, 1989). The mechanisms underlying the above age-related changes are not known. We sought to model some of these changes using acrylamide, a neurotoxin, in brainstem MNs of the awake adult cat (methods in J. Neurophysiol. 44: 349, 1980). Jaw closer MNs were impaled with glass micropipettes (3M KCl, 5-10 MΩ) and their electrical properties were determined. Masseter MNs were also antidromically activated to evaluate their CV. Following the collection of these control data, administration of acrylamide began (10mg/Kg/day, i.p.). After one week, data collection resumed and continued for approximately one month while the cats continued to receive the drug. Only cells with action potentials (AP) ≥55 mV were analyzed (12 control cells, 25 acrylamide cells). Comparisons between control and treated cells were made with one tailed t-tests (α=0.05). Significant changes occurred in the onset latency of the antidromically activated AP (13.6% increase) and in Rin (40% increase). The Tm increased by 33% (p=.055). There were no significant changes in the amplitude of the AP or the duration of the afterhyperpolarization. While it is not known if acrylamide affects the same mechanisms as the aging process, this drug may provide a method for assessing contributing factors to the electrophysiological changes that are observed in aged MNs. Supported by AG04307 & NS09999.

440.10

SYMPATHETIC PREGANGLIONIC NEURONS IN NEONATAL RAT BRAINSTEM-SPINAL CORD *IN VITRO*. C.T. Schulteis*, A. Lev-Tov & J.L. Feldman. Systems Neurobiology Lab., Dept. of Physiological Science, UCLA, Los Angeles, CA, 90024-1527.

Sympathetic preganglionic neurons (SPNs) were characterized using extracellular and sharp-electrode intracellular recordings from *in vitro* brainstem-spinal cord and hemisected spinal cord preparations of neonatal rats. Extracellular recordings from the T1-T5 segments of 3-5 day old rats, revealed spontaneous activity of single units in the intermediolateral cell column (IML). The firing frequency of units varied between 0.5-4 Hz at 27°C. Dye injection showed that the recordings were confined within the intermediolateral and intercalated nuclei. Sharp-electrode intracellular recordings from the T1-T6 segments of hemisected spinal cord preparations isolated from 8-14 days old rats, revealed high impedance (30-50 MΩ) low rheobase (0.02-0.04 nA) neurons located in the IML region. Neurons could be activated antidromically and orthodromically by stimulation of the ventral and dorsal roots respectively. Antidromic activation produced long duration, high amplitude action potentials, followed by delayed depolarization. Orthodromic activation evoked long latency polysynaptic potentials. Based on these criteria, these IML neurons were classified as SPNs. All the presumed SPNs exhibited spontaneous activity at variable frequencies, which could be modulated by shifting membrane polarization. One SPN exhibited clear pacemaker-like activity characterized by short (1-5 spikes at 0.5-10 Hz) repetitive bursts with 5-7s interburst intervals. Our findings suggest that intrinsic properties of SPNs may contribute to generation of the sympathetic tone and its modulation under different physiological conditions. Supported by NIH Grant NS 28805. CTS is supported by an NSF graduate fellowship.

441.1

HEAD AND TRUNK DYNAMICS OF YOUNG AND ELDERLY ADULTS DURING GAIT. R.L. Cromwell*, and E.A. Keshner. Dept. of Kinesiology, Univ. of IL at Urbana-Champaign, Dept. of Physical Therapy, Univ. of IL at Chicago. *Supported by grant DC01125.*

Changes in gait with age have produced a more cautious and stable pattern (Winter et al., Phys Ther, 1990). Mechanisms underlying these changes may be found in intersegmental relations. This study recorded the angular velocities of the head and trunk, and step cadence in four young adults (YA) and one elderly (ELD) subject during self-paced normal, slow, and fast walking. Neck velocity was calculated as the difference between head and trunk velocities. For the purpose of analysis, cadences were categorized as 95-120 steps/min for normal, <95 steps/min as slow and >120 steps/min as fast. (Normal cadences for the ELD subject fell within the slow range.) Predominant frequencies of both the head and trunk velocity responses ranged from 1.2-2.3 Hz over all conditions for YA. Head frequencies ranged from 0.08-0.4 Hz, whereas trunk frequencies remained in 1.2-2.0 Hz range for all conditions. Thus the 1:1 head to trunk frequency ratio found in YA was not maintained in the ELD subject. A time domain analysis of velocity revealed that the slow and normal ELD gait was similar to the YA slow gait in that the head, neck, and trunk velocity responses were in phase. A compensatory response was seen in the head, neck, and trunk of the YA normal and fast conditions; trunk flexion occurred with head and neck extension. In the ELD subject however, the fast cadence resulted in an uncoupling of the head, neck, and trunk where peak velocities of each segment occurred independently of the other segments. These data suggest that the ELD subject focused her control on keeping the head stable in order to better utilize vision. The coordinated phasing and the unity ratio of head to trunk in the YA implies that the upper body is controlled as a coordinative structure. Uncoupling of these segments in the ELD subject show a decrease in the ability to control these segments as a unit.

441.2

SHIFTING DYNAMICS OF THE HEAD AND NECK. B.W. Peterson*, E.A. Keshner, and R. Cromwell. Dept. of Physiology, Northwestern Univ. Med. School and Dept. of Physical Therapy, Univ. of IL at Chicago, Chicago, IL 60611. *Supported by grants NS22490 and DC01125.*

Rotations about the vertical and horizontal axes have revealed well delineated operating frequencies for mechanisms that control stability of the head (Keshner et al., Soc. Neurosci. Abstr., 1988, 1991). Here we explored how to independently manipulate these mechanisms through differing instructions and weights added to the head. Head velocity and neck EMG responses were recorded in 7 seated subjects receiving pitch rotations in the dark about a horizontal, bitemporal axis with a random sum-of-sines stimulus having 5 frequencies ranging from 0.35 to 3.05 Hz. Subjects were instructed to: 1) voluntarily stabilize the head with and 2) without vision, 3) perform mental arithmetic (MA) to minimize voluntary intervention, 4) simply relax the head, and 5) hold the neck stiff. The first 3 conditions were done with and without a 4.55 kg weight on the head. Voluntary stabilization of the head, seen below 1 Hz, was strong in the first two conditions with and without weights to the head, and was suppressed when the neck was held stiff. Greatly increased neck muscle EMG gains when stiffening suggested that subjects were locking the head to the trunk rather than compensating for the trunk rotations. Reflex stabilization of the head was strong throughout, with the stiffening, relax, and MA conditions exhibiting improved stability at frequencies above 1 Hz. Rising gains and large phase shifts at frequencies above 3 Hz indicated the beginning of head resonance (the mechanical contribution to stability) in the relax and MA conditions. With voluntary stabilization, particularly when the head was weighted, stabilizing gains decreased and phases became more variable above 2 Hz. More consistent compensatory responses were seen in the stiffening condition at the highest frequencies, but increasing neck muscle activation in other conditions implied that neural components continued to exert a compensatory effect.

441.3

THE CONTRIBUTION OF MOTOR UNIT RECRUITMENT TO SURFACE EMG DIRECTION DEPENDENCE. L.E. Miller *, M. Theeuwen, C. Doorenbosch, C.C.A.M. Gielen. Dept. Med. Physics, Univ. of Nijmegen, 6525 EZ Nijmegen, The Netherlands.

We have studied the direction dependence of mean rectified surface EMG and motor unit recruitment while isometric forces were applied at the wrist. Previous studies have shown that recruitment thresholds of motor units for forces in various directions can be fitted by a single straight line or by several line segments (van Zuylen et al., J. Neurophysiol. 60, 1988). In a number of muscles, several sub-populations of motor units have been found, each with different motor unit recruitment characteristics (ter Haar Romeny et al., Exp. Neurol. vol. 85, 1984). Similarly, surface EMG for forces in various directions may be fit by a circle for many muscles (Miller et al. Exp. Brain Res. Series, 22, 1992) , while for some muscles, more complex interpolating functions were required (Flanders and Soechting, J. Neurophysiol., 1990). The aim of our study was to examine the relation between motor-unit activity and surface EMG.

Recruitment contributes primarily to EMG amplitude, while rate modulation affects its spectrum. The effect on the direction dependence of EMG, of these parameters and the inhomogeneous distribution of motor units, has not been tested directly. We have simultaneously measured surface EMG and intra-muscular motor unit activity from several arm muscles while human subjects exerted isometric force in many directions within a horizontal plane. In most cases, the EMG directional dependence could be fit with a circle, and that of motor unit recruitment by a straight line. The orientation of the EMG response was generally consistent with that of the motor units. The few cases of disparity between EMG and motor unit orientation may be explained by sub-populations of motor units within a given muscle, each having a different orientation.

441.4

KINESTHETIC COORDINATION OF MOVEMENT SEQUENCES: COMPARISON OF ACTIVE *VS.* PASSIVE MOVEMENTS. L. Bevan* and P. Cordo. R. S. Dow Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, OR 97209.

In previous studies of passive elbow rotations, we have shown that the nervous system uses kinesthetic input to coordinate sequential joint rotations during movement sequences. To test the hypothesis that voluntary movement sequences are similarly coordinated, we have compared the errors in target attainment using a movement sequence that begins with either a passive, or an active joint rotation.

Subjects were asked to perform a movement sequence similar to "frisbee" throwing–they opened their right hand when they perceived that their elbow had rotated through a prescribed target angle. In half the trials we passively rotated the elbow at variable velocities. In the remaining half of the trials, subjects actively rotated their elbow at velocities within the range used in the passive trials. The arm and the target location were screened from the subject's view, and information about the target and the elbow rotation was presented on a graphics screen. To also test the effect of visual information on target attainment, subjects were given three types of visual feedback in separate blocks of trials: 1) continuous visual representation of the relative location of their arm with respect to the target, and knowledge of results, 2) knowledge of results only, or 3) no information.

The results suggest that the similarity between kinesthetic coordination of passive *vs.* active movement sequences is dependent on the visual information available. When visual information about the ongoing movement is not available, passive and active movement sequences are kinesthetically coordinated in a similar way.

441.5

SINGLE-TRIAL ESTIMATION OF THE MECHANICAL PROPERTIES OF THE ELBOW JOINT DURING A MOTION TRACKING TASK. Y. Xu, J.M. Hollerbach* and I.W. Hunter. Department of Biomedical Engineering, McGill University, Montreal, Quebec, Canada H3A 2B4.

To determine the time-varying dynamic stiffness parameters (inertia, viscosity, stiffness) of the elbow joint during movement, an ensemble averaging method must ordinarily be used (Bennett et al., Exp.Brain Res., 1992)). Because of the many trials needed in ensemble methods and the difficulties in aligning the movements, more recently we have been investigating how well time-varying dynamic stiffness parameters may be inferred from a single movement trial. To this end, we have proposed a tracking method based on exponentially weightedleast squares (Xu et al., Proc. 13th EMBS Conf., 1991,pp. 2020-2021). The dynamic stiffness parameters must vary slowly for the tracking method to work properly, and movements must necessarily be slow. We have found that subjects have some difficulty in making smooth slow motions, when the only constraint or aid is an endpoint target, and the lack of smoothness could be adversely affecting the estimations. In the present studies, we seek to improve trajectory smoothness by a mechanical aid: an artificial elbow joint and forearm which subjects are to track. In the experiments, the artificial forearm is placed next to and parallel to the subject's arm, and the subject is instructed to track a point on the artificial forearm at the wrist level. The target movement spans 60 degrees in the vertical plane, beginning from a vertical forearm position and then extending. The movement is very slow, 12 deg/s. There are five phases in each trial, three posturephases divided by two movement phases. Pseudorandom binary force perturbations are applied to the wrist by an airjet system (Xu et al.,IEEE Trans. Biomedical Eng., 38, 1991, pp.~1111-1122), and we record force, wrist position and target position. Our results indicate that the stiffness parameter is lower during movement phases than that in posture phases. The inertial and damping parameters do not have linear trends. This research is supported by the Medical Research Council of Canada.

441.6

Neck Muscle Motor Evoked Potentials (MEPs) Elicited by Transcranial Magnetic Motor Cortex Stimulation

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MEPs following transcranial magnetic motor cortex stimulation through a 9 cm coil centered over the Cz (10-20 EEG placement system) were recorded from the sternocleidomastoid (SCM), the splenius capitis (SC), the upper trapezius (UT) and deltoid muscles of four healthy adult male volunteers. Surface electrodes were used to record MEPs during relaxation, voluntary head rotation and antero- to lateral flexion.

MEPs in the neck muscles occurred with latencies of 10.5 $\pm$ 0.94 ms in the SCM, 10.2 $\pm$ 1.05 ms in the SC, 11.6 $\pm$ 0.98 in the UT and 13.1 $\pm$ 0.63 ms in the deltoid. There are at least two different, responses recordable from the SCM in which a later response is seen with a latency of around 60 ms. The amplitude of the early response in the SCM was increased during volitional lateral flexion to the to the side ipsilateral to the recorded muscle. Also, all muscles showed increased early MEPs during voluntary contraction. Our findings show that short latency MEPs can be recorded in neck muscles. They also suggest that motor control for movement can modify MEP amplitudes in neck muscles.

441.7

EFFECTS OF RADIAL NERVE STIMULATION AND ANAESTHESIA ON INDEX FINGER MOVEMENT CONTROL. W.G. Darling*, K.J. Cole, S. Ellison, C. Steyers. Dept. of Exercise Science, University of Iowa, Iowa City, IA 52242.

The role of cutaneous and joint afferents in index finger movement control has received little study, although reports from patients undergoing surgery indicate that finger kinaesthesia is strongly affected by hand anaesthesia (Moberg 1983, Brain 106:1). We have studied the EMGs and kinematics of isolated 3-joint index finger movements during non-noxious radial nerve stimulation and anaesthesia of the index finger to determine whether control of its motion is affected by altered cutaneous and joint afferent sensations.

EMGs were recorded using indwelling hooked wire electrodes inserted into 4-6 muscles of the index finger in 10 subjects. Movements were recorded optoelectronically with infrared emitting diodes placed over the base of the 2nd metacarpal and the centers of the 3 joints. Subjects performed 4 different types of discrete motions (with vision permitted) that involved flexion or extension of all 3 joints and flexion or extension at the interphalangeal (IP) joints while the metacarpophalangeal (MP) joint was voluntary held in a flexed position. Kinematic data showed no consistent changes in movements as a result of radial nerve stimulation (train of 6 pulses at 30 Hz beginning near movement onset), however anaesthesia of the index caused decrements in movement velocities and amplitudes. EMG analysis indicated increased cocontraction of antagonists during anaesthesia and some effects on muscle activity as a result of radial nerve stimulation. Thus, these data suggest that cutaneous and joint afferent information play a relatively small role in index finger movement control in comparison to central programs and, possibly, muscle proprioceptive sensations at least when vision is allowed.

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441.8

VISUAL PROPRIOCEPTION IN AGING: EFFECTS OF INAPPROPRIATE CUES ON POSTURE. H. Sveistrup* and M.H. Woollacott, Exercise and Movement Science, University of Oregon, Eugene OR 97403

The ability to regulate posture is a function of the detection and integration of inputs from the somatosensory, visual, and vestibular systems, and activation of appropriate muscle responses to correct body sway. The challenge of maintaining balance increases when information from two or more systems is in conflict, eg. when standing in a train while the adjacent train begins to move. Visual field motion is perceived as self motion and conflicts with the somatosensory and vestibular information indicating stability. The ability to reweight the input strength from the visual system to the somatosensory/vestibular systems is required. It is possible that a loss of this ability is observed in some older adults.

We recorded the response of normal young adults (YA: aged 20-29) and normal older adults (OA: aged 65+) to visual perturbations. Subjects stood inside a "moveable room" consisting of a front wall, two side walls and a ceiling which moved TOWARD or AWAY (60 cm) from the subject. Sway was recorded via video through a one-way mirror. Surface electromyograms (EMG) were recorded from the gastrocnemius (G), tibialis anterior (TA), hamstrings (H), quadriceps (Q), trunk extensors (TE) and abdominals (A). Following an AWAY movement of the room, the perceived sway would indicate a backward fall and the corresponding EMG correction would be to activate the TA, Q, and A muscles to pull the body back to vertical.

Minimal sway was recorded in both groups. However, in the AWAY condition, the OA subjects activated the TA, Q, and A muscles more frequently than the YA. A similar increase in frequency of activation of the postural muscles has also been observed in children (aged 5 months to 6 years). The increased frequency of response may reflect a loss of sensory integration ability with aging.

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441.9

VECTORIAL COMBINATION OF CONTROL MODULES IN THE FROG'S SPINAL CORD. FA Mussa-Ivaldi, SF Giszter and E Bizzi*. Dept. of Brain & Cognitive Sciences, MIT, Cambridge, MA 02139.

In recent experiments on the spinalized frog (Bizzi, Mussa-Ivaldi and Giszter, Science, 253, 1991)we have found that the focal microstimulation of a site in the premotor layers in the lumbar grey matter results in a field of forces acting on the frog's ankle and converging to a single equilibrium position. These experiments suggested that the interneuronal circuits in the spinal cord are organised into a set of control modules that "store" a few limb postures in the form of convergent force fields acting on the limb's end-point. The goal of this investigation is to understand how such postural modules can be combined by the CNS for generating and representing a wider repertoire of motor behaviors including but not limited to a large number of postures. In a theoretical study we have found that the vector summation of the convergent force fields generated by a set of control modules is a powerful mechanism for generating and combining a variety of control patterns. The most crucial issue regarding this computational framework is whether or not the fields generated by each spinal module are actually combined by the CNS according to a principle as simple as vectorial summation. To address this issue we have considered the force fields evoked by the microstimulation of two distinct sites in the spinal cord. First, we considered the fields elicited by the stimulation of each site, separately. Then, we compared the vector sum of these two fields with the field obtained by stimulating the two sites simultaneously Our current evidence indicates that the simultaneous activation of two interneuronal sites generates a field of forces that corresponds to the vector sum of the fields generated by the stimulation of each site. However, we also found that the simultaneous stimulation of two sites within the afferent fibers may result in a field that differs significantly from the vector summation of the fields obtained at each site. For example, we have observed "winner-take-all" responses to double stimulation of afferent systems. These results suggest the vector superposition of the motor output is a specific feature implemented by the interneuronal circuits in the frog's spinal cord.

This work was supported by NIH grants NS09343 and AR26710, and ONR grant N00014/K/0372.

441.10

A COMMON MOVEMENT PROFILE IS PRESERVED BY EMG CHANGES UNDER DIFFERENT GRAVITATIONAL LOADS. N. Virji-Babul, S.H. Brown* and J.D. Cooke. Dept. of Physical Therapy, University of Western Ontario, London, Ontario, Canada N6G 1H1

In many single joint movements in the horizontal plane a triphasic pattern of muscle activation is observed. Little is known, however, about the pattern of muscle activation producing movements in the vertical plane. We examined the kinematics and muscle activation patterns during movements made in the vertical plane. Subjects made elbow flexion (against gravity) and extension (with gravity) movements of different amplitudes (5-40 deg) during a visual step-tracking task.

Movement durations and peak velocities increased with movement amplitude. In addition, all movements, regardless of gravitational loads (i.e. flexion and extension) had time symmetric velocity profiles. However, changes were observed in the muscle activation patterns. Movements with gravity were initiated by an agonist burst followed by overlapping antagonist and second agonist bursts. In movements against gravity the initial agonist and antagonist bursts occurred simultaneously.

These findings suggest that the EMG pattern is modified in order to preserve a common temporal structure for movements made under different gravitational loads.

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441.11

SENSING THE PHYSICAL DYNAMICS IN RHYTHMIC MOVEMENTS. N. G. Hatsopoulos, W. H. Warren, Jr., and J. N. Sanes*. Dept. of Cog. & Ling. Sciences and Center for Neural Science, Brown Univ., Providence, RI 02912.

We present evidence for the view that the motor system takes advantage of the physical dynamics of the limb system during rhythmic movements. In particular, we tested the hypothesis that subjects choose a preferred rate of forearm swinging which corresponds to the resonant frequency of the physical system.

Human subjects were asked to oscillate their forearms in the vertical plane at their preferred frequencies under conditions of added mass and external spring loading. They were also asked to oscillate at frequencies above and below the preferred frequencies by synchronizing their movements to a metronome. These trials were performed in order to generate a phase transfer function under each loading condition which relates the phase between the displacement of the forearm and the torque generated by the biceps and triceps as a function of frequency. This phase transfer function was used to estimate the resonant frequency and to compute the stiffness of the joint (Viviani et al., J. Phys., Paris, 72, 45-58, 1976).

The results demonstrate that the preferred frequency corresponds to the resonant frequency of the muscle-limb system under each condition, a strategy that minimizes energy expenditure. This agrees with other studies which suggest but do not directly show that the preferred rate of oscillation corresponds to the natural frequency of the pendular limb and elastic joint (Kugler & Turvey, 1987; Holt et al., Hum. Mov. Sci., 9, 1990). We also show that the internal joint stiffness remains relatively constant across added mass conditions but is modulated so as to match the impedance of the external springs, a strategy that maximizes the elastic energy stored in the joint (Goldfield et al., subm. for pub., 1992; Hogan, IEEE TAC, 29, 681-90, 1984). These findings imply that the central nervous system can sense and tune the physical dynamics of the body and environment in order to optimize its performance. The results also provide insights as to how the central nervous system may solve the degrees of freedom problem.

441.12

CONVERGENCE OF EXCITATORY INPUTS FROM THE CENTRAL GRAY MATTER VOCAL CENTER AND INFERIOR COLLICULUS TO A SINGLE RETICULAR NEURON IN THE RAT Y. YAJIMA*, and Y. HAYASHI Dept. of Physiology Hyogo College of Med., Nishinomiya, Hyogo 663, Japan

Two distinct phases of vocal communication behavior in animals, sound production and sound hearing, are assumed to be integrated in the brain. Present study was conducted to know an interface mechanism for sound communication in rat brain stem. Under Ether anesthesia a bipolar electrode was implanted stereotaxically into the ipsilateral central gray matter(PAG) vocal center using audible vocal sound yielded by pulse train stimulation as a guide. Under alpha-chloralose urethane anesthesia the other electrode was placed in the contralateral inferior colliculus(IC) on the basis of auditory field potentials evoked by acoustic stimuli presented at an either ear. A grass micropippet filled with Fast green dye was introduced into the midline dorsal medulla to search for neurons activated by electrical stimulation of PAG and /or IC. Sixty-four reticular neurons sampled so far responded exclusively to PAG and 37 to IC stimulation(Non-convergent cells). Seventeen reticular neurons, on the other hand, were activated by electrical stimulation of both PAG and IC (Convergent cells). In many convergent cells, responses to PAG stimulation were augmented remarkably when PAG stimulation was preceded approximately by 5 msec to IC electrical stimulation or by 20 msec to noise burst stimulation, indicating the presence of modulatory convergence onto a single reticular cell. Histological verification revealed that most of convergent and non-convergent neurons were found in the ventromedial part of medulla.

441.13

HUMAN FORCE CONTROL: MODELLING AND DATA. J.R. Flanagan* and A.M. Wing. MRC Applied Psychology Unit, Cambridge, UK. CB2 2EF.

Models of force control should be able to account for the both the mean form and the variability of force trajectories. Whereas a number of studies have focused on the former, little attention has been given to the pattern of variation of the force-time function. In this study, we examine the mean and variability of force trajectories produced by squeezing a force transducer between the thumb and index finger. The amplitude, duration, initial force level, and direction (loading, unloading) of the force were varied. Step changes in force and force pulses were recorded.

These data are used to test and extend the Parallel Force Unit Model of Ulrich and Wing (1991, Psych. Rev., 98, 268-294). In PFUM, force buildup and decline reflects the summation of a large number of force units with variable onset times. The number and duration of the force units are controlled. This model accounts reasonably well for the mean form and variability of brief force pulses. In this report, we assess the ability of PFUM to account for force trajectories under a range of conditions. The model is elaborated to include antagonist "muscles" and additional motor unit-like properties.

A model based on the equilibrium point hypothesis of motor control (Feldman, 1986, J. Mot. Behav., 18, 17-54) is also presented. According to this hypothesis, central commands regulate force by specifying the equilibrium position of the limb and the level of co-contraction of opposing muscles. Simulations are used to assess the form of central commands underlying force generation. In addition, we examine how variation in these commands are related to variability in observed force trajectories. The equilibrium point model proposes a uniform account of unrestrained and isometric movements.

Finally, these two models of force control are not mutually exclusive and we consider how they may be related.

441.14

MODULATION OF SOLEUS H-REFLEX DURING HEAD-BODY TILTS IN MAN. N. Paquet* and C.W.Y. Hui-Chan. School of Physical and Occupational Therapy, McGill University, Montreal, Canada H3G 1Y5.

In a previous study, we found that the soleus H-reflex amplitude increased in a sinusoidal manner with ***static*** changes in forward or backward head-body tilts, in 11 of 13 subjects (Chan and Kearney, *Neurosc. Lett.* 33:333-338, 1982). The purpose of the present investigation was to determine whether soleus H-reflex was modulated in a similar or opposite manner during ***dynamic*** forward head-body tilts.

Eleven young normal subjects stood blindfolded, and were fixed on a tilting apparatus with neck collar and appropriate straps. Sudden forward head-body tilts of 20° were applied through an axis co-linear with the ankle joints. The mean peak acceleration was constant at about 0.7 g, as measured with a linear accelerometer mounted on a dental bite. Ten H-reflexes were elicited at five intervals ranging between 30 and 250 ms after the onset of head acceleration. Only the trials in which (1) the M response was within ±10% of the control amplitude, and (2) the soleus EMG activity was at a constant low level prior to tilt onset, were kept for analysis.

The main finding is that in the majority (8 of 11) of subjects, the soleus H-reflex was inhibited ($p<0.05$) by a mean of 24% (range = 5 to 53% of control H) at 150-250 ms after tilt-onset. In the remaining subjects, the tilt had no effect on the H-reflex amplitude in 2 subjects, and was facilitated by 30% at 110-200 ms after tilt-onset in one subject. These results appeared to reflect real changes, because two subjects tested on different occasions showed a similar (inhibitory) response pattern.

Our results indicate that the effect of dynamic head-body tilts on soleus H-reflex is opposite to that of static tilts, at least in a majority of subjects. Among other possible causes, the opposite modulation may reflect a difference in the stimulus given to the vestibular apparatus under the two tilting conditions, i.e. a transient acceleration during the dynamic tilt, and a change in the orientation of the gravity vector during the static tilt.

This project was supported by McGill University and an MRC studentship for N.Paquet.

441.15

MODIFICATIONS IN THE SPEED-INSENSITIVE STRATEGY FOR MOVEMENTS REQUIRING LOW LEVELS OF FORCE. D. M. Corcos*, G. L. Gottlieb, C. -H. Chen. University of Illinois at Chicago, Chicago, IL, 60608 and Rush Medical Center, Chicago, IL 60612.

The dual strategy hypothesis of motor control suggests that human elbow movements performed over different distances and against different inertial loads are controlled by excitation pulses to agonist motoneuron pools that are modulated in width (Gottlieb, Corcos and Agarwal, 1989). The antagonist muscle is delayed for longer movements and movements against larger loads. In addition, there is less myoelectric activity for longer movements than for shorter movements. The work of Hoffman and Strick (1990) suggests that wrist movements performed over short distances are controlled by a combination of height and width modulation and that the antagonist is not delayed for longer movements. To investigate this discrepancy, seven subjects performed movements over seven different distances that encompassed the range of movement distances employed in both sets of previous experiments. Position, acceleration and EMGs from flexor and extensor muscles were measured. In addition, velocity and impulse were calculated. The findings revealed that the myoelectric, kinematic and kinetic variables for the shorter movements at the elbow joint do not rise at the same rate as longer movements and that the antagonist is scaled with distance for the shorter movements but not for the longer movements. The relationship between antagonist EMG and impulse can be improved for the longer movements by including a term corresponding to a viscous force in the calculation of impulse.

These findings and the work of Hoffman and Strick (1990) suggest that short movements, which require brief pulses of force, are generated by pulses of excitation that have reached a minimum width. The only way to perform these movements is by using lower levels of intensity than are used for longer movements. As such, the dual strategy hypothesis has been reformulated so that when movements of short distance and/or with very light loads are generated, intensity is reduced when distance or load reaches very low levels.

This work was supported by NIH grants NS 23593, AR 33189 and NS 28176.

441.16

THE INFERIOR OLIVARY NEURON AS AN ERROR DETECTOR IN THE MOVEMENT DISORDER INDUCED BY INTRACEREBROVENTRICULAR INJECTION OF PROPIDIUM IODIDE IN THE RAT. S. Chen and Z.-C. Peng (SPON: EBBS). Institute of Anatomy, University of Verona, Italy.

Intracerebroventricular (ICV) injection of propidium iodide (PI) in the rat results in a movement disorder characterized by nystagmus, ataxia and shaking (Borges et al., Science 228, 346, 1985; Chen and Su, Brain Res 483, 379, 1989) and induces *c*-Fos expression in discrete brain areas, including the inferior olivary complex and cerebellar nuclei (Chen and Bentivoglio, Soc Neurosci Abstr 17, 864, 1991). In the present study, in order to investigate whether the *c*-Fos expression is induced directly by ICV injection of PI or indirectly by the subsequent movement disorder, we studied the *c*-Fos expression induced by ICV injection of PI under urethane anesthesia, in which the animals do not show any movement disorder. PI (10 µl, 0.1% in saline) was injected under urethane anesthesia (1.2 g/kg, i.p.) into the lateral ventricle through a previously implanted cannula. The animals were sacrificed 3 hrs after the PI injection. *C*-Fos immunoreactivity was distributed in the cerebellar nuclei as in the cases in which PI was injected without anesthesia. However, in contrast to the latter cases, in the inferior olivary complex only a few neurons in the medial nucleus were *c*-Fos immunoreactive. In the control cases injected with saline no *c*-Fos immunoreactitvity was observed in the cerebellar nuclei or in the inferior olivary complex. The present data, together with our previous findings, suggest that after the ICV injection of PI the activation of inferior olivary neurons results from the subsequent movement disorder, whereas the cerebellar nuclei are directly activated by PI administration. This supports the hypothesis that the inferior olivary neuron functions as an error detector or 'teacher' in motor control.

441.17

PROPRIOCEPTIVE AND VESTIBULAR INPUTS TO NEURONS OF THE CENTRAL CERVICAL NUCLEUS IN THE RAT
B. Ragnarson, L.B. Popova, G.N. Orlovsky and G. Grant* Department of Anatomy, Karolinska Institutet, Box 60 400, S-104 01 Stockholm, Sweden.

Activity of neurons in the central cervical nucleus (CCN) of the rat was recorded extracellularly in decerebrate, immobilized animals. Neurons were identified by their antidromic response to electrical stimulation of the contralateral superior cerebellar peduncule.

Stimulation of the ipsilateral dorsal neck muscles (m. biventer cervicis [MBC] and m. splenius [MS]) by a single electric pulse evoked a short-latency (1.0–3.0 ms) response in the majority of CCN neurons. The response was usually followed by a period of inhibition of the resting discharge of CCN neurons lasting for about 30 ms. Stimulation of the contralateral MBC and MS usually produced only inhibition of the resting discharge (lasting up to 30 ms), though in a few cells a short-latency excitation was also observed. Mechanically induced stretching of these muscles resulted in an up to 100% increase in tonic activity, comprising a static component during prolonged stretching and usually also a dynamic component with greater increase of discharge in the beginning of the stretch and inhibition at the end.

Stimulation of vestibular receptors by roll tilt of the animal (±20°) resulted in modulation of the discharge of CCN neurons. When sinusoidal movements (0.5–1Hz) were applied, neurons exhibited a maximal activity either at the maximal contralateral deflection (contralateral ear down, in relation to a neuron) or during transition from the ipsilateral (ipsilateral ear down) to the contralateral position. Usually, neurons also showed a static response, i.e. their tonic activity at continuously maintained contralateral tilt was 30–40% higher than at ipsilateral tilt.

It can thus be concluded that the CCN of the rat is a center of integration of afferent signals of two different modalities (proprioceptive and vestibular), as it was found earlier in experiments in the cat (Hirai et al. 1979). We have also found that the CCN responds not only to dynamic but also to static vestibular stimuli. Neurons of the CCN transmit to the cerebellum information about various aspects of head movement, as well as about maintained orientation of the head in space and in relation to the body.

441.18

Compliant Motion During Surface Following by the Human Arm
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Our goal is to understand what strategies are used by the CNS to control forces during movement when in contact with a surface. We have adapted the classic robotics task of "peg in hole" to study this form of movement experimentally. Subjects grasped a joystick with two degrees of freedom and used movements of the arm and wrist to control the movement of a "peg" on a video screen. Motors attached to the joystick were used to simulate the forces of contact between the peg and a flat, rigid surface. The subject was required to slide the peg along the surface withan applied normal force until the peg fell into the hole.

We considered two hypotheses about the control algorithm used to accomplish this task. In the first, called *passive compliance*, the CNS commands the position of the limb, specifying a nominal trajectory for the endpoint based on *a priori* knowledge of the surface. To generate a force, a *virtual* trajectory that passes inside the suface is commanded. This, coupled with a compliant endpoint impedance, results in a movement along the surface with an applied normal force. In contrast we considered an *active compliance* model, in which the normal force is measured continuously during the movement, and compared with a reference, to generate a movement step which maintains the desired value.

For an unexpected change in suface orientation, the virtual position control model predicts ans increesease in force for upward tilt, while the active force control model predicts a constant force output. Subjects executed the task using a contact force of 5 to 10 Newtons. In all subjects tested so far (n=4) the normal force averaged over the entire sliding movement increased for upward tilts of the surface, and decreased for downward tilts, as compared to nominal horizontal movements. The force does not, however, continue to increase throughout the trial. These results suggest that for short time scales the system acts as a passive compliance, but that over longer time intervals force information may be used to modify the virtual trajectory.

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441.19

ASSOCIATION BETWEEN RHYTHMICAL AND DISCRETE COMPONENTS OF MOVEMENTS. S.V. Adamovich, M.F. Levin, D.J.Ostry* and A.G. Feldman. Inst. for Problems of Information Transmission, Acad. of Sci., Moscow 101447, Russia; McGill Univ., Centre de Recherche, Inst. de réadaptation de Montréal, Inst. de génie biomédical, Univ. de Montréal, Montreal, Canada H3S 2J4.

Single-joint discrete movements are likely produced by monotonic shifts in the equilibrium point (EP) of the system. On the other hand, oscillatory shifts of EP may underlie rhythmical movements. We tested the hypothesis that the two types of control signals can be superimposed to produce both movements simultaneously (Feldman 1980). Subjects performed fast rhythmical elbow flexion and extension movements about a target position indicated by a light on a horizontal surface. When the target position was shifted by 60°, the subjects made a fast movement to the new target while continuing the oscillation. In other experiments, subjects made isolated discrete movements from one target position to the other. Arm position was recorded on a Watsmart system, and velocity and acceleration were calculated. EMG signals were recorded from two elbow flexors and two elbow extensors. The onset of discrete movement was associated with a deflection in the EMG signal and used as a synchronisation point for averaging of kinematic and EMG data. After averaging, the rhythmical component of the movement was eliminated and the residual discrete component was comparable to the isolated discrete movement made to the same target position. Fast discrete movements arose at any phase of the rhythmical movement, although some phases were preferred. Discrete movements performed in combination with rhythmical ones displayed multi-phasic EMG patterns and kinematics similar to those associated with isolated discrete movements. The data is consistent with the hypothesis of superposition of control signals underlying discrete and rhythmical movements when they are performed simultaneously. However, the discrete component obtained by averaging displays a greater number of terminal oscillations and EMG bursts indicating that there is an interdependency of the two motor components at the executive biomechanical level. (Supported by NSERC Canada).

441.20

THROWING IN THREE DIMENSIONS. J. Hore*, S. Watts and D. Tweed. Dept. of Physiology, Univ. of Western Ontario, London, Canada, N6A 5C1

What are the rules by which skilled multijoint movements in 3 dimensions are controlled? Which factors determine speed and which determine accuracy? As a start to answering these questions we studied the kinematics of overarm throwing. Simultaneous 3-D rotations of the hand, forearm, upper arm and clavicle were recorded at 500 Hz using search coils as 6 subjects sat with fixed trunk and threw balls.

Results: 1. When throwing at targets in different directions, the orientation of the hand at the moment of ball release remained relatively constant with respect to gravity, i.e. the hand was oriented approximately as if mounted on a Fick gimbal, as was the case for pointing movements. 2. Accuracy did not depend on hand orientation at the moment of release nor on the timing of release relative to any kinematic parameter. The reason, revealed by photography and hand translation reconstructed from search coil signals, was that the hand path in space to the point of release was approximately straight, and so the variation in the timing and location of the release point along the straight path made little difference to the direction of the ball's flight. 3. Tight linear relations, which held for all throwing speeds, were found between the angular positions in space of adjacent limb segments, i.e. the straight hand path, which could geometrically have been produced by many different patterns of joint motion, was always generated the same way, and faster throws were achieved by running the same joint motions faster.

441.21

PERCEPTION OF VISCOSITY: SENSORY THRESHOLDS. L.A. Jones*[1] and I.W. Hunter[2], School of Physical & Occupat. Therapy[1] and Dept. Biomed. Eng.[2], McGill University, 3654 Drummond St., Montreal, Canada H3G 1Y5.

Sensory thresholds have been calculated for a number of different aspects of the human proprioceptive system including limb position, movement, force, and stiffness. Most of this research has focussed on differential thresholds, that is, the amount a stimulus must be incremented or decremented in order for the subject to discriminate that two stimuli differ. For the proprioceptive system, there is a remarkable consistency in the differential thresholds measured for position, movement and force with the values varying between 6% and 10%. In contrast, subjects are much less consistent in discriminating changes in stiffness, for which the threshold is 23% (Jones & Hunter, 1990).

The objective of the present experiment was to extend these findings to an analysis of the sensitivity of human subjects to changes in viscosity. This was measured using the matching procedure in which eleven subjects adjusted the viscosity of a servo–controlled linear motor connected to their left arm (via a foot pedal containing an angular position transducer) until it was perceived to be the same as that of the motor connected to their right arm. The servo–system was under computer (IBM 320) control and force and position were recorded from each motor via the computer's A/Ds. The viscosities ranged from 2 N.s/m to 1024 N.s/m, and there were 8 repetitions of each of the 10 viscosities.

The matching function obtained for viscosity was linear, with a very small deviation from linearity at the lowest viscosity (i.e. 2 N.s/m).The differential thresholds calculated for viscosity ranged from 83% at the lowest viscosity to 26% at the highest, and averaged 34% over the range of 32 to 1024 N.s/m. These values are almost 50% higher than those reported for stiffness, suggesting that the human proprioceptive system is less efficient at integrating force and velocity signals to perceive changes in viscosity, as compared to force and displacement cues for stiffness.

CIRCUITRY AND PATTERN GENERATION II

442.1

MODULATION OF THE RELATIVE ROLES OF SPIKING AND GRADED SYNAPTIC TRANSMISSION BETWEEN GASTRIC PATTERN-GENERATING NEURONS. R.C.Elson and A.I.Selverston*, Department of Biology, UCSD, La Jolla CA 92093.

Neurons of the gastric central pattern generator (CPG) in the lobster stomatogastric ganglion release transmitter in response to graded depolarizations as well as spikes. The lateral gastric neuron (LG) inhibits and is inhibited by 2 LPG neurons. Without neuromodulators, the gastric CPG is quiescent. LG is silent while the LPGs fire tonically. Depolarizing LG evokes graded inhibition of the LPGs with little contribution by its high-threshold spikes. The muscarinic agonist, pilocarpine, initiates rhythm generation in the gastric CPG, modulating both cellular and synaptic properties. At the LG-to-LPG, it: (1) potentiates graded and spike-evoked synaptic potentials; (2) depolarizes LG; and (3) reduces LG's spike threshold. Whereas in control conditions, small depolarizations of LG elicit small, graded inhibition, under muscarinic modulation they elicit spikes, driving large IPSPs, producing dramatic inhibition. LG can now evoke large postsynaptic effects with little baseline depolarization, via spike generation and spiking transmission. These effects are important during rhythm initiation and burst transitions. In contrast, spiking transmission is not important when cells produce large plateau potentials (partly due to synaptic saturation); here pattern-generating interactions depend largely on graded transmission. Thus, the relative roles of spiking and graded transmission vary with modulatory conditions and the biophysical states of pre- and postsynaptic cells.

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442.2

PROPERTIES OF A RELAXATION-OSCILLATOR-BASED MODEL OF THE LOBSTER GASTRIC MILL CPG P.F.Rowat* and A.I.Selverston Biology Department, U.C. San Diego, La Jolla, CA 92093–0322.

The purpose of this work is to develop a model of the lobster gastric mill CPG that is as simple as possible yet is biologically plausible, and which captures significant network properties such as the emergence of patterns from network connectivity rather than endogenous cellular burst capability, continued pattern generation when cells are removed, and changes in the pattern after application of modulatory substances. The cell model, derived from the relaxation oscillator, has one fast current and a slow current that can be regarded as the combination of a slow inward and a slow outward current. It displays plateau potentials, postinhibitory rebound, postburst hyperpolarization, and endogenous oscillations for suitable settings of two parameters. One parameter controls whether the fast current has a region of negative resistance and the other controls the gain of the slow current. When the region of negative resistance is present, endogenous oscillations occur. A network model of the complete gastric circuit was made, using a model of graded synaptic transmission but excluding spike-based transmission. For a wide range of parameter values, the model generates phase-relationships that are approximately correct. Adjusting the gains of the slow currents in different cells causes small changes in phase relationships. A slow excitatory synapse causes a significant phase delay. Thus, a close match to *in vitro* recordings is possible. Also, some phase changes caused by neuromodulator application can be reproduced. The model network oscillates even when no component cell is an endogenous oscillator. When cells are killed, the network continues to generate a pattern provided at least one pair of reciprocal inhibitory cells remains. Current pulses were injected into cells in the network. Phase response curves and the limits of entrainment were computed for the model network, and compared with the biological data.

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442.3

AMINE MODULATION OF MIXED CHEMICAL-ELECTRICAL SYNAPSES IN THE PYLORIC NETWORK OF THE LOBSTER STOMATOGASTRIC GANGLION. B.R. Johnson*, J.H. Peck and R.M. Harris-Warrick. Section of Neurobiology and Behavior, Cornell University and Department of Psychology, Ithaca College, NY 14853.

Dopamine (DA, 10^{-4}M), serotonin (5HT,10^{-5}M) and octopamine (Oct, 10^{-5}M) each produce a unique motor pattern from the pyloric network in the stomatogastric ganglion of the lobster *Panulirus interruptus*. We are examining how amines modulate the pyloric rhythm. Here we describe amine effects on mixed graded chemical-electrical transmission between two different pairs of pyloric neurons. The AB and VD and the LP and PY neurons are electrically coupled; in addition, the AB chemically inhibits the VD, while the LP and PY are reciprocally inhibitory. Modulation of mixed synaptic interaction was examined in isolated neuron pairs in TTX-saline (20° C). DA and 5HT reduced electrical coupling and enhanced chemical inhibition between the AB and VD. DA also reduced electrical coupling and enhanced the reciprocol chemical inhibition between the LP and PY. Oct enhanced while 5HT reduced PY chemical inhibition of the LP with little effect on electrical coupling. As a consequence of these differential effects on chemical and electrical transmission, the amines could reverse the sign of synaptic interactions between these cells. For example, depolarization of the LP depolarizes the PY in control conditions; this reverses to hyperpolarization during DA perfusion. In preparations where the AB to VD chemical synapse is weak and electrical coupling dominates, DA and 5HT change the synaptic interaction from weakly excitatory to strongly inhibitory. Modulation of mixed synaptic interactions contributes to the structure of the motor patterns produced by these amines. Supported by NRSA NS07859 and NIH grant NS17323.

442.4

AMINE MODULATION OF ELECTRICAL COUPLING IN THE PYLORIC NETWORK OF THE LOBSTER STOMATOGASTRIC GANGLION. J.H.Peck* B.R. Johnson AND R.M. Harris-Warrick, Section of Neurobiology and Behavior, Cornell University and Dept. of Psychology, Ithaca College, Ithaca,N.Y. 14853.

We examined the I/O characteristics and amine modulation by dopamine (DA), serotonin and octopamine of 5 electrical synapses in the pyloric network in the STG of *Panulirus interruptus*. Some synapses (AB-PD and PD-PD) were non-rectifying, while the others (AB-VD, PD-VD, and LP-PY) all show significant rectification. The amines altered the strength of electrical coupling, but this varied with the different pairs of neurons and with the direction of current flow. For example, DA enhanced PD-AB but reduced AB-PD coupling. We modeled these results by an input resistance increase in the AB and decrease in the PD with no change in junctional resistance. DA causes these input resistance changes. For other synapses (such as DA reduction in AB-VD coupling), our model predicts that the amine reduces the junctional coupling in addition to altering cellular input resistance. Modulation of electrical coupling is a new mechanism by which amines modify the pyloric network to generate multiple motor patterns. (NRSA NS07859 and NIH NS17323)

442.5

MECHANISMS FOR DOPAMINE-INDUCED PHASE SHIFTS IN THE PYLORIC MOTOR PATTERN IN THE LOBSTER STOMATOGASTRIC GANGLION. R. M. Harris-Warrick*, A.J. Tierney and L. Coniglio. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

In the triphasic pyloric motor pattern, the pacemaker neurons inhibit the LP and PY neurons; these cells show different rates of post-inhibitory rebound, causing them to fire at different phases in the cycle. Dopamine (10^{-4}M) excites the LP and PY cells and causes a significant phase advance in their activity. This is explained in part by dopamine inhibition of the pacemaker PD neuron. Two additional mechanisms for phase advance are intrinsic to the post-synaptic PY and LP cells. 1) Conductance decrease of I_A. Both LP and PY express a transient K^+ current (I_A) that is selectively sensitive to 4-aminopyridine (4-AP). This current plays an important role in the rate of rebound after hyperpolarization in synaptically isolated LP and PY neurons. Both DA and 4-AP reduce the delay to first spike after 200 msec hyperpolarizing pulses, and increase the frequency of firing in response to tonic depolarization. Blockade of I_A by 4-AP occludes the effect of DA on the PY cell, suggesting that reduction of I_A is the major action of DA in this cell. 2) Enhancement of I_h. The LP cell displays a Cs^+-sensitive depolarizing sag during tonic hyperpolarization, caused by the hyperpolarization-activated inward current, I_h. DA enhances this sag, specifically by shifting its voltage dependence for activation in the depolarizing direction. This effect is eliminated by low concentrations of Cs^+, which eliminate I_h. This suggests that DA acts on the LP by both reducing I_A and enhancing I_h. We are now using voltage clamp of cultured LP and PY cells to try to directly measure dopamine modulation of these currents. Supported by NIH NS17323 and NS 25915.

442.6

INWARD CURRENTS UNDERLYING BURSTING IN CULTURED STOMATOGASTRIC NEURONS. G.G. Turrigiano* and E. Marder, dept. of biology, Brandeis University, Waltham, Ma 02254

Stomatogastric (STG) neurons *in situ* express slow regenerative conductances that underlie bursting and plateau behavior. We use primary cultures of STG neurons from the spiny lobster *Panulirus interruptus* to identify two inward currents that contribute to these properties. STG neurons in culture switch from tonic firing to bursting in TEA. In most neurons these oscillations, as well as fast spikes, can be blocked by TTX. In other neurons TTX has little effect on oscillations; these oscillations are blocked by Mn^{++}. Under two electrode voltage clamp we have identified two persistent inward currents, one that is blocked by TTX, and one that is blocked by Mn^{++}. The TTX-sensitive current begins to activate above -40 mV, peaks at -20 mV, and has an extrapolated reversal potential of +20 mV. The Mn^{++}-sensitive current activates above -40 mV, peaks at +20 mV, and reverses at +50 mV; the magnitude and reversal potential of this current depend on the external Ca^{++} concentration, suggesting that the current is carried by Ca^{++} ions. Most STG neurons have both inward currents, but their relative magnitudes, and thus the pharmacological sensitivity of bursting, vary from cell to cell. We are currently investigating whether the ratio of these conductances is a function of neuron type. Supported by NS-08971 to G.G.T. and BNS-9009251 to E.M.

442.7

REAL TIME INTERACTION BETWEEN A MODEL NEURON AND THE CRUSTACEAN STOMATOGASTRIC NERVOUS SYSTEM.

G. LeMasson, S. Renaud-LeMasson, A. Sharp, L.F. Abbott, and E. Marder* Biology Dept. Brandeis Univ. Waltham, MA 02254.

Model neurons are useful in understanding the complex interactions between single currents in a cell, or between different cells within a network. To study the dynamics of both synaptic influences and intrinsic properties of neurons we connect a conductance-based model neuron to a real neural network. We used a DSP board and the MAXIM software package to run the model and control the Input-Output interface. The system allows the model neuron to run and respond in real time to artificial synapses created between real neurons and the model neuron. The model neuron was constructed using data from the LP neuron of the stomatogastric ganglion. We make reciprocal inhibitory connections between the model LP neuron and a biological PD neuron and study the effect of changes in ih and iA current on the hybrid network, as well as the effect of changing the strength of the synapses between the model and the biological neuron. The results suggest that the model LP firing phase in the pyloric period depends critically on the characteristics of the synaptic inhibition coming from the PD neuron. The burst duration and the firing frequency of the LP model depends on ih and iA.

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442.8

Dynamic Clamp: A method to construct artificial synapses and assess the role of voltage-dependent and synaptic conductances. A.A. Sharp*, M. O'Neil, G. LeMasson, L.F. Abbott, E. Marder. Dept. of Biology, Brandeis University, Waltham, MA 02254.

Conventional voltage-clamp techniques provide data describing how the conductances that shape neuronal excitability depend on voltage and time, but do not allow the investigator to determine the participation of individual currents in the dynamic behavior of a neuron. We have devised a method, called a dynamic clamp, that allows us to increase, decrease or alter conductances in neurons during their normal dynamic behavior. This method is used to study the effects of a given voltage-dependent or synaptic conductance on a cell's intrinsic behavior and the emergent properties of a network. It is also used to create new networks by generating artificial synapses.

To construct the dynamic clamp, an Axoclamp in DCC mode is used to monitor the cellular potential and to inject current. Based on this potential and a computational model describing the dynamics of the conductance under investigation a computer determines the current to be injected. When constructing synapses both the pre- and post-synaptic potentials are monitored. The investigator controls the relevant thresholds, reversal potentials and time constants. We use this method to create synapses between dissociated neurons in culture, in the intact stomatogastric ganglion and/or to modify the intrinsic properties of neurons within these networks. Supported by MH 46742 and NSF BNS 9009257.

442.9

CRUSTACEAN CARDIOACTIVE PEPTIDE ACTIVATION OF THE PYLORIC NETWORK IN THE STG OF THE CRAB, *Cancer borealis*. J.M.Weimann*, H.G.Heinzel, and E.Marder. Biology Dept., Brandeis Univ., Waltham, MA 02245 and Institute of Zoology, Univ. of Bonn, Bonn, Germany.

The highly conserved crustacean cardioactive peptide (CCAP) activates the pyloric rhythm of the stomatogastric nervous system in a dose-dependent manner with a threshold at 10^{-10}M. The number of spikes/burst in the lateral pyloric (LP) neuron, a major target, increases from 2-5 spikes/burst in control saline to >50 spikes/burst in 10^{-6}M CCAP. This increase spikes/burst is associated with the induction of plateau potentials in the LP neuron. Bath application of CCAP ($>10^{-8}$M) causes oscillations in the membrane potential of the pharmacologically isolated LP neuron. The phase of the oscillations can be reset with brief current pulses into the LP soma. This is responsible for the change in the LP duty cycle from 10% to over 40% of the phase in 10^{-7}M CCAP.

Simultaneous endoscopic observations of the valve between the gastric mill and the pyloric chamber and extracellular recordings of the major STG motor nerves in intact crabs suggest that changes in the LP neuron's activity dramatically alter the movements of this valve.

Supported by NS17813 (EM), GRF HE1118 (HGH) and Human Frontiers Science Program.

442.10

SELECTIVE ENHANCEMENT OF RHYTHMIC PACEMAKER ENSEMBLE ACTIVITY BY A NEWLY IDENTIFIED MODULATORY NEURON. M.P. Nusbaum*, M.J. Coleman and B.J. Norris. Neurobiology Research Center and Dept. of Physiology & Biophysics; Univ. of Alabama at Birmingham; Birmingham, AL 35294.

The pyloric neural network, in the crustacean stomatogastric ganglion (STG), produces a three-phase motor pattern. Variants of the pyloric rhythm are produced in the presence of different modulatory inputs, some of which have been identified (Nusbaum et al., J. Neurosci. 12:In press). Recently, we identified a neuron, Modulatory Commissural Neuron 5 (MCN 5), that increases the cycle frequency and decreases the number of active phases in the pyloric rhythm of the crab, *Cancer borealis*. The MCN 5 soma is located in the commissural ganglion and projects to the STG via the inferior oesophageal nerve and stomatogastric nerve.

Intracellular stimulation of MCN 5 increased the pyloric cycle frequency, at least partly by exciting the pyloric pacemaker neuron AB. Each action potential elicited in MCN5 produced a fixed latency EPSP in the AB neuron. MCN 5 stimulation also enhanced the activity of the PD and LPG dilator motor neurons, which are electrically coupled to AB and fire their impulse bursts during the AB phase of the pyloric rhythm. The remaining pyloric components, which would be active during the two cycle phases following AB activity, are all inhibited during MCN 5 stimulation. With increasing stimulation of MCN 5, the activity of these pyloric components could be completely suppressed, producing a faster pyloric rhythm with only a single active phase. MCN 5 activity thus is capable of sculpting an active, single phase motor pattern by selective enhancement and suppression of individual neural network components.

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442.11

A motor basis for the radula movements underlying ingestion and rejection in *Aplysia*. D. W. Morton[2*], H. J. Chiel[1,2], Depts. of [1]Biology and [2]Neuroscience, Case Western Reserve Univ., Cleveland, OH 44106

We are studying the neural basis of the radula movements underlying *Aplysia's* consummatory feeding behavior. During ingestion, the radula is open as it protracts and closed as it retracts. During rejection, the radula is closed as it protracts and open as it retracts. We have identified several buccal ganglion motor neurons that produce specific radula movements and observed their activity during ingestion-like and rejection-like motor patterns in a reduced preparation. Stimulation of identified neuron B10 produces a sphincter-like contraction of the anterior buccal cavity and retraction-like movements when the radula is held in a protracted position. Stimulation of neurons B8a and B8b produces radula closure. During the ingestion-like pattern, B8a, B8b, and B10 are active during the retraction portion of the pattern, consistent with observations that during ingestion, the radula is closed as it retracts. During the rejection-like pattern, B8a and B8b are active during the protraction portion of the pattern, while B10 is active during the retraction portion, consistent with the observation that during rejection, the radula is closed as it protracts. In addition, the extracellular activity produced by these cells is consistent with neural activity observed *in vivo* during ingestion and rejection. Thus, our results suggest that B10 contributes to radula retraction, B8a and B8b contribute to radula closure, and the differential activation of these neurons may contribute to the difference between ingestion and rejection. Studying the mechanisms underlying these two feeding-like patterns could reveal how a neuronal circuit can generate more than one behaviorally relevant pattern.

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442.12

IONIC MECHANISM FOR SWITCHES IN ACTIVITY OF INTERNEURONS THAT CONTROL HEARTBEAT IN THE LEECH

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During the heartbeat of the leech, *Hirudo medicinalis*, the rhythmic activity of the segmental heart motor neurons is shaped by inhibitory input from heart interneurons (HN), a pair of which is located in each of the seven anterior ganglia (Thompson and Stent (1976) J Comp Physiol 111; Calabrese (1977) J Comp Physiol 122). The basic rhythm is produced by an oscillator comprising the HN cells in the four anterior ganglia, while the HN cells of the fifth ganglion, HN(5), have a different function. Both cells receive alternating rhythmical inhibitory inputs from the oscillator, but at a given time, only one of the two HN5 cells is active. This cell discharges high frequency bursts of action potentials between the rhythmical inhibitions, while the other cell produces only few or no action potentials (Calabrese 1977). Due to the different activity states, only one of the HN(5) cells effectively influences postsynaptic premotor neurons, which are connected to both of them. The activity of the HN(5) cells causes one of the bilateral heart tubes to contract peristaltically, the other synchronously.

Every 20 to 40 heartbeat cycles occurs a simultaneous switch between the active and the inactive state in the HN(5) cells. So far, the underlying mechanism of this switch is not known. The switch is not associated with a change of synaptic potentials. In this study, we investigated the ionic mechanisms of the HN(5) cells using switching single electrode voltage clamp. During the active state the membrane conductance is lower than in the inactive state, suggesting that the permeability of a hyperpolarizing ion is increased during a switch to the inactive state. The reversal potential of this current is close to that of Cl^-.

(supported by NIH NS24072)

442.13

FUNCTIONAL CHARACTERIZATION OF A NEURAL NETWORK FROM THE CNS OF THE SNAIL *LYMNAEA STAGNALIS*. N.S. Magoski*, N.I.S. Syed and A.G.M. Bulloch. Dept. Med. Physiology and Neuroscience Research Group, Univ. Calgary, Faculty of Medicine, Calgary, AB T2N 4N1 Canada.

We have identified a network of chemically and electrically connected neurons in the CNS of the pond snail *Lymnaea stagnalis*. Three large identified neurons termed right parietal ventral one - three (RPV1-3) (Symp.Biol.Hung.1976:41) were found to be electrically coupled to one another. These neurons send projections through nerves which innervate musculature involved with whole-body withdrawal and cardio-respiratory behavior. Neurons RPV1-3 were found to be inhibited by respiratory pattern generator interneurons, VD4 and IP3I (J.Comp.Physiol.A 169:557). Conversely, a pair of interneurons, L/RPeD11, that coordinate locomotor and withdrawal behavior (J.Exp.Biol.158:37) excite cells RPV1-3. Using isolated brain, semi-intact and cell culture preparations we have characterized the in situ and in vitro properties of this network. The connectivity and morphological characteristics of this neural circuit suggest a possible role in whole-body withdrawal; however, the functional significance of these neurons remains to be fully determined.

Supported by MRC (Canada) and AHFMR.

442.14

BIFURCATION DYNAMICS OF MULTIFUNCTIONAL NETWORKS ARE AFFECTED BY ERRORS THAT THE NETWORKS THEMSELVES GENERATE. G. J. Mpitsos*, M. A. Andrade, J. C. Nuño, F. Morán, F. Montero. Mark O. Hatfield Marine Science Center, Newport, OR 97365, and the Depts. of Biochemistry-Molecular Biology and Mathematics at the Univ. of Madrid, Spain.

Catalytic networks have been used as models to examine many biological systems, from prebiotic evolution to neural networks and population biology. Biological systems are inherently variable and error-prone, as shown for example by work on the multifunctional and variable motor systems of the sea slug *Pleurobranchaea* (Mpitsos and Cohan, 1986, J. Neurobiol.). In this regard, catalytic networks may provide rapid insights into the effect that error itself may have on the dynamics that a system can generate; i.e., error may not only be a product of a system, it may also feed back onto the system's self-organizing potential. Schnabl et al. (1991, Physica D) examined catalytic networks in which error arises by intermutations between reciprocally interconnected elements of the network. We have examined the more biologically plausible condition when error arises in chemical systems through the generation of mutant species (error-tail) that compete for substrate with the catalytic species but have no enzymatic effects on them. This allows for both reciprocal and one-way connections among the elements of the network, as occurs in all biological systems. Both models show that error affects system dynamics, but complex behavior in the error-tail model is more stable in the face of variation or noise in the error-related parameters. Catalytic networks may also provide insight into network architectures that permit bifurcation into rich repertoires of patterns of activity relating to multifunctionality. We discuss the application of catalytic networks to neural systems.

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442.15

BURST QUANTIFICATION IN CULTURED NEURONAL NETWORKS. J.C. Weil*, S.P. Fracek, B.K. Rhoades and G.W. Gross. Department of Biological Sciences, University of North Texas, Denton, TX 76203.

Cultured mammalian neuronal networks can be pharmacologically induced to exhibit a range of stable temporal spike activity patterns extending from quiescence, through isolated spiking, irregular and regular bursting, and burst fusion, making them ideal preparations for evaluating methods of burst detection and quantification.

Isolated networks of 50-200 neurons were grown on multimicroelectrode plates (MMEPs) from dissociated tissue of 14 day fetal mouse spinal cords or olfactory bulbs. Single unit spike trains for 15-60 minute periods were extracted from the extracellular recordings and digitally stored as time series records. In addition two types of artificial data sets were constructed - "bursting" spike trains of bursts generated with a semi-Markov model and "non-bursting" spike trains with interspike intervals (ISIs) randomly sampled from Poisson, beta, or gamma distributions.

Five burst identification algorithms are implemented and applied to this data: an ISI threshold based on a first-order statistic (Habets *et al., Exp. Brain Res.* 69:43, 1987), an ISI threshold for burst onset with a "Poisson surprise" statistical rule for burst offset (Legéndy & Salcman, *J. Neurophys.* 53:926, 1985), an ISI threshold chosen as the first local minimum following the first mode of the smoothed ISI histogram (Cocatre-Zilgien & Delcomyn, *J. Neurosci. Meth.* 41:19, 1992), a slope threshold applied to a cumulative sum function of the spike train (David Tam, personal communication, 1992), and our own dual-threshold method which performs fast and slow integrations of the spike train with one threshold applied to the fast integration and the second threshold applied to the fast-slow integration difference. Burst identification methods are evaluated on their relative abilities to produce parametric measures and descriptive statistics which compare favorably with hand measurements, distinguish artificial bursting and non-bursting spike trains, allow both temporal and spatial network analysis and comparisons across treatment conditions in the burst domain, and extend to multiunit records.

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442.16

PATTERNED ELECTRICAL STIMULATION OF CULTURED NEURONAL NETWORKS ON MULTIMICROELECTRODE PLATES. B.K. Rhoades* and G.W. Gross. Dept. of Biol. Sci., University of North Texas, Denton, TX 76203.

Cultured neuronal networks derived from the embryonic mammalian spinal cord display spontaneous activity which is characterized by the temporal consolidation of spikes into bursts and a variable degree of burst synchrony across the network. The multimicroelectrode plate (MMEP), a culture substrate incorporating an $1mm^2$ array of 64 photoetched extracellular microelectrodes, provides a uniquely stable and interactive system for studying activity dynamics in an isolated neuronal network.

Networks of 50-100 neurons were cultured onto MMEPs from dissociated spinal cord tissue of 14-day mouse embryos, and activity was recorded after 20-150+ days in culture. Monophasic and biphasic stimulation pulses (0.2 - 1.0V, 0.1 - 1.0 msec.) were applied across a 10MΩ resistor in front of the preamplifier FET of one or more recording channels (average impedances @ 1kHz: 1.3MΩ electrode, 12MΩ amplifier input, 40MΩ conductor-bath shunt).

Single pulses delivered to a single microelectrode can trigger network-wide burst responses. The threshold strength-duration relationship for this response can be fit by standard physiological curves, with a unique rheobase and chronaxie for each stimulation site. Repetitive, periodic stimulus pulses can entrain network bursting to the temporal stimulation pattern. Repeated high-intensity and high-frequency stimulus trains can induce an epoch of intense, repetitive bursting which continues for seconds to minutes after stimulation is discontinued and may be considered the culture equivalent of an electrically-induced epileptic seizure.

This is the first demonstration of effective electrical stimulation of a cultured network via photoetched electrodes, and opens several intriguing avenues for further research. These include training networks to exhibit novel spatio-temporal activity patterns with repetitive patterned stimulation, testing the Hebb rule at the network level in both spike and burst domains by using spike- or burst-triggered stimulation to drive network activity, and developing a cultured network model of kindling.

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442.17

REPRODUCIBILITY OF GABA-INDUCED ACTIVITY SUPPRESSION IN CULTURED SPINAL CORD NETWORKS. R. S. Jordan*, B. K. Rhoades, and G. W. Gross. Dept. of Biological Sciences, Univ. of North Texas, Denton, TX 76203.

Cultured monolayer networks grown on photoetched multimicroelectrode plates have been used for pharmacological studies of patterned neural activity. These preparations allow spatially uniform and temporally constant exposure to pharmacological agents, are isolated from outside neural influences, are not damaged by invading electrodes, and allow simultaneous, multi-site recording (64 channels). However, cultures vary in cell number, distribution of cell types and sizes, apparent density of neurite interconnections, and native activity patterns. Therefore, the reproducibility of pharmacological responses is an important consideration. Studies were performed on networks grown from dissociated cells of 14 day fetal mouse tissues from either whole spinal cord (WC) or ventral cord (VC). From each culture, five successive dose response curves were obtained reflecting exponential decreases in burst rate with the incremental accumulation of GABA until burst shut off at a total inhibition concentration (TIC). Burst duration, amplitude, temporal pattern, and synchrony remained stable until shutoff. The GABA TIC was highly reproducible within n=12 cultures (WC range: 14±2 to 26±4, μM±SD) and suprisingly constant across these cultures (WC mean: 18±5). A comparison of networks cultured from WC and VC revealed different pattern dynamics, with VC networks displaying characteristically shorter bursts with less variability in burst duration. However, TIC values showed no tissue specificity (WC mean: 18±5, n=12; VC mean: 19±7, n=12). These results indicate that (1) GABA influences burst initiation without major effects on spike patterns within the bursts, (2) different cultures display similar network responses to GABA, and (3) burst suppression has a generally constant dose dependency both across and within cultures. (Supported by grants from the Texas Advanced Research Program and the Hillcrest Foundation of Dallas, TX.)

442.18

EMERGENT INFORMATION PROCESSING STRATEGIES IN A NETWORK OF NEURONS S. Shah, W.E. Faller and M.W. Luttges*. Departments of Electrical and Aerospace Engineering Sciences, University of Colorado, Boulder, Colorado 80309-0429.

Neurobiological information processing is presumably mediated by the spatiotemporal interactions among neurons in a network of cells. To study these complex spatiotemporal interactions with regard to structure/function relationships simultaneous single-unit recordings were obtained from the mesothoracic ganglion of the house cricket (Atheca Domestica) both before and after electrical stimulation of the interganglionic connectives. Each individual spiking record obtained presumably reflects the superimposition of EPSP's and IPSP's onto the intrinsic cellular physiology of each neuron. To discriminate between hard-wired and "emergent" responses of the neurons in this network both individual cell firing patterns and short-time scale, transient, "functionally" inhibitory and excitatory connections between neurons were analyzed before and after the stimulus presentation. Analyses indicated that some cells consistently respond in a predictable fashion to the stimulus, while other cells show more complex changes in both firing behavior and functional connectivity patterns with other cells in the network. Cells which "follow" the electrical stimulation, have predictable responses, may be involved in the hard-wired/mechanistic processing of information in the ganglion. Those cells which show dynamic changes in firing behavior and functional connectivity patterns as a delayed response to the stimulus, on the other hand, appear to contribute to more "emergent" information processing capabilities within this network of neurons. The structure/function relationships underlying both these hard-wired and emergent responses are being studied with respect to both the underlying spatial architecture and individual neuron size.

442.19

MULTIPLE SITE OPTICAL RECORDING OF SYNCHRONIZED BURSTS IN NEOCORTICAL AND HIPPOCAMPAL DISSOCIATED CELL CULTURES USING VOLTAGE-SENSITIVE DYES.
D. D. Cummings[1*], S. Rohr[1,2], A. L. Obaid[1,2], M. A. Dichter[1,3], and B. M. Salzberg[1,2]. [1]Institute of Neurological Sciences, [2]Dept. of Physiology, [3]Dept. of Neurology, University of Pennsylvania School of Medicine, and Graduate Hospital, Philadelphia, PA 19104.

Primary cultures of neocortical and hippocampal neurons exhibit synchronized bursts detectable by multisite calcium fluorometry (Ogura et al., Neurosci. Lett., 78 (1987), 69-74). The use of DI-8 ANEPPS (L. Loew; Mol. Probes Cat. # D-3167), a fluorescent potentiometric probe with a microsecond response time, allowed us to monitor rapid voltage transients, as well as the slower voltage oscillations associated with changes in $[Ca]_i$. Continuous multiple site recordings were obtained for approximately two minutes before excessive photobleaching occurred and reproducible recordings were obtained for at least one hour with each culture, using numerous experimental fields. NMDA and non-NMDA receptor antagonists, and GABAergic agonists and antagonists produced reversible perturbations in the synchronous bursting activity.

Multiple site optical recording of transmembrane voltage fluctuations in small networks combined with electrophysiological recordings should facilitate studies of the relationship between synaptic properties of CNS neurons and the origin and maintenance of emergent oscillatory behavior characteristic of networks formed in neuronal cultures.

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442.20

OPTICAL AND PHARMACOLOGICAL STUDIES OF PROPRIOSPINAL NEURONS INVOLVED IN RHYTHMIC MOTOR ACTIVITY IN THE EMBRYONIC CHICK SPINAL CORD. Stephen Ho* and Michael O'Donovan. Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892.

Our previous studies have identified a class of propriospinal neurons whose axons travel in the ventrolateral white matter tracts (LT) and are involved in synchronizing motoneuron discharge during rhythmic motor activity. The properties of these LT neurons were studied further in E9-10 chick embryos using optical and pharmacological methods. LT neurons were labelled retrogradely with a Ca indicator (Ca-green dextran), and were found on both sides of the cord following an unilateral LT injection. Ipsilateral cells were distributed dorsal and medial to the lateral motor column. The contralateral group was more discrete and was located in the medial part of the cord, near the central canal. During episodes of motor activity, both groups expressed synchronized, rhythmic fluorescence signals in phase with motoneuron discharge.

LT stimulation resulted in a brief synchronized discharge in flexor and extensor nerves followed by a more prolonged burst. The synchronous discharge and the prolonged potentials were abolished respectively by CNQX (5 μM) and AP-5 (50 μM). Depolarizing potentials persisting in the presence of kynurenic acid (1-2 mM) were blocked by strychnine (50 μM) or bicuculline (100 μM). LT evoked potentials are not mediated by electrical coupling or by cholinergic transmission because they were blocked by Co^{2+} and were unchanged during bath application of curare (20 μM). These findings indicate that LT axons have both excitatory and inhibitory connections with motoneurons. In addition to their role in intersegmental coordination they may also participate in contralateral synchronization and in activating the shunting inhibition that underlies the alternation of flexor and extensor motoneurons.

443.1

SELECTIVE IMPAIRMENT OF DELAYED NON-MATCHING-TO-SAMPLE (DNMTS) FOLLOWING FRONTAL CORTICAL LESIONS IN THE RAT. L. M. Harrison* and R. G. Mair. University of New Hampshire, Durham, NH 03824

Two experiments were conducted to determine the effects of lesions of the lateral internal medullary lamina of thalamus (L-IML) and of frontal cortex on three measures of spatial memory: DNMTS, radial arm maze (RAM), and serial reversal learning (SRL). In Exp. 1, RAM was trained prior to surgery. While both RAM and SRL were disrupted by lesions of the L-IML, neither task was affected by destruction of frontal cortical areas along the medial wall (MW) or dorsal to the rhinal sulcus (RS). In Exp. 2, DNMTS was trained prior to surgery. Rats with lesions of MW or RS showed impairments comparable to those seen following L-IML lesions. Anatomical studies were conducted using Wheat Germ Agglutinate - Horseradish Peroxidase (WGA-HRP) to map out projections of the mediodorsal nucleus (MDn) of thalamus in controls and in animals with RS and MW lesions. These analyses showed that the RS and MW lesions produced near-complete destruction of MDn projections to cortex, and that neither lesion (MW or RS) disrupted pathways leading from the MDn to the other frontal target area (RS or MW).

443.2

IBOTENIC ACID LESIONS OF THE HIPPOCAMPUS FACILITATE ACQUISITION OF AN APPETITIVE SIGNALLED BAR-PRESSING TASK. S. F. Logue*, E. M. Klamo, D. P. Miller, and J. E. Steinmetz. Program in Neural Science and Department of Psychology, Indiana University, Bloomington, IN 47405.

The role of the hippocampus in various spatial and temporal tasks has been widely studied. In a variety of aversive conditioning tasks (i.e. rabbit eyelid conditioning and passive and active avoidance tasks) hippocampal lesions have been shown to facilitate acquisition. On the other hand, hippocampal lesions have disrupted acquisition of appetitive conditional discrimination, but facilitated acquisition has been demonstrated for odor discrimination in rats with entorhinal cortex lesions. Reported here is a demonstration of facilitated acquisition of a single-cue signalled bar-pressing task in rats with hippocampal lesions. One group of rats was given ibotenic acid lesions in the hippocampus and a second group served as unlesioned controls. After training the animals to bar-press and establishing a baseline rate of responding the two groups of rats were divided into tone and no tone control groups and given daily sessions of 100, 1 sec tone (2 kHz, 90 dB) presentations. A bar-press during the tone (or during the 1 sec silent tone period for the controls) delivered a food reinforcement followed by a 10 sec intertrial interval and a variable pre-tone period (range 1-8 sec). A bar-press during the pre-tone period reset the time interval before the tone period. The lesioned tone rats reached asymptotic performance in fewer sessions than the non-lesioned tone rats although both groups reached the established criterion for reinforced responses. There was no lesion effect on the response latencies of either the tone or no tone groups. The tone and no-tone rats given hippocampal lesions after learning the task showed no impairment of performance. The effect of hippocampal lesions on a parallel signalled avoidance task are currently being assessed. [Supported by NSF Research Training Grant to S.F.L. and J.E.S.]

443.3

HIPPOCAMPAL LESIONS DO NOT IMPAIR NEGATIVE PATTERNING: A CHALLENGE TO CONFIGURAL ASSOCIATION THEORY. L.E. Jarrard*[1], M.G. McKernan[1] and T.L. Davidson[2]. [1]Dept. Psychol., Washington and Lee Univ., Lexington, VA. 24450, and [2]Dept. Psychol. Sci., Purdue Univ., West Lafayette, IN. 47907.

In a critical test of the configural association theory of hippocampal function, Sutherland and Rudy (Psychobiology, 17:129, 1989) reported that rats with neurotoxic (colchicine + kainic acid (COL+KA)) lesions of the hippocampus were unable to learn a negative patterning problem. In the present replication and extension of this research rats with either ibotenic acid (IBO) or COL+KA lesions of hippocampus, together with controls, were trained on a negative patterning discrimination where responding in the presence of either a tone or light was reinforced while responding when tone and light were presented in compound was nonreinforced. Following acquisition, transfer trials were given to see if the negative patterning problem had been learned using configural associations.

The results indicated that (1) rats in both COL+KA and IBO hippocampal groups learned the negative patterning discrimination, (2) COL+KA rats responded at higher rates, and (3) all groups learned the problem using a configural solution. Since neither type of hippocampal lesion impaired the ability of rats to solve the negative patterning problem, and transfer tests indicated that the groups learned the problem using configural associations, these results fail to support the configural association theory.

443.4

EFFECTS OF PERIRHINAL CORTEX AND MEDIAL EXTRASTRIATE VISUAL CORTEX LESIONS ON MEMORY ASSOCIATED WITH AN OBJECT CONTINUOUS RECOGNITION TASK. A. Ravindranathan*, P. Jackson-Smith and R.P. Kesner. Program in Neuroscience and Department of Psychology, Univ. of Utah, Salt Lake City, Utah 84112.

The perirhinal cortex is a major convergence site for afferents from the neocortex. Thus, it may play an important role in linking the neocortex to allocortical areas thought to be involved in recognition memory, including the hippocampus. In order to ascertain whether the perirhinal cortex and the medial extrastriate visual cortex play a role in recognition memory for 3-dimensional objects, lesions of the areas in question were effected in trained rats. The training procedure involved the sequential presentation of eight novel objects, and four repeated objects (chosen from the eight) within a session. These were selected from 120 different 3-dimensional objects in various shapes, sizes, textures and degrees of brightness. Repeated objects had lags ranging from 0 to 4 (from 0 to 4 different objects were presented between the first and the repeated presentation). An object was presented on one side of a long table divided in half by an opaque Plexiglas guillotine door, and the latency between opening the door and the rat moving the object was measured. Only the initial presentation of an object resulted in food reinforcement. Rats were assigned to groups and received surgery upon reaching criterion performance. Prior to surgery, latencies were significantly longer on repeated object presentations than on non-repeated presentations for all rats. There was no deficit following sham lesions or medial extrastriate visual lesions, whereas post-surgery performance was significantly worse than pre-surgery performance for the perirhinal group. Thus, the perirhinal cortex plays an important role in memory associated with this object continuous recognition task, whereas the medial extrastriate visual cortex does not.

443.5

HIPPOCAMPAL CHOLINERGIC AND SEROTONERGIC LESIONS: EFFECTS ON WORKING AND REFERENCE MEMORY. S.J. Murtha*, B.A. Pappas. Psychology Dept., Carleton University, Ottawa, Ontario K1S 5B6.

Combined loss of hippocampal acetylcholine (ACH) and serotonin (5-HT) occurs in Alzheimer's disease and may interact to impair memory. To examine this hypothesis, we tested rats pretrained in a 12 arm radial maze for the separate and combined effects of NMDA induced lesions of medial septal neurons and 5,7-DHT induced lesions of the fimbria/fornix plus cingulate gyrus, on reference (RMeM) and working (WMeM) memory. NMDA and 5,7-DHT reduced hippocampal choline acetyltransferase and 5-HT to 48% and 82% of control, respectively. Neither treatment alone nor their combination affected RMeM. The 5,7-DHT treatment by itself did not affect WMeM. NMDA caused an increase in WMeM errors. The combined NMDA and 5,7-DHT effect did not differ from the NMDA-only effect on early postoperative WMeM. However, the combined lesion did appear to retard the post-operative recovery of WMeM that was observed for the NMDA-only treated rats. In Alzheimer's disease, hippocampal 5-HT deficits may prevent compensation for the disruption of working memory that is caused by loss of hippocampal Ach function.

443.6

EFFECTS OF HIPPOCAMPAL CHOLINERGIC AND FOREBRAIN NORADRENERGIC LESIONS ON REFERENCE AND WORKING MEMORY. B.A. Pappas*, B.A. White, S.J. Murtha, G.A.S. Park and K. Hewitt. Dept. of Psychology, Carleton University, Ottawa, Ontario K1S 5B6.

Combined loss of hippocampal acetylcholine (ACH) and norepinephrine (NE) terminals occurs in Alzheimer's disease and may interact to impair memory. To test this hypothesis, we determined in maze pre-trained rats, the separate and combined effects of NMDA-induced lesion of the medial septal neurons and DSP4-induced lesion of forebrain NE terminals on reference and working memory in the radial arm maze. The NMDA and DSP4 treatments reduced hippocampal choline acetyltransferase activity and NE levels to 53% and 45% of control respectively. The DSP4 treatment by itself did not affect either reference or working memory. The NMDA treatment caused temporary disruption of reference and a long lasting disruption of working memory. DSP4 did not exacerbate the immediate effects of the NMDA lesion on working memory. It did prevent complete recovery of working memory in the NMDA lesioned rats, however. In Alzheimer's disease, forebrain NE deficits may potentiate over the long term, the effects of hippocampal ACH loss on working memory.

443.7

POST-TRAINING REVERSIBLE INACTIVATION OF THE AMYGDALA ATTENUATES BEHAVIORAL CONTRAST. L.F. Cahill*, J.A. Salinas, M.G. Packard & J.L. McGaugh. Center for the Neurobio. of Learning & Memory and Dept. of Psychobio., U. of Calif., Irvine, CA 92717.

Rats trained to run a straight alley for a large food reward display sharply increased latencies when shifted to a small reward. This behavioral phenomena, referred to as the Crespi effect (1942) or behavioral contrast, is often interpreted as an aversive emotional reaction to a reduction in reward magnitude. The behavioral contrast paradigm has been used extensively in studies examining anxiolytic effects of drugs. The present study used the behavioral contrast paradigm to examine the role of the amygdala in memory for a reduction in reward magnitude. Male Sprague-Dawley rats (175-200g) were implanted with bilateral intra-amygdala cannulae and trained to run a straight alley (6 trials/day) for either ten or one 45 mg food pellets. On day ten of training, half the animals in the high reward group were shifted to a one pellet reward. Immediately following shifted trials, the animals received an intra-amygdala injection of either a 1% lidocaine solution or phosphate buffer (0.5 ul/side). Shifted training continued for two more days and no further injections were given. Shifted animals that received a buffer injection displayed a characteristic increase in response latencies on the 2nd day of shifted training. In contrast, animals that received lidocaine injection showed no increase in latency on either the 2nd or 3rd day of shifted training. The findings indicate that post-training inactivation of the amygdala attenuates behavioral contrast, suggesting that the amygdala is involved in regulating memory for reduction in reward magnitude.

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443.8

LESIONS OF THE AMYGDALA INHIBIT FEAR REACTIONS IN THE SHOCK-PROBE BURYING TEST BUT NOT IN THE ELEVATED PLUS-MAZE TEST. D. Treit*, S. Rotzinger, and C. Pesold. Department of Psychology, University of Alberta, Canada, T6G 2E9.

The purpose of the present experiments was to investigate the role of the amygdala in anxiety, using two different, pharmacologically validated animal models of anxiety: the elevated plus-maze test and the shock-probe burying test. Experiment 1 showed that animals with complete amygdaloid lesions made significantly more contacts with the electrified probe than sham-lesioned controls, but were not significantly different from controls in the duration they buried the probe or the amount they explored the open arms of the elevated plus-maze. Experiment 2 replicated these results, and showed that rats with larger amygdaloid lesions contacted the electrified probe more than rats with smaller amygdaloid lesions. Experiment 3 showed that amygdaloid lesions did not alter the ability of diazepam to increase open-arm activity in the plus-maze test or to decrease burying behavior in the shock-probe test. Taken together, these results suggest that 1) the amygdala does not mediate the anxiolytic effects of diazepam in these tests, and 2) the amygdala may be involved in the inhibition of some fears [e.g., fear of an electrified probe] but not others [e.g., fear of an elevated, open arm].

443.9

PAIRED ASSOCIATE LEARNING IN RATS: CRITICAL INVOLVEMENT OF THE PERIRHINAL-ENTORHINAL CORTEX. M. Bunsey* & H. Eichenbaum, Dept. Psychology & Neurobiogy Curriculum, UNC Chapel Hill, N.C. 27599.

Olfactory-guided learning in rodents can serve as a useful model system for the study of the neurobiological bases of higher order processes in learning and memory. The present experiment involved the development of an odor-guided paired associate task (PA) analogous to the verbal PA task frequently used to assess human memory.

In the present version of the PA task, rats are trained to sniff at an odor port while two different odors are presented in succession, separated by a brief blank interval. If the stimulus sequence involves any one of four arbitrarily assigned paired associates the subject is rewarded for responding at a nearby water port. Alternatively, if the sequence consists of any other combination of the same eight odors ("mispairings") the subject must withhold a response. In addition, the rat must withhold responses on interspersed "non-relational" trials involving one of the eight odors paired with one of four odors from a second set; these odor sequences are never rewarded.

Intact rats learn to discriminate the non-relational trials rapidly, and more gradually differentiate mispairings from paired associates. Rats with bilateral ablation of the perirhinal-entorhinal cortex were profoundly impaired in learning to distinguish mispairings from paired associates. However, the same lesioned rats showed only a mild and transient deficit on non-relational trials. These results support the hypothesis that the hippocampal system is critical to PA learning across species and, more generally, that this system supports memory representations based on arbitrary relationships among perceptually independent cues across stimulus modalities.

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443.10

MEMORY-BASED LEARNING DEFICITS PERSIST INTO ADULTHOOD AFTER X-IRRADIATION-INDUCED HIPPOCAMPAL GRANULE-CELL HYPOPLASIA IN EARLY INFANCY. N. J. Lobaugh*, J. L. Diaz-Granados, P. L. Greene, & A. Amsel. Department of Psychology & Institute for Neuroscience, University of Texas, Austin, TX, 78712.

Our laboratory has shown that electrolytic hippocampal lesions in infant rats (Lobaugh, et al., *Behav. Neurosci.*, 103:1159, 1989), postnatal exposure to ethanol (Greene, et al., *Behav. Neurosci.*, 106: 51, 1992), and early postnatal hippocampal x-irradiation (Diaz-Granados, et al., *Soc. Neurosci. Abs.*, vol. 16, 1990) all disrupt the acquisition of patterned (single) alternation (PA), a form of memory-based learning, in infant rats. After x-irradiation exposure, pups tested at 17-18 days of age showed a severe deficit in PA with a 60-s intertrial interval (ITI) but not a 30-s ITI. In the present experiment, we examined the effect of early postnatal exposure to x-irradiation on memory-based learning in adult rats at 60-86 days of age. The irradiated animals showed a significant deficit in PA learning at a 60-s ITI when compared to normals and sham controls. The specific reductions of hippocampal dentate granule cells at day 86 were comparable to the reductions in the younger animals of our earlier result. These results suggest that early insult to hippocampal granule-cells by x-irradiation results in lasting memorial and morphological deficits. Supported by NSF grant BNS-8609877.

443.11

A PRELIMINARY ANALYSIS OF THE BEHAVIORAL EFFECTS OF LESIONS OF THE SUPERIOR CEREBELLAR PEDUNCLE IN RATS. R. N. Leaton* and J. M. Kelso. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

A series of tests assessed the behavior of rats with knife-cut lesions of the superior cerebellar peduncle as compared with sham operated controls. In the first of two 20-min sessions in a brightly illuminated open field the lesioned rats were significantly more active than controls. This difference disappeared at the second session 24 hr later. In tests of the acoustic startle response the lesioned rats were significantly more responsive than controls. They showed significantly more robust within-session response decrements than controls. Both groups showed significant response decrements over days, i.e., long-term habituation, but the decrements appeared somewhat more variable in the lesioned animals. On the balance-beam test of motor stability, there was no significant difference between the groups. Neurological examinations revealed some deficit in the lesioned animals in hind-leg placing. Overall we were most impressed with the subtle nature of the behavioral effects in the lesioned animals. Histological analysis showed significant, near-complete bilateral section of the superior peduncle in 6 of the 7 lesioned rats.

443.12

INTACT PROJECTIONS FROM THE MEDIODORSAL NUCLEUS (MDn) OF THALAMUS TO FRONTAL CORTEX IN RATS FOLLOWING PYRITHIAMINE-INDUCED THIAMINE DEFICIENCY (PTD). S.M. Koger* & R.G. Mair. Psych Dept, Univ. New Hampshire, Durham, NH 03824.

Previously we have shown that PTD treatment in rats impairs working memory and produces consistent lesions of the internal medullary lamina (IML) surrounding MDn. Examination of Fink-Heimer stained tissue has shown signs of widespread layer IV cortical denervation, including all areas of frontal cortex. To determine the extent of frontal cortical denervation, we implanted WGA-HRP bilaterally into 10 rats recovered from PTD, aimed at areas of MDn typically spared by this treatment. Rats with IML lesions and partial destruction of MDn showed anterograde and retrograde transport of WGA-HRP to frontal cortex. Rats with near-complete MDn lesions did not. These findings indicate that areas of MDn spared in PTD rats remain connected to frontal cortex.

443.13

MK-801 IMPAIRS LEARNING BUT NOT RETENTION FOR SERIAL PATTERNS IN RATS. J.D. Rowan* and S.B. Fountain. Department of Psychology, Kent State University, Kent, OH 44242.

MK-801 is a N-methyl-D-aspartate antagonist that has been found to impair learning in some behavioral tasks. The effects of MK-801 exposure on learning and retention of serial patterns were examined. Rats were trained in a serial pattern learning task to anticipate elements of one of two patterns. The task required rats to anticipate the correct sequence of leverpresses in an array of levers mounted on the walls of an octagonally shaped operant chamber. Both patterns were highly structured, but one pattern contained a violation of pattern structure. In the first phase of the experiment, male hooded rats were exposed to 0.0625/kg MK-801 by i.p. injection 30 minutes prior to testing each day during acquisition. Rats exposed to MK-801 during acquisition were significantly impaired in learning the patterns compared to saline injected controls. The rats showed impairments primarily in learning the pattern containing the violation of pattern structure. In the second phase of the experiment, saline control rats from the first phase that had already learned their patterns were injected with 0.0625 mg/kg MK-801 to assess its effects on retention of the serial patterns. Compared to saline controls, rats exposed to MK-801 were not significantly impaired in retention. These results support the view that MK-801 impairs the acquisition of new serial patterns but does not affect retention of previously learned serial pattern information.
(Supported by NIH BRSG S07RR7208 and NIMH MH48402.)

443.14

POTENTIATION OF ODOR AVERSION BY TASTE IS DIFFERENTIALLY AFFECTED BY 6-OHDA OR QUISQUALATE INJECTIONS. J. Fernández-Ruiz,[2] R. Guzmán,[1] M.I. Miranda,[2] F. Bermúdez-Rattoni*,[2] and R. Drucker-Colín.[1,2] [1]Depto. de Fisiología, Facultad de Medicina and [2]Instituto de Fisiología Celular, UNAM, 04510 México DF.

It has been demonstrated that Parkinson's disease patients have an olfactory deficit. One possible cause may be that dopamine depletion of the olfactory structures leads to an olfactory dysfunction. To test this hypothesis we submitted two control groups, three quisqualate and three bilateral 6-OHDA lesioned rat's groups to a modified potentiation of odor aversion by taste paradigm. The regions tested were the insular cortex, the amygdala and the hippocampus dorsalis. At the end of the experiment the 6-OHDA brains were prepared for HPLC. The behavioral results showed that both the injection of 6-OHDA or quisqualate in the insular cortex produce the disruption of taste, but not odor aversions. On the other hand, the lesion of the amygdala with 6-OHDA or quisqualate, caused the disruption of the potentiated odor, but not the taste aversions. Finally, the quisqualate, but not the 6-OHDA lesions in the hippocampus dorsalis produced a disruption of both taste and potentiated odor aversions. The HPLC showed a decrease in catecholaminergic contents of the lesioned sites. These results indicate that the injection of 6-OHDA in the amygdalar region, but not in dorsal hippocampus nor in the insular cortex, produced a severe olfactory deficits. Supported by FIIRESIN and DGAPA-IN-204689.

443.15

DIFFERENTIAL BEHAVIORAL EFFECTS OF SINGLE OR COMBINED LESIONS TO THE INFRA- AND SUPRACALLOSAL SEPTO-HIPPOCAMPAL PATHWAYS IN THE RAT: WORKING MEMORY DEFICITS ARE NOT AMELIORATED BY OXOTREMORINE OR PILOCARPINE. **P.L. Greene, J.C. Cassel, C. Kelche, H. Jeltsch & B.E. Will*, L.N.B.C. UPR 419 du CNRS, Université Louis Pasteur, 67000 Strasbourg (France).**

Female Long-Evans rats sustained single electrolytic infracallosal (group IN, n=13) or aspirative supracollosal (group SU, n=12), or combined lesions (group INSU, n=12) of the septo-hippocampal pathways at 90 days of age. Sham-operated animals (group SH, n=13) served as controls. Infra- and supracallosal pathways were chosen because of their differential content of cholinergic fibers. There was an infracallosal lesion-induced (groups IN and INSU) increase in open-field and home-cage activity, and decrease in spontaneous alternation and habituation to novel stimuli 18 days post-lesion. Of these effects, only increased open-field activity and decreased spontaneous alternation were again observed at 75 days post-lesion. Performance in an 8-arm radial-maze task was impaired as well, again only in groups IN and INSU. These differences in radial-maze performance between groups became more robust with a one-minute delay imposed between the 4th and 5th choices. Oxotremorine (0.1 & 0.03 mg/kg) and Pilocarpine (1.0 & 0.32 mg/kg) were ineffective at improving the performance of impaired animals either with or without a delay between the 4th and 5th choices in the radial-maze task. There were no differences between groups SH and SU among the behaviors observed in the present study. The results are discussed in relation to the importance of septo-hippocampal cholinergic pathways in working memory and in terms of the possible relevance of interactions between acetylcholine and other neurotransmitters in memory function.

443.16

IBOTENIC HIPPOCAMPAL LESION IN RAT DO NOT DISRUPT RETRIEVAL: IS PRIOR CUING IN ANIMAL EQUIVALENT TO HUMAN PRIMING? P. GISQUET-VERRIER*[1] AND F. SCHENK[2] [1]Laboratoire de Neurobiologie de l'Apprentissage et de la Mémoire, C.N.R.S., URA-1491, 91198 Gif-sur-Yvette, France & [2]Institut de Physiologie, 7 rue du Bugnon, 1005 Lausanne, Switzerland.

Retrieval processes can be promoted by an exposure to some training features, delivered shortly before the retention test. The effectiveness of a particular cue depends on the nature of the feature and on the length of the training-to-test interval (TTI) (Anim. Learn. & Behav. 1989, 17: 394-408). The aim of the present study was to determine a possible involvement of the hippocampus in the promotion of retrieval processes, either shortly (1 day) or distant to training (21 days). Rats receiving bilateral ibotenic acid lesions of the hippocampus were compared to sham-operated animals. They were trained for 15 trials in an avoidance brightness discrimination. After a 1-day and a 21-day TTI separate groups of animals were submitted to a retraining session. Before the testing session, cued animals were briefly exposed either to the CS or to the experimental context. The facilitation of the performance induced by pretest cuing was qualitatively and quantitatively identical in hippocampal and in sham-operated animals. After a 1-day TTI, animals in both conditions showed an improvement of their retention performance subsequent to a CS exposure. After a 21-day TTI, a pretest exposure to the experimental context was the only effective procedure promoting retrieval in both lesioned and sham groups. However, all the lesioned animals showed a characteristic deficit in performance when submitted to a radial maze task, demonstrating that the lesions successfully impaired learning in tasks requiring an intact hippocampus.

In sum, these results demonstrate that the hippocampal formation does not seem to be implicated in the promotion of the retrieval processes by prior cuing in rats. Given that human priming is also preserved following hippocampal lesions, the present results suggest a possible similarity between the processes involved in human priming and in animal prior cuing. Supported by ETP twinning grant 9040.

443.17

RF LESIONS OF THE LATERAL INTERNAL MEDULLARY LAMINA (L-IML) OF THALAMUS IN THE RAT INCREASE THE RATE OF TEMPORAL DECAY OF A DELAYED NON-MATCHING TO SAMPLE TASK. E.E. Kivlahan* & R.G. Mair. Dept. of Psychology, University of New Hampshire, Durham, NH 03824.

36 rats were matched for performance on a DNMTS task and then randomly assigned to control or to 1 of 3 lesion groups: L-IML, mediodorsal nucleus of thalamus (MDn), or fornix (Fnx). After recovery, rats were trained to stability on DNMTS at 6 delays from 1.8 to 8.8 s. L-IML animals performed significantly worse than all other groups, exhibiting significantly faster rates of decay up to 6.3 s (when their performances reached chance level). MDn and Fnx rats were not significantly impaired on DNMTS. All 3 lesion groups were significantly impaired compared to controls when subsequently trained on an 8 arm radial maze task.

444.1

CORTICAL CYTOARCHITECTURAL ABNORMALITIES CORRELATE WITH COGNITIVE DEFICITS IN MICE. C.F. Hohmann*, J.E. Sweeney, E.Bachman, and J.T. Coyle. Kennedy Krieger Institute, Baltimore, MD; Wellesley College, Wellesley, MA; Harvard/ MGH, Boston, MA.

In neonatal mice, lesions of the basal forebrain (nBM) projections to cortex result in transient cholinergic denervation which lead to persistent changes in cortical connectivity and cytoarchitecture. Because these cytoarchitectural abnormalities resemble morphological changes observed in human mental retardation, this is a model for developmental disabilities. We have shown previously that nBM lesions in neonates result in increased motor activity, decreased passive avoidance retention latencies and increased swim maze latencies in adult mice. The purpose of this study is to determine whether there are correlations between histological abnormalities and behavioral impairments in these mice. 25 Balb/cByJ mice received lesions to the nBM 12-24 hours after birth. Behavioral testing began at 8 weeks and included assessments of motor activity, retention (passive avoidance task) and cognition (swim maze task). Following behavioral testing, 9 mice were killed for Nissl and AChE histology. Morphological abnormalities were evaluated and scored. Lesions varied in size, anterior/posterior and medial/lateral extent of damage and severity of resulting cortical abnormalities. Independent of lesion location and size, cortical AChE intensity and distribution were comparable to controls. Motor activity did not correlate with passive avoidance or swim maze latencies. Additionally, lesion location, size and cortical cytoarchitectural abnormalities did not correlate with motor activity or passive avoidance retention latencies. Cortical histology did, however, correlate with swim maze latency($r^2 = 0.85$; $p = 0.003$), i.e., severely abnormal cortical morphology predicted high swim maze latencies. These data indicate that cortical cytoarchitectural abnormalities resulting from nBM lesions in neonates correlate with impairments on a cognitive task, but not on activity-related measures in adult mice. Thus in this lesion model and, by extrapolation, in mental retardation structural changes in the cortex which result from ontogenic abnormalities could lead to functional changes later in life.

444.2

GENDER DIFFERENCES IN BEHAVIORAL STRATEGIES AND RESPONSES TO CHOLINERGIC DRUGS. J.E. Sweeney*, R.T. Richardson and C.F. Hohmann. Wellesley College, Wellesley, MA; Johns Hopkins University, Baltimore, MD; Kennedy Krieger Institute, Baltimore, MD.

We have shown previously that basal forebrain lesions in neonatal mice lead to persistent behavioral impairments in adult mice. We now intend to test whether cholinergic reconstitution therapy with physostigmine (PHY) can ameliorate the lesion-induced deficits. Our behavioral studies include both female and male mice because lesions are performed in pups when gender is not readily detectable. Gender differences have been reported in other behavioral paradigms, thus, we decided to assess the performance in the control female and male population on a passive avoidance and spatial navigation task before and after administration of PHY. In passive avoidance, mice were injected with either saline or PHY (0.1 mg/kg, i.p.) after acquisition, then retention latencies were measured 24 hours later. In the spatial navigation task, mice were trained to swim to a hidden platform and then were injected with either saline or PHY (0.1 mg/kg, i.p.) 15-20 mins before testing. Small sex-related differences in performance existed in the absence of drug treatments, most notably, in acquisition of spatial navigation. Analyses of swim paths and patterns suggest that females and males use different strategies. The most dramatic differences in performance, however, occurred in response to PHY. The same dose of PHY significantly increased passive avoidance latencies (76.9 ± 26 to 168 ± 12 s) and decreased swim latencies (19.9 ± 2 to 11.1 ± 3 s) in males but did not significantly alter performance in females. Full dose response curves are currently being generated to characterize gender differences in response strategies and sensitivity to PHY and other cholinergic drugs.

444.3

INTERACTIONS BETWEEN EMOTION AND MEMORY FORMATION. A PROPOSED HYPOTHESIS. D. Galey*, Laboratoire de Neurosciences comportementales et cognitives, CNRS URA 339, Université Bordeaux I, avenue des Facultés, 33405 Talence Cedex France.

Using two inbred strains of mice (BALB/C and C57BL/6), we have attempted to elucidate the relationships between memory processes and the modulation of activity of the septo-hippocampal cholinergic pathway.

At first, we have shown in BALB/c mice that medial septal stimulation (30 µA, 100 Hz) applied 30 sec after the partial acquisition of an operant lever press conditioning increased subsequent retention performance 24 hours later with a temporal gradient of the stimulation effect less than 15 min. This result suggests that the stimulation improved consolidation processes. In these conditions, C57BL/6 which display the greater increase in septo-hippocampal cholinergic activation evidenced poor consolidation abilities (GALEY et al., submitted for publication). Additional analysis revealed in fact that these two parameters are negatively correlated. Thus it is suggested that the consolidation of informations would be possible only after a decrease of the level of cholinergic activation.

Finally, our data suggest the systems by which emotional states could act on information processing and storage through the modulation of the septo-hippocampal cholinergic pathway.

444.4

LEARNING-ASSOCIATED CHANGES IN MONOAMINE RELEASE IN THE RAT BASAL FOREBRAIN. K. Kariya*, J. Tanaka, K. Hori, M. Oda, M. Iwaki and M. Nomura. Sect. of Pharmacol. Res. Lab., Torii & Co., Ltd, Chiba 272, *Dept. of Physiol., Saitama Med. Sch., Saitama 350-04, Japan

Extracellular levels of monoamines and their metabolites were measured by the microdialysis method combined with high performance liquid chromatography in the region of the nucleus basalis (NB) in rats before, during and after a learning test to examine whether inputs from brainstem monoaminergic system to the basal forebrain are involved in the learning process. In male Fischer 344 rats (n = 12) which have acquired the operent-type brightness discrimination learning, dopamine (DA), dihydroxy-phenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindole acetic acid (5-HIAA) levels during the learning test showed a trendancy to increase as compared with the pre-learning period. The percent increases in the monoamine and metabolite concentrations were then compared with various parameters of learning. There were significant positive correlations between the percent increases in DOPAC, HVA and 5-HIAA concentrations and the correct response ratio. The percent increases in the concentrations of metabolites, on the other hand, were significantly correlated with neither the total responses nor the reinforcements. These results imply that the monoaminergic inputs to the basal forebrain may play an important role in the learning process.

444.5

EXPRESSION OF IEGs DURING ODOR DISCRIMINATION: ELEVATION OF C-FOS LEVELS OBSERVED IN HIPPOCAMPAL SUBFIELD CA3. U.S. Hess*, R. Nabi, U. Staubli, C. Gall[1], and G. Lynch. Center for the Neurobio. of Learning & Memory, [1]Dept. of Anatomy and Neurobio., Univ. of Calif., Irvine, CA 92717.

The IEGs c-fos and zif-268 are rapidly and transiently expressed in response to increases in neuronal activity in the CNS following various experimental manipulations. Proteins encoded by these rapid response genes may act as transcription factors that regulate the expression of downstream target genes, and so therefore function as mediators of long-term responses such as those necessary for the consolidation of memory. In support of this idea, Campeau *et al.* (1991) showed that conditioned fear dramatically elevates c-fos mRNA levels in the amygdala. We used c-fos and zif-268 ^{35}S cRNA probes and *in situ* hybridization to investigate the effect of odor discrimination learning on IEG expression in the rat brain. Initially, we found that handling rats for the first time and/or placing them in a novel environment (the training apparatus) elevates c-fos and zif-268 mRNA levels significantly above control levels in regions such as hippocampus and rostral piriform cortex. This effect was more pronounced for c-fos than zif-268, and was greater in the CA1 than in the CA3 subfield of the hippocampus for both IEGs. These responses were decreased significantly by repeated handling and immersion in the training environment. To determine if specific brain regions are activated in experienced animals performing a novel odor discrimination, we compared c-fos and zif-268 expression levels in two groups of rats. Rats in both groups had been previously trained to solve seven odor discriminations between two simultaneously presented odors. On the last day, some of these animals (n = 19) performed on an eighth odor discrimination (for 30 min) and the remainder (n = 15) were simply placed in the training apparatus (for 10 and 30 min) as "context controls." Animals were perfused 30 min after their last experimental treatment. Animals actively performing the task had significantly higher c-fos levels in CA3 when compared to context controls ($p = 0.03$, unpaired t-test). Such a change in c-fos expression was not found in area CA1 or three other regions measured, nor was it found for zif-268 expression levels in the same regions in adjacent tissue sections. These results suggest that selective areas of the brain exhibit enhanced IEG induction/activity during odor discrimination behavior.

444.6

AGE-RELATED PERFORMANCE ON ODOR-GUIDED DELAYED NONMATCH TO SAMPLE AND WATERMAZE LEARNING. D. Zyzak, H. Eichenbaum*, T. Otto, and M. Gallagher. Dept. of Psych. Univ. of North Carolina, Chapel Hill, N.C. 27599

Recent work from this lab has shown substantial variation in the spatial learning abilities of aged rats, such that some aged rats are impaired in the Morris water maze task, while others perform as well as young rats. In the present study we assessed the effects of aging on nonspatial cognitive performance in odor-guided continuous delayed nonmatch to sample (cDNM). Rats were initially trained to perform the cDNM task under minimal delay and interference conditions, then challenged with longer memory delays and increased inter-item interference. These same rats were then tested in the Morris water maze to compare performance across the two tasks.

As in the maze task, we found a substantial variation in aged rats' ability to acquire the cDNM task. Aged rats were significantly retarded in acquisition of the cDNM task, however they were only slightly, if at all, impaired in later testing under increased interference and memory delay conditions. Furthermore, cDNM acquisition scores of aged rats were related to performance in spatial learning. Thus, when aged rats were distinguished according to their maze performance, only maze impaired rats required significantly more trials to learn the cDNM task. These results reveal a relationship between the effects of aging on spatial and non-spatial tasks that may reflect a more broadly based deterioration in cognitive function than that commonly ascribed to a spatial memory impairment.

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444.7

VIGILANCE AND SELECTIVE ATTENTION IN RATS USING AUDITORY STIMULUS DETECTION. P.J. Bushnell*[1] and K.L. Kelly[2]. [1]Neurotoxicology Division, US EPA and [2]ManTech Environmental Technology, Inc., Research Triangle Park, NC 27711.

Vigilance involves maintaining attention to repeated stimuli over time, and selective attention may be inferred from changes in behavior due to manipulation of the likelihood (expectancy) of discriminative stimuli. To study vigilance, rats worked in operant chambers in which an auditory signal (a 20-msec increase of 1 to 7 dB in the intensity of continuous white noise) was presented on half of the trials. A food pellet was delivered if the rat pressed one of two retractable response levers on a signal trial, or if it pressed the other lever on a blank (no signal) trial. Signal detection analysis showed monotonic increases in both sensitivity (sensitivity index, SI) and bias (response index, RI) with increasing signal intensity, showing that the accuracy of signal detection improved and the criterion for responding "signal" became more lenient as signal intensity increased. Acute exposure to toluene vapor (2000 ppm) reduced SI at all signals greater than +1 dB without changing RI. Selective attention was studied with a similar procedure which required rats to detect either a 50 msec increase (+7 dB) or a 50 msec decrease (-6 dB) in continuous white noise. The probability of signal type (increase or decrease) did not affect SI when both signal types required the same response (e.g., press the left lever) and blank trials required the other response (e.g., press the right lever). However, the probability of signal type greatly affected response accuracy when each signal required a different response. These results suggest the importance of the response in demonstrating effects of expectancy on selective attention in rats.

444.8

EFFECT OF LESIONS IN THE MFB AT THE DIENCEPHALIC-MESENCEPHALIC BORDER ON THE REWARDING EFFICACY OF LH SELF-STIMULATION. M. I. Leon* and C. R. Gallistel. Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563. Electrolytic lesions in the medial forebrain bundle at the junction between the posterior hypothalamus and ventral tegmental area were found to attenuate the rewarding efficacy of stimulation at the level of the lateral hypothalamus, as measured by the shift in the rate-frequency functions at several different currents. These results are consistent with findings by Waraczynski and her collaborators, that MFB knife cuts attenuate the rewarding efficacy of stimulation at sites rostral to the cut, but often have no effect on the rewarding efficacy of stimulation at sites caudal to the cut. A surprising finding was that our lesions had to be reasonably large (300 μA cathodal current for 200 s) before any decrease in rewarding efficacy of stimulation became evident.

444.9

MATCHING BEHAVIOR IN RATS SELF-STIMULATING IN THE MEDIAL FOREBRAIN BUNDLE. T. A. Mark*, J. C. Sim, and C. R. Gallistel. Dept. of Psychol., UCLA, Los Angeles, CA 90024-1563.

Rats pressing two levers for brain stimulation reward on concurrent variable-interval schedules allocate their time between the levers in accord with relative reward abundance (subjective reward magnitude multiplied by rate of reward) - the matching law. This preparation may therefore provide a model system in which to study the neurobiology of elementary computational processes. We studied the kinetics of matching after step changes in the relative reward magnitudes, the relative rates of reward, or both. Rats very rapidly altered their relative time allocation (time on one lever/time on the other lever) in response to a change in either component of relative reward abundance. Transitions were commonly complete after one interreward interval on the leaner schedule. Relative time allocation tracked the random short term fluctuations in relative interreward intervals inherently present in two concurrent Poisson processes; hence, the extent to which rats conformed to the matching law depended on the method used to estimate overall relative reward rates and overall time allocations. [Supported by NSF Grant BNS 89-96246.]

444.10

REWARD SATURATION IN ELECTRICAL SELF-STIMULATION OF THE MFB. Janine M. Simmons* & C. R. Gallistel. Interdepartmental Neuroscience Program, UCLA, Los Angeles, CA 90024-1563.

Rats press a lever to deliver a train of brief electrical pulses to the medial forebrain bundle (MFB). The time the animal spends on the lever depends on the remembered effect of the stimu-lation (the subjective magnitude of the reward). Increases in either current or pulse frequency increase the subjective reward magnitude, but only up to a frequency called the saturation frequency, above which reward magnitude no longer increases. We used a two-lever choice paradigm with variable interval schedules on both levers to determine the subjective reward magnitude as a function of pulse frequency at currents ranging from 1000 mA down to the 'current wall.' We find that the saturation frequency depends strongly on current, varying from 251-631 pps at the lowest currents (100-251 mA) to 63-200 pps at a 1000 mA. Increasing current shifts the function for reward-magnitude-versus-pulse-frequency to the left and sometimes increases its asymptote. These results make it unlikely that the saturation is due to a failure of frequency following in the first-stage axons.

444.11

EVIDENCE THAT HIPPOCAMPAL PLACE CELL REPRESENTATIONS OF MULTIPLE ENVIRONMENTS ARE NOT STRICTLY TOPOGRAPHIC. J.L. Kubie*, R.U. Muller, E.S.Hawley and C.P. Jia. Depts of Anatomy and Physiology, S.U.N.Y. Brooklyn, Brooklyn, NY 11203

The discovery of hippocampal place cells led to the hypothesis that the rat hippocampus is involved in spatial representations of the environment (O'Keefe and Nadel, 1976). Two questions that arise are: 1) How are multiple environments represented? and 2) Are the environments topographically mapped within the hippocampus? Previous work implied that there are independent representations of different environments (Muller and Kubie, 1987), but left open the question of topographic organization (Eichenbaum et al, 1990).

In this study we trained rats to chase food pellets in a square chamber and a cylinder. To date, we have recorded 7 sets of at least two cells in each environment. When two cells have firing fields in one environment, the relationship between the fields is called either overlapping or disjoint. We often see that a pair of cells with overlapping fields in one environment has disjoint fields in the second. These observations corroborate the idea of independent representations of different environments. They also strongly imply that the hippocampal representation of space is not topograhic. In addition, we find that temporal cross-correlations for pairs of simultaneously recorded cells are greatly affected by the animal's current environment. This result has consequences for theories of how the representation of a particular environment is established and maintained. (Supported by NIH NS20686).

444.12

A RAT MODEL FOR ENDOGENOUS ATTENTION DEFICIT-HYPERACTIVITY DISORDER (ADHD). J.G. Kohlert*, S. Shillingford, R.Mills, D.E.Fleming and G.J. Bloch Dep't Psych., Brigham Young U., Provo,UT, 84602.

Animal models for ADHD have been proposed; however, they are not "pure";i.e., these "hyperactive" animals have been made so by electrical/chemical brain lesions, by pharmacological agents, or by genetic breeding but coupled with hypertension or an abnormal response to stress (Anderson, Dev Pharm,'83; Hendley, Am Phys Soc, '91; Kerr, Pharm Biochem,'91; Sagvolden, Neurosci,'91). Based on our observations that some male rats are hyposexual and also hyperactive, we determined that they portray characteristics representative of ADHD. These rats, which have not been rendered abnormal by any external manipulation, respond as do humans: 1) they are significantly more active than controls on open-field testing; 2) they respond in a paradoxical fashion to the psychmotor effects of amphetamine; 3) they show deficits in selective attention by failing to block irrelevant information in a conditioned avoidance response; and 4) preliminary evidence suggests they are more distractable. These data indicate that these rats may be an excellent model for the study of Attention Deficit-Hyperactivity Disorder.

444.13

LESIONS OF THE NUCLEUS ACCUMBENS IN ADULT RHESUS MONKEYS RESULT IN A DEFICIT OF MOTOR LEARNING BUT NOT IN S-R ASSOCIATIVE LEARNING OR MEMORY. R. Killiany* and H. Mahut, Department of Psychology, Northeastern University, Boston, MA 02115

Nearly 45 years ago, Ryle postulated that memory was not a unitary phenomenon but rather consisted of at least two components he identified as "Knowing How" and "Knowing That" (Ryle, 1949). Hirsh (1974), among others, has further modified these components into a primitive S-R learning system (habit) and a hippocampal memory system, respectively. The nucleus accumbens (NA) by virtue of its suggested role in reinforcement systems appeared as a promising component of the habit system. Therefore, we tested the notion that lesions of NA would result in an impairment of S-R types of learning. Accordingly, 8 adult monkeys, 4 with bilateral ablations of NA, 2 sham operated monkeys and 2 unoperated control monkeys, were tested on two versions of the concurrent object discrimination task (COD), a delayed recognition memory task (DNMS) and a motor skill task which required the monkey to remove a lifesaver from different patterns of bent wire.

Contrary to our prediction, monkeys with NA lesions were not impaired on the COD tasks or the DNMS task. Surprisingly however, they were significantly impaired on the lifesaver task. Ongoing histological verifications of the lesion will clarify whether this impairment of motor skill learning can be attributed directly to NA damage or is the result of inadvertent damage to adjacent subcortical structures.

444.14

A METHOD FOR THE SEPARATION OF ATTENTION AND MEMORY IMPAIRMENTS FROM MOTIVATIONAL AND NON-SPECIFIC DEFICITS DUE TO WORK SCHEDULE SHIFTS. **W.N. Tapp*, R.J. Servatius, T.A. Pritzel, S.D. Drastal, M.T. Bergen, V.A. Medical Center, E. Orange, NJ 07019 & New Jersey Med. Sch., Newark, NJ.**

Circadian rhythms have profound effects on arousal, memory, and attention. Using rhesus monkeys, we previously explored the effects of different light:dark schedules, including 6 hr phase-shifts, on task performance. However, our vigilance task could not discriminate between attention deficits and motivational or other non-specific changes which caused monkeys to not attempt to work on the task. Further, the previous task limited our ability to examine effects on memory versus cognitive effects on task performance. The current report describes a new chained vigilance-delayed same-different (DSD) task which vastly improves our ability to identify the type of deficit produced by light schedule shifts. The new task employs 3 levers and a tricolored cue light at the face of the cage. The monkey must begin each trial by making a check-in response indicating that it is willing to work on the trial. This specific "willingness-to-work" measure can be used to separate motivational changes (e.g. due to satiation) and non-specific effects. Once a monkey has indicated that it will work on a trial, we test "attention" using a 10 sec vigilance trial with a 0.2 sec light stimulus. Signal detection methods are used to examine changes in sensitivity and bias. Finally, the DSD task can be used to test the monkey's ability to perform a rule-based same different task and memory can be tested by imposing increasing delays between the test and target stimuli. Thus, this task increases our ability to discern motivational or non-specific effects of circadian and pharmacological treatments from attention-, cognitive-, and memory-based effects. Supported by V.A. Medical Research funds.

444.15

BASAL FOREBRAIN (BF) LESIONS IN MONKEYS PRODUCE IMPAIRMENTS IN ATTENTION. M.L. Voytko*, D.S. Olton, R.T. Richardson and D.L. Price. Neuropathology Laboratory, The Johns Hopkins University Sch. of Medicine, Balto., MD 21205.

We have shown that BF lesions in monkeys do not produce severe impairments in learning or memory [*Soc. Neurosci. Abstr.* 16:617, 1990]. Because the BF may be involved in attention processes, a covert orienting paradigm was used to examine attention in these monkeys. To obtain rewards, monkeys with BF lesions (n=3) and controls (n=2) were required to detect a target at one of two lateral positions. A cue directed attention to either the target location (valid trials) or an alternate location (invalid trials). Time to release a central panel and time to hit the target were measured as a function of cue location. For both groups, time to release was more rapid on valid trials. However, lesioned monkeys were slower to release for both types of trials, particularly invalid trials. Motor impairments cannot account for these group differences because time to hit the target was similar between groups. These findings suggest that, although BF lesions may not directly disrupt mnemonic processes in primates, attentional processes may be affected.

444.16

TEMPORAL ORDER MEMORY IN MONKEYS IS IMPAIRED BY MEDIAL THALAMIC LESIONS. E.C. Gower.* Boston VAMC and Boston University School of Medicine, Boston, MA 02130.

Given a list of objects to remember, normal monkeys can later retrieve information about both object identity, and - as we have recently demonstrated - object order. In the order recognition task, several objects are presented individually and in succession; two of them are then paired for a forced-choice test in which the object that had appeared earlier in the list is correct. Monkeys can discriminate the order of any pair of objects from a list of at least five, and like human recency judgments, their accuracy depends on the lag of both objects in the probe. Here we show that monkeys with lesions in the limbic thalamus who have only a modest object recognition impairment do not discriminate object order.

On any trial, a sample list of 2, 3, 4 or 5 randomly-selected objects appeared at 10 s intervals. Each list was followed by a single probe trial in which two objects from the previous list were presented simultaneously. Probes contained either two adjacent objects (eg., AB, BC or CD in a 4-object list) or two end objects (eg., AD), with the earlier object the correct and reinforced choice. Forty trials were collected in each of the 13 conditions in the experiment. Two normal monkeys selected the earlier object in the probe 72.5 and 72.7 % of the time and their performance was lag-dependent. In contrast, two monkeys with medial thalamic lesions made correct choices 63.3 and 64.6 % of the time, which is above chance by Chi-square tests of significance but also impaired relative to controls. Only in those conditions where the last object in the list appeared in the probe were their choices nonrandom, and in contrast to normal performance accuracy in discriminating the order of end objects did not increase with list length. Whereas normal monkeys possess a complete and ordered mnemonic representation of the list, the experimental monkeys appear to be be capable only of assigning a list position to the most recent object. Korsakoff alcoholic amnesics and patients with lesions in the frontal lobes are impaired in processing information sensistive to the time of occurrance of events. The present results suggest that the medial thalamus may play a specific role in these disorders of temporal memory.

444.17

LOCUS COERULEUS UNIT ACTIVITY IN RESPONSE TO NOVELTY AND CHANGE ENCOUNTERED DURING ENVIRONMENTAL EXPLORATION. S.J. Sara*, A. Vankov and A. Hervé, Inst. A. Fessard, CNRS, Gif/Yvette, FRANCE.

During learning, marked but short-lived phasic responses occur in Locus Coeruleus (LC) units when stimulus-reinforcement contingency is changed. LC units also respond robustly to novel stimuli and habituate rapidly (Sara & Segal, 1991). To further investigate the role of the noradrenergic (NA) system in learning and attention processes, LC cell activity was monitored in behaving rats, by means of movable microelectrodes, during free exploration of a cue controlled environment. The testing chamber was a closed arena with holes in the floor under which various objects were placed. The precise temporal pattern and duration of inspection of individual holes was monitored by photocells such that LC spikes could be displayed as peristimulus time histograms around the visits to particular holes. The LC unit tended to fire in bursts at the first encounter with a novel object, the response habituating during subsequent visits. Some cells fired on the first encounter with an empty hole, but most empty holes did not elicit the bursting activity. Control for the NA nature of the cells was achieved by injection of alpha$_2$ receptor agonist or antagonist which inhibited or enhanced, respectively, spontaneous firing. The results further demonstrate the engagement of the LC in the presence of change in environmental stimuli, as seen in the conditioning experiments. The NA released at this bursting activity promotes gating and tuning of forebrain responses necessary for stimulus selection and cognitive adaptation.

Sara, S. J. & Segal, M., Prog Brain Res, 88, 571-585, 1991.

444.18

PREFRONTAL CORTEX COGNITIVE DEFICITS IN EARLY-TREATED PKU (ET-PKU) DUE TO DOPAMINE DEPLETION: RESULTS OF A LONGITUDINAL STUDY IN CHILDREN & OF AN ANIMAL MODEL A. Diamond[*1,2], V. Ciaramitaro[2], E. Donner[2], W. Hurwitz[1], E. Lee[1], W. Grover[3], & C. Minarcik[4]. [1]Dept. of Psychology & [2]Inst. of Neurological Sci.s, U. of Penn., Phila., PA. [3]Neurology, St. Christopher's Hospital for Children, Phila., PA. [4]Pediatric Neurology, Cooper Medical, Camden, NJ.

Clinical opinion has considered (1) diet sufficient treatment for PKU & (2) phenylalanine (phe) levels of $\leq$10 mg/dl safe. Results of longitudinal study of 44 infants, toddlers, & young children treated since birth for PKU or hyperphenylalaninemia show otherwise: These children performed poorly on a variety of prefrontal tests at all 3 age ranges, though normally on control tasks, including those requiring parietal or medial temporal lobe function, relative to matched controls, siblings, & a normative sample. Children with higher phe levels (e.g., 6-10 mg/dl) performed worse on prefrontal tests than those with lower levels. *In the same child* over time, performance covaried inversely with blood levels of phe. Cognitive deficits appeared reversible at least through 1 year.

An animal model of ET-PKU tested our hypothesis that the effect on prefrontal function is mediated by a selective dopamine depletion: Rat pups received daily injections of α-methyl-phe (24 μmol/10g body wt) & phe (12 μmol/10g body wt); their littermates received saline injections. A 3rd group was exposed to α-methyl-phe & phe prenatally & postnatally. Relative to controls, the 2 experimental groups had elevated phe levels (like those in ET-PKU & hyperphe), showed impaired performance on the prefrontal task (delayed alternation), & had lower DA & HVA levels specifically in medial prefrontal cortex & anterior cingulate cortex. (Support: NIMH #MH41842, MRRC, March of Dimes, BRSG #RR07083-26).

445.1

LEARNING-INDUCED INCREASE OF TONE-EVOKED RESPONSE IN THE AUDITORY THALAMUS DURING PARADOXICAL SLEEP.

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We have previously provided behavioral (Behavioural Brain Research, 18:241, 1985) and electrophysiological (Psychobiology, 19:193, 1991) evidences that a relevant stimulus can be detected during paradoxical sleep (PS). We report here that when an acoustic stimulus has acquired significance by conditioning during waking, its processing in the auditory system is enhanced during PS. After 1 session of habituation to a tone, waking rats underwent 3 conditioning sessions with the tone as CS preceding a footshock. After each session, the same tone was presented during PS phases, without any awakening of the animal. Multiunit activity in the medial division of the medial geniculate body was recorded at each tone presentation during waking and during PS. After conditioning, tone-evoked response was increased both in the waking and PS states. No such changes were observed in pseudoconditioned animals receiving unpaired tone-shock presentations. Increased responses to meaningfull stimuli, occurring in the sensory pathways on a background of limited responsiveness, could explain how an organism in PS is able to discriminate a relevant stimulus from all the other inputs.

445.2

THE FORMATION OF LONG-TERM HABITUATION TO SPATIAL NOVELTY MODIFIES THE STRUCTURE OF POST-TRIAL SLEEP IN RATS.

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To investigate the role of post-trial sleep in the elaboration of memory traces (Physiol.Behav., 51:217, 1992), a series of experiments was run to study its role in the formation of long-term habituation (LTH) to spatial novelty. Adult male Sprague-Dawley rats with chronically implanted cortical electrodes for EEG recording were exposed to a Làt-maze, and horizontal (HA) and vertical (VA) activity were counted during two 10min test trials at a 3 h or 24 h interval. EEG conventional recordings taken during 3 h under baseline conditions (day 0), and after exposure to the maze (day 1), were analyzed as to the number (n) and mean duration (d) of paradoxical sleep (PS) and synchronized (SS) sleep episodes followed by PS (*SS-->PS*) or by wakefulness (*SS-->W*). A significant LTH of HA and VA associated with LTH of emotionality index (defecation score: LTH-E) was accompanied by an increase in the (n) and (d) of post-trial SS-->PS (but not SS-->W), concerning episodes longer than 4 min. Further, correlative analyses among behavioral and sleep parameters showed that SS-->PS (and not SS-->W) (n) and (d) covaried positively with LTH-HA in all three hours, and with LTH-VA in the 2nd and 3rd h. In contrast, LTH of HA and VA dissociated from LTH-E, was accompanied by a lower SS (n) in the 1st h and a higher SS (d) in the 2nd h, with no change in SS-->W episodes. Correlative analyses showed that LTH-HA and VA covaried positively with SS-->PS, and negatively with SS-->W episodes, whereas LTH-E covaried negatively. The different time-dependent correlative profiles for HA, VA and E, with a prevailing cognitive or non cognitive content, respectively, suggest their sequential/parallel processing during both sleep phases, thus confirming the validity of the *sequential hypothesis* of sleep function.

445.3

INHIBITION OF DAY-TIME MEMORIES DURING NON-REM SLEEP AND SUPPRESSION OF MEMORIES OF DREAMS IN THE HIPPOCAMPUS.

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A "tracing circuit association" model has been proposed in which multimodal memory traces in cortical cell assemblies (tracing circuits) are consolidated by prolonged tracing through cortico-hippocampal projections and associated by LTP in intra-hippocampal connections (Kuroda,Y.: *Concepts in Neurosci.*2:221, 1991). However, such multimodal associative memory connections in hippocampus would be easily saturated. Here, we propose a hypothesis that erasing of weaker connections occurs during non-REM sleep. Repetitive low-frequency stimulation (more than 1000 pulses at 1-2 Hz) of excitatory input cause depotentiation(DP) of LTP(not PTP) in CA1 neurons in the slice, as well as long-term suppression of LTP (LTS; LTP cannot be formed for approx.60 min.). LTD is not induced by the stimulation. (Fujii,S.et al.: *Brain Res.*,555:112, 1991). Such low frequency(1-2 Hz) activity of hippocampal neurons is observed as the slow wave during non-REM sleep in rat (Suzuki,S.et al.:*Electroencephal. Clin. Neurophysiol.*, 67:348,1987). Therefore, LTP of intrahippocampal connections can be reversed by DP with only strong LTP persisting after sleep. During sleep, especially REM-sleep, random associations of cortical activities (dreams) should be memorized by the association model, however, LTS caused by previous non-REM sleep may have inhibited formation of associative memory by LTP in REM-sleep. Some dreams may occasionally be stored by PTP and by strong LTP in the hippocampus which LTS fails to inhibit.

445.4

SUPPRESSION OF LTP INDUCTION IN THE DENTATE GYRUS DURING ALERT WAKEFULNESS BUT NOT DURING "ENHANCED" REM SLEEP AFTER AVOIDANCE LEARNING

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Rapid eye movement (REM) sleep is thought to play a critical part in memory formation. Learning events, such as active avoidance conditioning, are followed by a transient increase in the amount of REM sleep which is necessary for normal memory retention.

Rats were chronically implanted for unilateral stimulation of the perforant path and recording of evoked potentials in the dentate hilus. Test potentials were collected during REM sleep and a still-alert (SAL) behavioral state. After baseline recording rats were trained on a 40 trial shuttle-box avoidance task. Conditioned rats exhibited a significant increase in the duration of REM episodes relative to pseudoconditioned, yoked controls. High-frequency trains were applied during the second, third and fourth post-trial REM episodes. Another group was tetanized at the same time points during SAL. LTP was reliably induced during both SAL and REM following pseudoconditioning. In the learning group, however, tetanization in SAL failed to elicit LTP of the EPSP slope (8% increase) while normal LTP developed after tetanization in "enhanced" REM (32% increase). There was no difference in the magnitude of population spike LTP.

We conclude that avoidance learning affects subsequent LTP induction in a state-dependent manner, allowing normal induction during post-trial REM but suppressing it during alert wakefulness.

445.5

SACCADES MODULATE UNIT ACTIVITY IN MEDIAL TEMPORAL LOBE IN THE DARK

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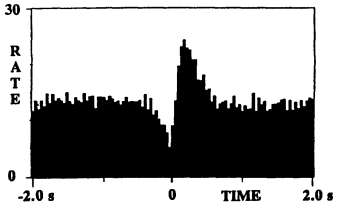
In primates, saccadic eye movements frame the visual input. They may then serve to enforce synchrony onto visual and mnemonic processing. Thus, we thought to look in the hippocampus and nearby temporal cortical areas for unit activity coupled to eye movements. Such responses turn out to be common, strong, and widespread, in both light and dark. It was found through 12 of 16 implanted guide tubes aimed at hippocampus, inferotemporal and medial ventral cortex.

In our sample analyzed to date (n > 300, 3 monkeys), over 25% of the single units showed clear responses coupled to eye movements in the dark. For some cells, the altered activity begins 100ms before the saccade. Both increased and decreased activity were seen. Responses were usually dependent upon the eye movement's direction.



Post-stimulus time histogram taken in the dark, triggered on each saccade (0 on abscissa). The ordinate is in spikes/sec.

445.6

A HEAD-FIXED PREPARATION FOR ELECTROPHYSIOLOGICAL STUDIES OF LEARNING AND MEMORY IN THE BEHAVING RAT.

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A number of electrophysiological investigations of learning and memory could be more directly controlled and more easily accomplished in behaving rats if one could fix the animal's head in space and utilize recording techniques conventional in acutely anesthetized preparations. The successful development of a head-fixed behaving rat preparation depends on a long-lasting rigid head mounting system, techniques for adapting the rat to the fixed-head training environment, and selection of learning tasks in which rats can perform well during brief recording sessions. We have been developing such a preparation, adapting head-fixing and training techniques from recent successes with similar preparations by C.D. Woody and T. Ono, and exploiting rats' superb learning abilities when guided by olfactory cues.

Male Long-Evans rats were pre-trained on an odor discrimination task then, under ketamine-nembutal anesthesia, implanted with multiple miniature self-tapping skull screws, a bipolar MFB electrode, and two horizontally-mounted cannulae on the skull surface. After recovery, they were habituated under successively smaller doses of acepromazine to stereotaxic fixation of the head using pins inserted into the cannulae. Then they were shaped to lick a water spout in order to receive sugar-water plus 400uA MFB stimulation. Later they were re-trained on the odor discrimination for sugar-water rewards. Following these procedures rats could then be trained within a single session on novel odor discriminations. Futhermore, large single hippocampal complex spike and other cell types were readily isolated with conventional microelectrodes and could be recorded with stable, large signal-to-noise ratio during behavioral performance.

445.7

RAT'S HIPPOCAMPAL UNITS HAVE BEHAVIORAL CORRELATES DURING THE PERFORMANCE OF AUDITORY WORKING AND REFERENCE MEMORY TASKS. Y. Sakurai* Dept. Psychol., Toyama Med. & Pharm. Univ., Sugitani, Toyama 930-01, Japan.

The present study examined roles of hippocampal neurons by recording their single units while the rat performed both auditory working and reference memory tasks. The working memory task was continuous nonmatching-to-sample (Sakurai, Behav. Neurosci., 104: 253, 104:856, 1990). The reference memory task was continuous discrimination. The apparatus, stimuli and sequence of the stimuli are identical for both the memory tasks. Around 10% of the units from CA1, CA3, and dentate gyrus showed behavioral correlates only in the working memory task. Behavioral correlates mean differences in unit's activity to the types of stimuli and/or responses in the tone presented and/or delay periods. Between 20% and 30% of the units showed behavioral correlates only in the reference memory task. About half of the units showed behavioral correlates both in the working and reference memory tasks. Types of those correlates were different between the tasks. These results suggest that there are different types of mnemonic neurons in the hippocampas and more of the neurons are related to both nonspatial working and reference memory processes. (Supported by Grants-in-Aid Nos. 02255208, 02610040 and 03251213).

445.8

LEARNING-DEPENDENT ENHANCEMENT OF DENTATE GYRUS GRANULE CELL RESPONSIVITY. T. Otto*, A. Kitzmiller, and H. Eichenbaum, Dept. of Psychology, University of North Carolina, Chapel Hill, NC 27599.

Previous studies have demonstrated that brief episodes of exploration are accompanied by a short-term enhancement of EPSPs in dentate gyrus (DG) evoked by perforant path (PP) stimulation. It remains to be determined, however, whether a similar enhancement of synaptic efficacy can be induced by formal training in tasks known to engage hippocampal function. The present, ongoing study examines whether these effects can be induced by explicit bouts of olfactory learning, and the extent to which such enhancement is learning-dependent.

Male Long-Evans rats were implanted with a stimulating electrode in the PP and a recording electrode in the hilus of the DG. Following recovery, 24 EPSPs evoked by PP stimulation (20 sec ISI, 50% max EPSP) were collected each day in the subject's home cage. Following establishment of a stable baseline (<10% variation in initial slope across 3 consecutive days), subjects were on successive days placed for 15 minutes into the chamber in which behavioral training would eventually take place, presented with a number of different odorants from a sniff port and received water reinforcers at a nearby water port ("shaping"), and trained on an odor-guided continuous delayed nonmatching to sample (cDNM) task. Fourty-four EPSPs were collected immediately after each day's behavioral session. Preliminary evidence indicates that no change in EPSP slope is observed following simple exposure to the behavioral chamber or following shaping. By contrast, an increase in EPSP slope is observed following daily cDNM training; the magnitude of this increase is significantly correlated with cDNM performance during acquisition (r's = .4 - .86). These data suggest that under these conditions enhanced synaptic efficacy in the PP-DG pathway reflects information processing in the entorhinal-DG system and is not due to simple sensory stimulation. Supported by ONR grant N00014-91-5-1881 to HE and TO.

445.9

FUNCTIONAL SIGNIFICANCE OF ANATOMIC CONNECTIONS BETWEEN CA1 AND CA3 HIPPOCAMPAL CELLS DURING DELAYED MATCH TO SAMPLE BEHAVIOR IN THE RAT

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Recent studies of the intrinsic anatomic connections between the principal cells of the hippocampus (CA1 and CA3 pyramidal cells) have proposed an intricate gradient of connectivity which traverses the longitudinal axis of the hippocampus (Amaral & Witter, Neuroscience 31:571-591, 1989; Ishizuka *et al.* J. Comp. Neurol. 295:580-623, 1990). The functional significance of these connections was investigated using many neuron recording and classification strategy to identify cells which appeared to be synaptically "coupled" along these gradients in freely moving rats performing a delayed match to sample task (Heyser *et al.* Soc. Neurosci. Abstr. 15:1170, 1989). Custom fabricated arrays consisting of 2 rows of 8 microelectrodes were implanted to traverse the longitudinal axis of the hippocampus, with the electrode tips in each row positioned in either the CA3 or CA1 cell layers. Spike triggered histograms were constructed from spikes on each of the 8 CA3 electrodes for the entire set of spikes on the CA1 electrodes. Extracellular spikes from individual cells were isolated via a multichannel online spike sorter (Spectrum Scientific) and cell firing correlated with various phases of the DMTS task. Several of the CA3 cells exhibiting task specific firing correlates also showed short latency "coupled" discharges associated with CA1 cell spikes. Both the position and the degree of coupling between pairs of CA1 and CA3 cells across these electrode arrays as well as their relation to hippocampal anatomy will be described.

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445.10

FUNCTIONAL ARCHITECTURE OF THE HIPPOCAMPUS STUDIED WITH THE OPTICAL METHOD T. Iijima*, M.Ichikawa S. Akiyama and G. Matsumoto Electrotechnical Lab. 1-1-4 Umezono , Tsukuba, Ibaraki 305, Japan

According to the lamella hypothesis, each lamella which is prepared by sectioning the hippocampus transversely to the longitudinal axis should involve basically the similar excitatory pathway, and may operate as an independent functional unit. We examined the hypothesis with the optical method using a voltage-sensitive dye by comparing the neuro-circuit involved in each slice preparation sampled from the various portion of a hippocampus. The results obviously showed the heterogeneity of the neuro-circuit in each " lamella ".

In vivo studies were performed along with the *in vitro* study to reveal the actual information processing in the three dimensional space of the hippocampus. The optical measurements applied to the *in vivo* study revealed the strong association of neuro-circuit in the longitudinal direction of a hippocampus.

In the optical measurement of neural activity, we also studied the expression of a long-term potentiation (LTP) *in vivo*. Based on the observation of the spread of activated area due to the establishment of LTP, we analized the change of the manner of information processing by the neural plasticity.

445.11

BLOCKADE OF MITRAL/TUFTED CELL HABITUATION TO ODORS BY ASSOCIATION WITH REWARD. D.A. Wilson* and R.M. Sullivan, Devel. Psychobiology Lab., Dept. Psychology, University of Oklahoma, Norman, OK 73019

Association of odor and reward during the early postnatal period modifies rat pup behavioral responses and olfactory bulb neural responses to subsequent presentations of that odor. Recent evidence from our lab has shown that olfactory bulb output neurons, mitral/tufted cells, receive convergent odor and reward inputs. Here, we examined the effect of reward input on M/T cell response to odors during associative training.

Single-unit recordings of M/T cells were made in PN11-12, urethane anesthetized Wistar rat pups. Responses of cells were examined to peppermint odor and to stimulation of the medial forebrain bundle/lateral hypothalamus. Odor responsive cells were then randomly assigned to either a PAIRED training group, receiving 4 sec odor pulses with MFB/LH stimulation at odor offset or ODOR-only training. For both groups odors were delivered every 30 sec. Response magnitude to the odor stimulus (pre-MFB/LH stimulation) was compared across 15-30 conditioning trials.

The results suggest that contiguous odor-reward pairings prevent M/T cell habituation to the odor that normally occurs to repeated odor-only stimulation.

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445.12

MODULATION OF THE EARLY AND LATE COMPONENTS OF EMG STARTLE ACTIVITY IN THE RAT

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The reflex pathway of the acoustic startle response is a simple, fast-latency system within the brainstem of the rat. Short-term habituation of startle occurs within the reflex pathway, but long-term habituation relies upon the inhibition of the response by neural mechanisms outside of the reflex circuit. The startle response also is modulated by other processes such as prepulse inhibition and potentiated startle. The short latency of the reflex places serious timing constraints upon these modulators of startle amplitude. The modulating effects of long-term habituation, prepulse inhibition, and potentiated startle on the startle reflex were studied using EMG recordings in the spinotrapezius muscle of 24 rats.

Acoustic startle stimuli provoked a bimodal EMG response with response latencies of about 8 and 18 ms. Preliminary evidence suggests that the late component may undergo stronger long-term habituation than does the early component. Both components, however, respond to changes within a test session, including manipulations of stimulus intensity and interstimulus interval. Furthermore, the amplitude of both components decreased during prepulse inhibition testing. Preliminary evidence suggests that the responses of the two EMG components are not altered within a potentiated startle paradigm when the startle stimulus serves as the conditioned stimulus paired with aversive footshock.

445.13

EFFECTS OF DORSAL CORTEX LESIONS ON HABITUATION TO A LOOMING STIMULUS IN TURTLES. A. S. Powers* and L. Wojcik. St. John's University, Jamaica, NY 11439.

Previous investigations in our laboratory have shown that dorsal cortex lesions impair learning in turtles. The present study was undertaken to determine whether habituation to a looming stimulus (LS) would be affected by such lesions. Painted turtles (Chrysemys picta) were given dorsal cortex (n=4) or sham lesions (n=5). The apparatus consisted of a platform on which the turtle was restrained in front of a rear-projection screen on which the LS was presented. Head withdrawals were detected by a photocell beam that was shadowed when the subject's head was extended. Before being presented with the LS, the turtles were habituated to the restraint being used. In the experiment proper, the LS was a circle projected on the screen, enlarging from 7.5 cm to 22.5 cm within 1 sec. The typical response of the turtle was head withdrawal. Turtles with sham lesions showed rapid habituation to the restraint in the pretraining and showed both within- and between-day habituation to the LS. Turtles with dorsal cortex lesions took significantly longer to habituate to the restraint, and 2 animals failed to show such habituation. There was a nonsignificant suggestion of an impairment in between-day habituation in the 2 turtles that were able to be tested on habituation to a LS. The results suggest that dorsal cortex lesions may produce a deficit in habituation in turtles.

445.14

A FREQUENCY ANALYSIS OF HABITUATION IN STATIONARY RESPONSIVE VISUAL CELLS IN THE AMPHIBIAN TECTUM. M. M. Nikoletseas.* Dept. of Anatomy, U. of Puerto Rico, Sch. of Med. San Juan, PR 00936.

Tectal cells of various sensory modalities have been reported to habituate. Habituation is traditionally defined as a decrement in the number of responses as a function of repeated stimulus presentation. In this study a quantitative analysis of habituation was undertaken with emphasis on the changes in interspike intervals. Frogs (Rana catesbeiana) were anesthetized and paralyzed. A light stimulus (duration 50 ms, ISI 1 sec) was presented in the center of the receptive field of stationary responsive (SR) cells (movement-sensitive or directionally-selective cells were excluded) for 1.5 min, and spikes were preamplified and fed into a computer for on-line acquisition and analysis. Use of various filters excluded stimulus artifacts and noise from the analysis. The main findings are: 1. These cells had very low spontaneous activity. 2. The increment in response rate as a result of stimulus presentation gradually declined with repetition but never reached spontaneous levels. 3. As habituation progressed interspike interval lengthened (coding by frequency). 4. In spontaneous recovery there was generally a return to high response rates and short interspike intervals. 5. The frequency coding mechanism is independent of that of spike count. In conclusion, during habituation SR tectal visual cells employ the same coding mechanisms as do somatosensory and auditory tectal cells previously reported.

INGESTIVE BEHAVIOR: TASTE AVERSION AND NEURAL MECHANISMS

446.1

ALTERED DIET SELECTION IN RATS WITH LESIONS OF AREA POSTREMA/ADJACENT NUCLEUS OF THE SOLITARY TRACT (AP/mNTS-LESIONS). G.L. Edwards*, B.J. Mullen, R.J. Martin and J.D. Power. Dept. of Physiol. & Pharm. and Dept. of Foods and Nutrition, Univ. of Georgia, Athens, GA 30602.

Recent diet selection studies have reported that rats fed dietary tallow consume increased amounts of high protein/low carbohydrate diet compared to control, corn oil fed rats (J. Nutr. 120: 1418, 1990). These studies suggest that some factor or condition associated with ingestion of saturated fats results in a shift in diet selection. In this study we examined the effect of AP/mNTS-lesions on selection of a high protein/low carbohydrate diet versus a low protein/high carbohydrate diet. We found that rats that received an AP/mNTS-lesion at least 12 weeks earlier selected a greater percentage of their daily diet from the high protein/low carbohydrate diet than unlesioned control rats (AP/mNTS-lesion, 41.5 ± 10.4% vs CONT, 15.1 ± 3.7%). Weight loss by the lesioned group does not appear to explain altered diet selection as weight matched control rats consumed only 6.1 ± 2.2% of daily intake from the high protein diet. These data provide support for a role of the AP in diet selection. (Funded by NIH DK42533)

446.2

LESIONS OF AREA POSTREMA (AP) ABOLISH CONDITIONED TASTE AVERSIONS BUT NOT ANOREXIA INDUCED BY LiCl IN RATS. K.S. Curtis, A.F. Sved, J.G. Verbalis, E.M. Stricker*. Dept. of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

AP lesions were produced by vacuum aspiration in adult male Sprague-Dawley rats. Consistent with previous findings, when water-deprived rats were allowed to drink novel flavored fluids just before treatment with LiCl (3 mEq/kg, ip), sham-operated (n=6) and non-operated control rats (n=7) demonstrated a pronounced aversion to the fluids whereas rats with AP lesions (n=12) did not decrease fluid consumption significantly. However, in a 30-min test period after overnight food deprivation, rats with AP lesions and control animals reduced food intake significantly and to an equivalent degree after pretreatment with either LiCl (3 mEq/kg, ip) or hypertonic saline solution (2-3 ml of 2 M NaCl, ip). These results are consistent with the traditional view that AP mediates the sensation of nausea produced by LiCl treatment (hence the loss of conditioned taste aversions after AP lesions), but suggest that neither nausea nor AP is critical for the marked disinclination to eat that is induced in rats by systemic treatment with LiCl or with hypertonic NaCl solution.

446.3

TRACKING LITHIUM-INDUCED SHIFTS IN SUCROSE PALATABILITY: ROLE OF THE AREA POSTREMA. L.A. Eckel* and K.-P. Ossenkopp. Neuroscience Program, Univ. of Western Ontario, London, Ontario, CANADA N6A 5C2.

The present study investigated the role of the area postrema (AP) in mediating the effects of lithium on taste reactivity (TR) responses. Rats received lesions of the AP (APX, N=12), or sham lesions (APS, N=14), followed by implantation of an intraoral cannula. On the first test day, half of the rats in each group were injected with either LiCl or equimolar NaCl (0.6 M, 5 ml/kg, i.p.). Ingestive TR responses (rhythmical mouth movements, tongue protrusions, and lateral tongue protrusions) and aversive TR responses (gapes and chin rubs), were videotaped during 30 sec intraoral infusions of 0.3 M sucrose. Infusions began immediately post-injection and at 5 min intervals for 30 mins. Three days later this procedure was repeated. Rats were tested 72 hours later for the expression of a conditioned taste aversion. APS rats given NaCl showed high levels of ingestive responses during all test days. APS rats given LiCl showed a decline in ingestive responses and an increase in aversive responses ($p<.001$). In comparison, APX rats treated with either LiCl or NaCl displayed high levels of ingestive responses similar to the APS/NaCl group ($p>.10$). It was concluded that lithium-induced activation of the AP is required to produce palatability shifts and the formation of CTAs. (Supported by a NSERC operating grant to KPO).

446.4

CONDITIONED TASTE AVERSION INDUCED BY INCREASED ACETYLCHOLINE IN THE NUCLEUS ACCUMBENS K.M. Taylor*, K. Davidson, G.P. Mark, P. Rada and B.G. Hoebel Department of Psychology, Princeton University, Princeton, NJ 08540.

Increased extracellular ACh in the nucleus accumbens (NAC) is associated with precipitated morphine withdrawal (Rada et al., 1991) and supression of feeding (Mark et al., 1991). If these findings indicate an aversive action of the transmitter, then ACh increase in the NAC may be sufficient to induce a conditioned taste aversion.

Male Sprague Dawley rats were water deprived and trained to drink during 30 min. daily access to water. After training, animals were presented 2.5 mM sodium saccharin in water, and the taste was paired with either 4 μg/μl neostigmine (n=7) or vehicle (n=7) injected bilaterally in the NAC (0.5 μl per side). A pseudoconditioning control group received NEO in the NAC paired with water presentation (n=7). In a two bottle preference test, the saccharin-neostigmine group showed a significant aversion to saccharin [$F(2,18)=15.57$, $p<0.001$] compared to the two control groups. There were no differences in overall fluid consumption. These results suggest that an increase in extracellular ACh in the NAC may be sufficient to produce an internally aversive state.

446.5

ETHANOL-INDUCED CONDITIONED TASTE AVERSIONS: EFFECTS OF CHEMICAL LABYRINTHECTOMY. K.-P. Ossenkopp*, J. Rabi and L. A. Eckel. Neuroscience Program, Univ. of Western Ontario, London, Ontario, CANADA, N6A 5C2.

Male rats were chemically labyrinthectomized by intratympanic injections of sodium arsanilate (Group VNX). Control rats were given intratympanic injections of isotonic saline (Group VNS). All rats were adjusted to a 23 hr/day water deprivation schedule and then exposed either to a conditioned taste aversion (CTA) procedure or a control procedure. The CTA technique involved the pairing of a novel saccharin taste (0.15% solution) with subsequent i.p. injection of ethanol (1.5 g/kg; 15% solution). The control procedure involved the pairing of the saccharin taste with an injection of isotonic saline (10 ml/kg). Following 2 conditioning trials and 3 days of water only, saccharin preference ratios were obtained (2-bottle choice test) on 4 consecutive days. VNS rats exposed to saccharin plus ethanol exhibited a strong CTA ($p<0.01$) relative to VNS controls. VNX rats given saccharin plus ethanol showed a strong CTA ($p<0.01$) if conditioning occurred 29-30 days post labyrinthectomy. However, CTAs were abolished in VNX rats conditioned 19 days post labyrinthectomy. Thus, ethanol-induced CTA formation varied across the post labyrinthectomy time period. (Supported by a NSERC operating grant to KPO).

446.6

ALTERED GASTRIC MOTILITY FOLLOWING AREA POSTREMA ABLATION (APX). M. Kathleen Gruver, Arash Aflatooni, and Nancy J. Kenney*, Dept. Psychology, University of Washington, Seattle, WA 98195.

Ablation of the area postrema and caudal and medial portions of the nucleus of the solitary tract (APX) decreases intestinal transit of rats independent of APX-induced hypophagia. This study extends our analysis of APX on gastrointestinal (GI) function by examining its effects on gastric motility.

Eight APX rats and 7 sham-lesioned rats (SHAM rats) were studied 4 days following ablation. Gastric motility of urethane-anesthetized (1.25 mg/kg) rats was monitored via a tension transducer attached to the anterior gastric corpus. Following a 1-hr baseline period rats were injected centrally with either 5 g/.6 l TRH or .6 l saline. Motility was recorded for 20 min following injection.

Baseline gastric motility of APX rats tended to be higher and more variable than SHAM rats. TRH increased motility of both APX and SHAM rats, but the TRH response of APX rats was significantly suppressed.

Thus, the AP/cmNTS region may be involved in both unstimulated as well as TRH-induced increases in gastric motility. Whether changes of GI motility underlie the altered eating behavior of APX rats requires further study.

446.7

EVIDENCE OF LEARNING IN THE ETIOLOGY OF POISON-ELICITED PICA. D. Mitchell* and J. B. Nast. Department of Psychology and Psychobiology Program, University of Southern California, Los Angeles, CA 90089-1061.

Both humans and other animals frequently engage in pica (the consumption of non-nutritive substances) when suffering from gastrointestinal malaise. We have previously shown that a specific form of pica, geophagia (clay consumption), can be used to quantify the severity of gastrointestinal malaise induced by a variety of toxins and emetics, including lithium chloride. Though a small dose of lithium chloride reliably elicits robust conditioned taste aversions, the same dose elicits only a modest pica response compared to other toxins and emetics. This apparent inconsistency between the robust effectiveness of lithium chloride as a UCS in conditioned taste aversions and its modest effectiveness in eliciting pica led us to examine the putative role of learning in the etiology of poison-elicited pica.

Rats maintained with food, water, and kaolin always available were administered repeated intraperitoneal injections of .15 M lithium chloride (127.2 mg/kg) every fifth day for a total of ten trials. Results showed that the animals learned to engage in pica; clay consumption gradually increased across the first five trials from 1.5 g on the first trial to 15.3 g on the fifth trial. Thereafter, it remained stable across the remaining trials. These results are consistent with clinical literature which suggests that learning plays an important role in the etiology of pica.

446.8

ADRENALECTOMY DOES NOT PRODUCE CONDITIONED FOOD AVERSIONS IN RATS. P.K. Green*, T. Moore, C.W. Wilkinson, I.L. Bernstein and S.C. Woods. Depts. of Psychol., and Psychiat. and Behav. Sci., Univ. of Washington, Seattle, WA 98195 and GRECC, American Lake VAMC, Tacoma, WA 98493.

Following adrenalectomy, rats consume less food than sham-operated controls. If adrenalectomy produces illness or nausea, it is possible that adrenalectomized (ADX) rats show reduced food intake because they have developed a conditioned aversion to the diet consumed following surgery. To test this hypothesis, rats were introduced to a novel diet (either C-21 or AIN) for a period of seven days immediately following adrenalectomy or sham-adrenalectomy. ADX rats consumed significantly less food than sham-ADX rats during this period. On the eighth day following surgery, a preference test was administered. Rats were 8-hr. food-deprived, and then provided 4-hr. access to both C-21 and AIN. Mean 4-hr. food intakes for each of the groups, expressed as grams of C-21 consumed divided by total consumption during the preference test, were: sham C-21-maintained, .86; ADX C-21-maintained, .95; sham AIN-maintained, .34; ADX AIN-maintained, .55. Statistical analysis by ANOVA revealed that ADX groups did not show significantly lower preferences than sham-ADX groups for the diet to which they were exposed during the week following adrenalectomy. ADX rats tended to consume a higher percentage of their food as C-21 than sham-ADX controls during the preference test, but this trend was not significant. These data do not support the hypothesis that decreased food intake following adrenalectomy is due to illness or malaise.

446.9

WHERE IS AVERSION: LATERAL HYPOTHALAMUS OR VENTRAL PALLIDUM/SUBSTANTIA INNOMINATA? H.C. Cromwell*, D. Karimipour and K.C. Berridge. The University of Michigan, Department of Psychology, Ann Arbor, MI. 48109

Many previous studies have reported that LH damage induces aversion (Teitelbaum et. al., 1961, Stellar et. al., 1974, Schallert et. al., 1978). However, a study of neuron loss restricted to the LH found aphagia without aversion (Berridge, 1989). The purpose of this study was to resolve where the crucial site for aversion inducing lesions is located. Small bilateral excitotoxin lesions (QUIN, 60nM in 0.5ul of PBS) or bilateral sham injections of the lateral hypothalamus (LH), nucleus accumbens (NAcc) or the ventral pallidum/substantia innominata (VP/SI) were completed to determine the exact locus related to aversion enhancement. The taste reactivity test (Grill and Norgren, 1978) using oral taste infusions of sucrose (1M) was completed and the number of aversive responses (gapes, chin-rubbing, head-shaking and forelimb flails) to the palatable sucrose were tallied. To identify the lesions, two lesion mapping techniques were used: 1) a conventional neuron counting procedure, in which an attempt is made to count all neurons within a brain region, and 2) a new modified 'fractionator' procedure consisting of accurate 400X magnification counts at many point locations within a brain region.

Results indicated that food aversion is produced following bilateral neuron loss exceeding 70% in a 500um diameter area of the caudal ventromedial VP/SI alone, and not from NAcc or anterior LH damage.. The crucial subregion is located ventral and medial to the globus pallidus, and dorsal and lateral to the lateral hypothalamus.

446.10

2,5-ANHYDRO-D-MANNITOL (2,5-AM) INCREASES FOS-LIKE IMMUNOREACTIVITY (Fos-li) IN THE AP/NTS REGION BY STIMULATING VAGAL SENSORY NEURONS. T.T. Dinh*, S. Ritter and M.I. Friedman. Washington State University, Pullman, WA 99164-6520 and Monell Chemical Senses Center, Philadelphia, PA, 19104.

The antimetabolic fructose analogue, 2,5-AM, stimulates feeding. Feeding induced by low but not high 2,5-AM doses is blocked by hepatic branch vagotomy (Tordoff et al., '91). To further evaluate the role of the vagus in the response to high 2,5-AM doses, we assessed the ability of 2,5-AM (300 and 500 mg/kg) to induce Fos-li in the AP/NTS in sham-operated, hepatic branch vagotomized (HBV) and total subdiaphragmatic vagotomized (TSV) rats. 2,5-AM or equimolar doses of 2DG or fructose were infused remotely for 20 min through atrial catheters in the absence of food and rats were killed 2 hr later. Both doses of 2,5-AM, but not fructose, induced Fos-li in the AP/NTS. The effect of the lower 2,5-AM dose on Fos-li was blocked by HBV. The high dose effect was blocked by TSV, but not by HBV. We also found that TSV blocked feeding induced by the high dose of 2,5-AM. As reported previously, 2DG also induced Fos-li in the AP/NTS and stimulated feeding, but these effects were not blocked by vagotomy. Although Fos-li may not reveal all activated neurons, results are consistent with the hypothesis that stimulation of feeding by 2,5-AM, but not 2DG, may be entirely dependent on the vagus. Hepatic branch neurons may have the lowest threshold for activation by 2,5-AM, while higher doses stimulate neurons in other vagal branches.

446.11

CHARACTERIZATION OF GASTRIC VAGAL AFFERENT MECHANORECEPTOR RESPONSES TO CLOSE ARTERIAL INFUSIONS OF CCK IN RATS. G.J. Schwartz*, P.R. McHugh, & T.H. Moran, Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

We have previously demonstrated that gastric vagal mechanoreceptors also respond to close arterial infusions of the brain-gut peptide CCK. To examine the pharmacological specificity of these CCK-elicited responses, we observed the electrophysiological activity of single rat cervical vagal afferent fibers sensitive to both gastric loads and CCK (N=14) before and after administration of the selective type A and type B CCK receptor antagonists, L-364,718 and L-365,260. Some of these fibers (N=9) were also monitored for their responses to the above stimuli after acute pylorectomy. In addition, we investigated the ability of JMV-180, an antagonist at pancreatic low affinity sites that inhibits CCK satiety, to suppress CCK-induced gastric vagal afferent activity. Celiac artery infusions of L-364,718 acted competitively to inhibit the the response to CCK, both before and after acute pylorectomy, without altering the response to gastric loads. L-365,260 (10 pmol-1 nmol) failed to attenuate the gastric vagal afferent response to either gastric saline loads or 100 pmol infusions of CCK. In intact rats, celiac artery administration of 100 nmol of JMV-180 completely blocked the ability of 100 pmol CCK to stimulate gastric vagal afferent fibers, again without attenuating the response to gastric saline loads. These results demonstrate that CCK-elicited responses in a population of gastric vagal mechanoreceptive fibers are mediated by non-pyloric, type A CCK binding sites similar to pancreatic low affinity CCK receptors. Supported by DK19302.

446.12

REFEEDING AFTER 48 HOUR FAST INDUCES C-FOS mRNA AND PROTEIN IN RAT BRAINSTEM AND HYPOTHALAMUS. T.A. Houpt, T.C. Wessel, T. H. Joh, and A.C. Towle*, The Bourne Lab. and the Burke Medical Research Institute, Cornell University Medical College, White Plains, NY 10605.

In order to identify specific brain regions stimulated at the transcriptional level by food ingestion, we examined the expression of the proto-oncogene *c-fos* in brainstem and hypothalamus by immunohistochemistry and *in situ* hybridization in three groups of rats: *ad lib.* fed controls, rats fasted for 48 hrs, and rats fasted for 48 hrs and allowed to eat Purina rat chow for 1 hr prior to perfusion (n=4 in each group). For *in situ* analysis, 40μm tissue sections were hybridized overnight with synthetic, [^{35}S]-labelled *c-fos* oligonucleotides. Sections were dipped in photoemulsion and developed 4-6 wks later. Adjacent sections were immunohistochemically stained for c-Fos-related proteins.

One hour of feeding following a 48 hr fast caused intense *c-fos* mRNA expression and immunoreactivity co-localized in several regions. In the brainstem, expression was very strong in the nuclei of the solitary tract; in the rostral hypothalamus, strong *c-fos* expression was seen in the paraventricular and supraoptic nuclei, and more diffuse expression was seen in the anterior hypothalamic nuclei and bed nuclei of the stria terminalis; at the level of the caudal hypothalamus, the dorsal medial nuclei and the thalamic habenular and paraventricular nuclei were labelled, as well as individual cells encircling the third ventricle. Control and fasted animals showed little or no expression compared to refed animals in brain stem and anterior hypothalamus, although *ad lib.* fed controls showed occasional *c-fos* expression around the dorsal medial nuclei in the caudal hypothalamus.

Our results show that feeding induces *c-fos* expression in specific brain nuclei at both the mRNA and protein level. Further studies are required to correlate *c-fos* expression in these neural areas to specific orosensory and postingestive effects of food intake.

446.13

RETROGRADE, TRANSYNAPTIC LABELING OF TRIGEMINAL PREMOTOR NEURONS USING PSEUDORABIES VIRUS. R. Fay* and R. Norgren. Neuroscience Program and Dept. of Behavioral Science, Col. of Medicine, Penn. State Univ., Hershey, PA 17033.

Following bilateral removal of the superior cervical ganglion, pseudorabies virus (15 ul, $8x10^8$ pfu/ml) was injected into the anterior digastric, masseter, or temporalis muscles of rats. The animals were allowed to survive from 72 to 120 hours. Primary infections were restricted to trigeminal motor neurons (Mo 5) in a predictable myotopic pattern. Secondary infections appeared in neurons in areas known to project to Mo 5, including the Kolliker-Fuse, parabrachial, and principal trigeminal nuclei, as well as the supratrigeminal and subcoeruleus zones. Within these areas, however, infections of different muscles produced different distributions of labeled cells. Similarly, differential labeling was observed in regions thought to be involved in the central pattern modulation of Mo 5 activity, such as the gigantocellular and paragigantocellular areas. In the brainstem, increasing survival times revealed polysynaptic connections to the subpeduncular and pedunculopontine nuclei in the midbrain, the lateral tegmental and the oral, ventral, and caudal pontine nuclei in the pons, and the paratrigeminal, solitary, and rostroventrolateral nuclei in the medulla. With longer survival times the infection also spread to the forebrain, particularly in the hypothalamus, amygdala, and paleocortex. Nevertheless, the neocortex, thalamus, and most of the striatum remained remarkably free of labeled neurons. Supported by PHS grants DC 00240 and MH 00653.

446.14

MEDULLARY INPUTS TO THE FIFTH, SEVENTH, AND TWELFTH CRANIAL NERVE MOTOR NUCLEI. E.T. Cunningham, Jr.* and P.E. Sawchenko, The Salk Institute, La Jolla, CA 92037.

Prior anatomical studies performed in the rat identified the region of the caudal medullary reticular formation immediately subjacent to the nucleus of the solitary tract (NTS) as the primary source of afferent inputs to the motor nuclei of the fifth (MoV), seventh (VII), and twelfth (XII) cranial nerves, which are involved directly in the control of oromotor behaviors such as deglutition and mastication. In this study, the sensitive fluorogold retrograde tract tracing technique was used to identify medullary areas innervating MoV, VII, and XII, and to characterize the cells of orgin of these projections. Discrete deposits of the anterograde tracer, *Phaseolus vulgaris*-leucoagglutinin, were then placed in implicated portions of the dorsomedial reticular formation, and the topography of labeled terminal fields within each of the cranial nerve motor nuclei were charted. The results may be summarized as follows: 1) The regions of the reticular formation innervating MoV, VII, and XII are largely overlapping, and extend from the level of the MoV to the rostral spinal cord. In each instance, the greatest concentration of retrogradely labeled cells lay at the level of the NTS. 2) Most inputs are bilateral with an ipsilateral predominance. The single exception is the largely contralateral input to motor neurons innervating the masseter and pterygoid muscles in the dorsolateral portion of MoV. This projection arises from a cluster of large multipolar neurons immediately ventrolateral to XII at the level of the area postrema. These large cells appear not to project to VII or XII. 3) By contrast, smaller, more dorsally situated neurons project to motor neurons innervating the anterior digastric and mylohyoid muscles in the ventromedial portion of MoV. They also project to the intermediate and dorsal portions of VII, and to all subdivisions of XII, regions that innervate a number of muscles involved in the initiation of deglutition. Thus, although the resolution of these techniques does not allow us to discount the possibility that a small proportion of neurons in the reticular formation might share characteristics of both populations, it appears clear that two distinct, though topographically contiguous, deglutitive and masticatory pathways arise from the reticular formation in the dorsomedial medulla.

446.15

ELECTROLYTIC AND IBOTENATE LESIONS OF THE DORSAL AND CENTRAL SUBNUCLEI OF THE LATERAL PARABRACHIAL NUCLEUS (IPBN) ABOLISH LIPOPRIVIC BUT NOT GLUCOPRIVIC FEEDING. F.H. Koegler*, N.Y. Calingasan, S. Ritter. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

Lesions of the area postrema/nucleus of the solitary tract (AP/NTS) abolish lipoprivic and glucoprivic feeding induced by mercaptoacetate (MA) and 2-deoxy-D-glucose (2DG), respectively. Since the IPBN is a major relay site for ascending visceral sensory input from the AP/NTS region, electrolytic and ibotenate lesions of IPBN subnuclei were made to investigate the involvement of the IBPN in the central pathways of metabolic control of food intake. Lesions were confirmed either by cresyl violet staining or by glial fibrillary acidic protein immunohistochemistry. Lesioned rats were tested for feeding in response to 0.9% NaCl (s.c. or i.p.), MA (400, 600, and 800 μmol/kg, i.p.) and 2DG (100 and 200 mg/kg, s.c.). Electrolytic lesions of the dorsal, central, superior, and external IPBN abolished lipoprivic but not glucoprivic feeding. Ibotenate lesions of the external and superior subnuclei did not impair lipoprivic or glucoprivic feeding, but ibotenate lesions of the dorsal and central subnuclei did abolish lipoprivic feeding. These results suggest that the cell bodies in the dorsal or central IPBN participate in the lipoprivic control of feeding. Electrolytic lesions of the superior and external subnuclei appear to have damaged fibers of passage important for this control, possibly those in transit to or from the dorsal and central subnuclei.

446.16

RESPONSES OF PARABRACHIAL TASTE NEURONS IN SHAM-FEEDING RATS BEFORE AND AFTER DUODENAL LIPID INFUSIONS. L. Foster*, K. Nakamura, and R. Norgren, Dept. of Beh. Sci., Col. of Med, Penn State Univ., Hershey, PA 17033

Duodenal lipid infusions rapidly suppress sham intake of liquid diets and sapid stimuli. Three rats were outfitted with gastric fistulas and duodenal cannulas, maintained on a food deprivation schedule, and trained to ingest fluids while restrained in a chronic recording apparatus. Parabrachial gustatory neurons were isolated using conventional recording techniques. These cells were tested with intraoral infusions (50 ul) of a concentration series of sucrose, as well as a single concentration of polycose, NaCl, citric acid, and quinine HCl before, during, and after duodenal infusions of a lipid emulsion (Intralipid) or saline. The neurons were tested during 5 different, 15-min periods -- 1 before and 4 after the duodenal infusions. The responsiveness of at least 7 taste neurons was assessed during each time period; 11 neurons were tested both before and after an infusion. During the first post-infusion interval (0-15 min), neural activity elicited by NaCl decreased to less than that produced by any concentration of sucrose. During the next time period (15-30 min), taste neurons responded less to the lower concentrations of sucrose, but increased their responsiveness to NaCl. During the last period (45-60 min), NaCl responses returned to pre-infusion levels, while sucrose responses increased. These data suggest that some neurons may switch their best response category depending on whether the animal is food deprived or satiated. Supported by DC-00047, DC-00240, MH-00653.

446.17

CELLS WITHIN PONTINE PARABRACHIAL NUCLEUS COLLATERALIZE TO INNERVATE HYPOTHALAMUS AND DORSAL MEDULLA IN THE RAT: IMPLICATIONS FOR FUNCTIONAL INTEGRATION OF PAIN AND SATIETY. L.L. Bellavance*, P.L. Faris, R.D. Hofbauer, and C. Cozzari, Division of Neuroscience Research in Psychiatry, Univ. of Minnesota Sch. of Med., Minneapolis, MN 55455.

The pontine parabrachial nucleus (PBN) receives input relevant to feeding and nociception, and may play an integral role in the synchronization of these different functional systems. In the first part of this study, we utilized the retrograde tracers rhodamine-conjugated latex beads and fluorogold to determine if a single cell in the PBN collateralizes to innervate two distinct brain areas involved in processing information relevant to feeding or nociception. After injection of rhodamine-conjugated latex beads into the dorsal medulla and injection of fluorogold into the hypothalamus, double-labeled cells were identified in the rostral part of the dorsal lateral parabrachial complex. This would suggest that some PBN cells collateralize to innervate both the dorsal medulla and the hypothalamus, structures involved in the regulation of feeding and/or nociception. The second part of this study was designed to look at the peptide content of the collateralizing PBN cells. We combined the retrograde fluorescence-immunofluorescence technique to determine whether the PBN cells that collateralize also co-localize peptides implicated in the transmission of information relevant to feeding and/or nociception: cholecystokinin (CCK), calcitonin gene-related peptide (CGRP), and enkephalins. The results of these studies suggest that the PBN may utilize collateralization as an integrating system in addition to the more traditional interneuronal networks. Furthermore, the PBN may play an integral role in the synchronization of different functional systems, i.e. feeding and nociception.

446.18

COMPLEX NEUROCHEMICAL PROPERTIES OF FEEDING-ASSOCIATED PALLIDAL NEURONS. Z. Karádi, A. Czurkó, B. Faludi, I. Vida, Cs. Niedetzky, A. Hajnal, J. Czopf and L. Lénárd. (SPON: European Neuroscience Association) Institute of Physiology, Pécs University Medical School, Pécs, H-7643 Hungary

The multibarrel microiontophoretic technique was applied to record extracellular single neuron activity and to investigate specific chemical characteristics in the globus pallidus (GP) of anesthetized rats and anesthetized and alert rhesus monkeys. In both species, approximately 10% of all cells tested exhibited, predominantly inhibitory, discharge rate changes to electrophoretically administered glucose. Many of the glucose-responsive and non-responsive neurons changed in activity in response to perioral and intraoral somesthetic stimulation as well as, in the alert monkey, to food objects. These feeding-related cells displayed distinct sensitivities to various chemicals (catecholamines, GABA, Ach, MAO-blockers and "NMDA"-enantiomers) applied microiontophoretically. The present findings, along with previous results, suggest that differentially organized subsets of pallidal neurons utilize complex and distinct neurochemical mechanisms to control integrative functions in the regulation of feeding.

446.19

HYPERPHAGIA AND OBESITY IN FEMALE RATS WITH AMYGDALOID LESIONS. B. M. King*, J. M. Kass, N. L. Cadieux, K. L. Neville, H. Sam, and A. C. Tatford. Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70148.

Hyperphagia and obesity in cats, dogs, and primates with lateral amygdaloid lesions were reported by several investigators during the 1950's, 1960's, and early 1970's (e.g., Bucy & Kluver, 1955; Fonberg, 1971; Green, Clemente, & de Groot, 1957). Interest in this extra-hypothalamic obesity syndrome disappeared when such effects were not observed after amygdaloid lesions in rats. In an initial part of the present study, we also found that large lesions of the amygdala resulted in no weight change or even a small loss of body weight. However, small bilateral lesions in a specific portion of the amygdala (detailed histological analysis had not been completed at the time of submission) resulted in weight gains of 15-25 grams during the first 2-4 postoperative days. Daily food intake nearly doubled in some animals. In a subsequent controlled study, adult female rats with amygdaloid lesions gained 20-30% of their preoperative body weight in 30 days, compared to only 5% for animals with sham lesions. The amygdaloid-lesioned animals were unaffected when switched from a standard pellet diet to a high-fat diet and back again. It is hypothesized that the amygdala plays a major role in appetitive mechanisms.

446.20

DAILY CALORIC INTAKE REGULATION IN CHRONIC DECEREBRATE AND INTACT RATS. R.J. Seeley*, J.M. Kaplan, and H.J. Grill, Dept. of Psychology, University of Pennsylvania, Philadelphia, PA 19104-6196.

The chronically maintained decerebrate (CD) rat demonstrates, as do intact rats, changes in both taste-elicited oral motor responses and the size of individual intraorally delivered meals in response to a variety of ingestive challenges. In this study, the ability of the CD rat to defend daily caloric intake was explored. In a baseline period, both CD and intact control rats received 3 intraorally delivered meals of milk diet per day for a week. Meal size was determined by measuring the amount of the milk diet stimulus consumed before the rat rejected the stimulus. Both CD and intact rats took substantial meals in this paradigm and gained weight over the baseline period. Rats were then challenged to maintain their 3 meal daily intake over a week in which only 2 intraoral intake tests per day were delivered. In response to the lost meal, intact rats increased the size of the remaining meals and continued to gain weight. CD rats, however, did not increase their meal size in the 2 meal condition and lost weight during the test period. Additionally, a separate group of histologically identified rats had transections that were incomplete. Despite substantial neural damage, these rats, like controls, increased their meal size in response to the lost opportunity to feed. So despite the ability of CD rats to respond to short term regulatory challenges, these results suggest that the caudal brainstem is not sufficient to regulate daily caloric intake without neural connections with the forebrain. Such a deficit may reflect that different physiological signals and/or different neural substrates are responsible for shorter and longer term intake regulation. Supported by NIH grant DK-21397.

446.21

DISTRIBUTION OF LICKING - AND SWALLOWING - RESPONSIVE CELLS IN THE MEDULLARY RETICULAR FORMATION OF THE AWAKE FREELY-MOVING RAT. J.B. Travers* and L.A. DiNardo. Oral Biology, Coll. Dent. Ohio State Univ. Columbus, OH 43210.

Neuroanatomical studies have implicated the reticular formation (RF) lateral to the hypoglossal nucleus (mXII) as a substrate interposed between brainstem orosensory and oromotor nuclei. This substrate may be important for producing ingestion (licking and swallowing) or rejection (gaping) responses to gustatory stimulation. In the present study, rats were implanted with a chronic microdrive that advanced a bundle of fine wire NiCr electrodes (17u). In addition, a subset of the oro-pharyngeal musculature was implanted with fine wire electrodes to record EMG activity and intraoral cannulae were implanted to deliver gustatory stimuli. A total of 80 single cells (and 8 multi-unit responses) were recorded from 18 preparations. Thirteen preparations had recording tracks through the nucleus of the solitary tract (NST) and the RF lateral to mXII from a level 0.2 mm rostral to obex to the rostral pole of mXII. Electrode tracks ranged from the lateral border of mXII to 0.5 mm lateral to the nucleus. Five preparations had electrode tracks extending from the rostral pole of mXII to a level 2.5 mm rostral to obex and from 0.4 - 1.0 mm lateral to the midline. Oro-rhythmic (licking) responses to intraoral fluid stimulation were recorded from all preparations (43/80 cells) except two, the most rostral and the most lateral cases. Responses specific to swallowing were recorded dorsal to oro-rhythmic sites in 5 preparations and were located in the NST at levels ranging from 0.4 - 1.4 mm rostral to obex. Thus it would appear that the caudal NST and the RF ventral to it (and lateral to mXII) mediate different aspects of the ingestive sequence. Supported by DC00417

446.22

LiCl-INDUCED TASTE AVERSION IN BULLHEAD CATFISH. T. Valentinčič[1], V. Pirc[1] and W. Silver[2]*. [1]Department of Biology, University of Ljubljana, 61000 Ljubljana, Slovenia, and [2]Department of Biology, Wake Forest University, Winston-Salem, NC. 27109.

To determine whether bullhead catfish (Ictalurus nebulosus) could learn to discriminate among different foods as a result of LiCl-induced taste aversion, anosmic catfish were fed cod muscle previously soaked in LiCl (50mg LiCl/100g body weight) ad libidum during a single session. These catfish became unresponsive to stimuli and remained sluggish on the bottom of the aquarium during the 1-5 hours subsequent to the LiCl poisoning. All tested catfish regurgitated the cod muscle within 1-2 hours of the LiCl ingestion. One day following the LiCl ingestion, the same catfish responded to cod muscle with unimpaired feeding behavior patterns: search swims, turning to the food, and biting and snapping. Although initially taken into the oral cavity, the cod muscle was rejected by the catfish within 1-3 masticatory movements; however, during this same time period, these catfish fed normally on other foods. In addition, no differences were observed between aversive conditioned and unexperienced bullhead catfish in the incidence of other behavioral patterns released by amino acids and meat juices. For both groups, L-alanine was more effective than either L-proline, L-arginine or L-lysine in releasing turning, biting and snapping behavior towards stimulus plumes of >10-4M amino acids. The results indicate that the LiCl-induced taste aversion to cod muscle was not based solely on the detection of water-soluble substances, such as amino acids, emanating from the cod muscle before it was taken into the mouth, but that the perception of the aversive nature of the meat included recognition by the catfish of the complex taste and tactile qualities of the food.

Supported by the Ministry of Science of Slovenia, grant P1-0114-487.

447.1

D_1 DOPAMINE RECEPTOR ACTIVATION INDUCES SOCIAL REACTIVITY IN MICE SELECTIVELY BRED FOR AGGRESSION. M.H. Lewis[1,2*], J.-L. Gariepy[3], P. Gendreau[3], M.A. Mayleben[1,3], D.E. Nichols[5], R.B. Mailman[1,2,4]. Brain and Development Research Center.[1], Depts. of Psychiatry[2] Psychology[3] and Pharmacology[4], University of North Carolina, Chapel Hill, NC, 27599 and School of Pharmacy[5], Purdue University, West Lafayette, IN, 47907.

Robust individual differences in social behavior have been obtained by selectively breeding ICR mice for high and low levels of aggression. As previously shown, when paired with a non-selected, group-housed partner mouse, NC900 mice exhibit isolation-induced aggression, whereas NC100 mice fail to attack, but rather freeze upon social contact. Previous studies have established that NC100 mice have lower dopamine concentrations in nucleus accumbens and caudate nucleus, with increased dopamine receptor densities in these same regions. Thus, we wished to determine the effect of administration of a dopamine receptor agonist on social behavior. Mice of both lines were administered 0, 1, 3, or 10 mg/kg (s.c.) of the selective, full efficacy D_1 receptor agonist dihydrexidine and their behavior assessed in a social interaction test. Dihydrexidine dose-dependently reduced aggression in NC900 mice. Instead of aggression, these animals displayed a marked reactivity to mild social stimulation, as measured by increases in escape behavior, reflexive kicks, and vocalizations. Dihydrexidine had no systematic effect on the freezing behavior characteristic of the low-aggressive line, but did induce social reactivity in these animals, albeit to a lesser degree. In independent experiments, mice were pretreated with either the D_1 antagonist SCH23390 (0.1 mg/kg) or the selective D_2 antagonist remoxipride (1.0 mg/kg), after which they received dihydrexidine (10 mg/kg) and were tested as above. The effects of dihydrexiidine on social reactivity (both NC900 and NC100) and attack (NC900) were significantly antagonized by SCH23390 but not attenuated by remoxipride. These studies suggest an important role for D_1 dopamine receptors in the emotional response to social stimuli (Supported, in part, by PHS Grants MH45371, MH40537, and MH42705).

447.2

MODULATORY EFFECTS OF ACUTE D-AMPHETAMINE AND CLOZAPINE ON EXPLORATORY BEHAVIORS IN RATS. T. Steele*, B. Haas, and B. Wilcox, Dept. of Psychology, Univ. of Wisconsin Oshkosh, Oshkosh, WI, 54901.

Rats administered dopamine (DA) agonists are frequently hyperactive and stereotypic. These behaviors have been found to oppose exploration and subsequent learning (1). To determine if exploratory impairments are due, in part, to stimulation of DA-1 receptors, clozapine (CLOZ), a DA-1 receptor blocker, was administered to rats given d-amphetamine (AMPH). It was predicted that CLOZ would reverse a subset of the exploratory deficits produced by AMPH.

Six groups of hooded rats were administered a combination of AMPH (0 or 3 mg/kg) and CLOZ (0, 2, or 4 mg/kg). Rats were then allowed 15 min to explore a familiar field containing novel and familiar stimuli. Locomotor activity, object interactions, and other indices of exploratory behavior were observed four times over 2 weeks.

Certain aspects of exploration were independent of the drug effects. All animals explored the novel objects more than the familiar objects, and generally habituated to the objects as each session progressed. However, AMPH enhanced locomotor activity and subsequent object contacts. Both effects showed evidence of sensitization. CLOZ failed to reverse AMPH effects, but altered other components of exploration without reducing locomotor activity.

Although DA appears to influence exploratory behaviors, the observation that the drug effects were generally independent of one another suggests that different features of exploration may be selectively modulated by different DA receptor subtypes.

(1) Steele, T.L., & Williams, M. (1991). Neurosci. Abs., 17, 879.

447.3

INTRACRANIAL SELF-STIMULATION ON FR15 PRODUCES A BEHAVIORALLY SENSITIZED RESPONSE TO LOW DOSES OF COCAINE HYDROCHLORIDE (COC) IN THE RAT. P.R. Hartley* and D.B. Neill. Department of Psychology, Emory University, Atlanta, GA 30322.

In a previous study *(Neurosci Abst, 1990)* we report that rats performing ICSS at LH electrodes on an FR15 schedule showed a complete cessation of responding when administered low-dose AMPH unlike animals trained on an FR1 schedule. The FR15 rats developed increasing levels of stereotypic behavior over the course of the 20 minute session and a dose-response analysis revealed a left shift in FR15 as compared to FR1 rats. To determine if the above behavioral augmentation occurred outside the operant environment, rats were assessed in their home-cage environments. No differences in stereotypy ratings between groups were observed.

In a similar study, the effects of low-dose COC were examined. A similar response suppression was observed in rats performing on the FR15 schedule though the stereotypy that was observed during the session was qualitatively different to the AMPH-induced stereotypy. Dose-response analysis of COC revealed a left shift in FR15 rats but, unlike AMPH, there was some response suppression at all doses. A home cage assessment between groups revealed no differences in the stereotypic response to COC and measures of locomotor behavior obtained immediately following ICSS were also no different.

We propose that the FR15 schedule induces an elevated DA release *only* within the context of the operant environment and that the addition of the AMPH or COC results in an elevated baseline resulting in the development of stereotypic behaviors that compete with the performance of the lever-press response.

447.4

EFFECTS OF REPEATED SKF 38393 AND APOMORPHINE TREATMENTS ON LOCOMOTOR ACTIVITY AND DOPAMINE SYNTHESIS J.K. Rowlett*, B.A. Mattingly and M.T. Bardo, Departments of Psychology, University of Kentucky, Lexington, KY 40506 and Morehead State University, Morehead, KY 40351.

Chronic treatment with the dopamine receptor agonist apomorphine results in a progressive increase in locomotor activity (ie., behavioral sensitization). This behavioral sensitization effect is accompanied by augmented dopamine synthesis. The present study was conducted to assess the role of D_1 dopamine receptor stimulation in the augmented dopamine synthesis effect produced by chronic apomorphine treatment. Rats were injected with vehicle, apomorphine (5 mg/kg) or one of two doses of the D_1 agonist SKF 38393 (8, 16 mg/kg) daily for 10 consecutive days. Behavioral sensitization of locomotor activity was observed in rats treated with daily apomorphine, but not SKF 38393. Dopamine synthesis, as measured by accumulation of dihydroxyphenylalanine (DOPA) following inhibition of DOPA decarboxylase with NSD-1015, was enhanced in the caudate-putamen of rats treated with repeated apomorphine, but not SKF 38393. Since apomorphine challenge in rats treated with repeated SKF 38393 results in behavioral sensitization, these data suggest that the augmented dopamine synthesis effect observed after repeated apomorphine treatment is neither mediated by D_1 dopamine receptors nor is likely to be the primary mechanism of behavioral sensitization. (Supported by a grant from the Kentucky EPSCoR committee).

447.5

EFFECTS OF DOPAMINE BLOCKADE ON THE HYPERACTIVITY PRODUCED BY DRUG INJECTIONS INTO THE MEDIAN RAPHE NUCLEUS. I.Shim*, J. Krebs and D. Wirtshafter, Dept. Psychol., Univ. Ill at Chicago, Box 4348, Chicago, IL 60680.

We have shown previously that injections of GABA and opioid agonists into the median raphe nucleus (MR) result in large increases in both locomotor activity and dopamine turnover within the nucleus accumbens. In the present study, we examined the possibility that the MR's influence on locomotion might be mediated by alterations in dopamine release. Hyperactivity was induced by intra-MR injections of either the GABA-A agonist muscimol (25 ng), the GABA-B agonist baclofen (62.5 ng), the mu opiate agonist DAGO (437.5 ng), or the delta opiate agonist DPDPE (7.5 ug). For comparative purposes, activity was also studied after systemic treatment with d-amphetamine (1.5 mg/kg). All of these treatments resulted in approximately equal degrees of hyperactivity. Pretreatment with the dopamine antagonist haloperidol, at a dose of 0.4 mg/kg, was able to abolish the hyperactivity induced by systemic amphetamine or by intra-MR injections of DAGO or DPDPE. In contrast, haloperidol was without effect on the response to intra-MR injections of muscimol or baclofen. These results suggest that dopamine may be involved in the response to intra-MR injections of opiates, but is unlikely to play a critical role in the rosponse to GABA agonists. These data also suggest that different populations cells may mediate the locomotor responses to intra-MR injections of GABA and opiate agonists.

447.6

FORCE LEVER PERFORMANCE OF RATS WITH METH-AMPHETAMINE-INDUCED DOPAMINE LESIONS AND PRE-TREATED WITH VITAMIN E. C.S. Myers and G.C. Wagner*. Psychology Dept., Rutgers Univ. New Brunswick, NJ 08903

Rats treated with methamphetamine (12.5 mg/kg for 4 SC injections at 2-h intervals) exhibited a 32% depletion of striatal dopamine. Pretreatment with vitamin E (2 g/kg for 4 SC injections at 24 h intervals) protected the rats against this lesion.

In a behavioral assessment of fine motor control, rats were trained (over the course of one year) to press a lever with 11-15 g of force for 1.0 sec to procure a water reinforcer. Lesioned rats earned fewer reinforcers/session than control or vitamin E rats and also made more force band entrances/reinforcer. However, lesioned rats and those pretreated with vitamin E were more sensitive to the disruptive effects of pre-session oxotremorine (dose range 0.025 - 0.2 mg/kg, administered 30 min presession). Therefore, vitamin E afforded protection against the dopamine lesion but not against the increase in sensitivity to oxotremorine.

447.7

DIABETIC RATS DISPLAY BLUNTED BEHAVIORAL RESPONSE TO d-AMPHETAMINE OR DARK PHASE; IMPLICATIONS OF ALTERED DOPAMINE (DA) FUNCTION. Q. AHMAD AND Z. MERALI* Sch. of Psychol. & Dept. of Pharm. Univ. of Ottawa, Ont. Canada. K1N 9A9.

The behavioral response of Spontaneously diabetic Wistar BB rats (SDR) (maintained on insulin) and matched controls (CTL), to systemically administrated d-amphetamine (d-AMPH) during early (0-2 months), intermediate (4-8 months) and long-term (8-12 months) stages of diabetes, was assessed. Each stage comprised of a different set of animals. Behaviors monitored for 1 hr periods included locomotion, general activity, rearing frequency and duration. During the early stage, d-AMPH (0, 0.3, 0.5, 1.0 mg/kg) induced a dose dependent increase in behavior of CTL rats. The SDR group only responded to the higher doses (0.5 and 1.0 mg/kg) of d-AMPH and with a smaller magnitude than the CTL group. At the intermediate and long-term stages, the SDR again displayed a shift to the right of the d-AMPH dose response curve. Similarly blunted behavioral activation was also noted in the SDR during the dark phase of the light cycle. Specifically, the SDR displayed lower levels of activity at light offset or before light onset. Thus the attenuated response of the SDR may be related to the impairment of synthesis and/or release of DA. This contention was tested in SDR (intermediate state), using microdialysis. Extracellular levels of DA at the nucleus accumbens were monitored every 20 min during the dark phase, in behaving animals. The SDR had a higher basal level of DA as compared to CTL. However, the SDR displayed blunted fluctuations in DA levels, as compared to CTL. Thus the diabetic state may be related to altered DA release pattern and/or altered post synaptic responses to DA.

447.8

DRUG INDUCED DOPAMINE RECEPTOR SENSITIZATION: A CRITICAL ROLE FOR CONTEXTUAL CUES. R.J. Carey.* SUNY and V.A. Medical Centers, Syracuse NY 13210.

The unilateral 6-OHDA lesion model is widely used as a behavioral assay for direct acting dopamine agonists. In the present report, behaviorally matched sets of rats with unilateral 6-OHDA lesions were given repeated injections of apomorphine (0.05 mg/kg) either paired or unpaired to test environment in which rotational behavior was measured using a video image analysis system. Subsequently the animals were given 10 mg/kg L-DOPA plus 1 mg/kg Carbidopa or Saline prior to testing. Importantly, only animals which had received apomorphine paired to the test environment exhibited contralateral rotation following L-DOPA treatment. Biochemical measurements using HPLC-EC indicated that L-DOPA produced equivalent increases in metabolites of L-DOPA and dopamine in the paired and unpaired groups. While dopamine levels were comparable in paired and unpaired groups the concentrations of L-DOPA were selectively elevated in the paired group. These findings indicate that contextual cues can have substantial impact upon the biochemical and behavioral expression of sensitization effects induced by dopamine agonists.

447.9

INTERACTION OF APOMORPHINE AND COPULATORY BEHAVIOR IN THE "PENILE-ERECTION/STRETCHING-YAWNING SYNDROME." B. D. Sachs*, K. Akasofu, and S. S. McEldowney. Univ. of Connecticut, Storrs, CT 06269-1020.

In two studies, freely moving male rats were observed after they had varying amounts of copulation followed by injection apomorphine (APO; 60 ug/kg sc; a dopamine agonist), or vehicle. As expected, APO increased erections and yawning in males that had no antecedent sexual contact. Surprisingly, three intromissions or one ejaculation potentiated erections even in vehicle-treated males, which did not differ from APO-injected copulating males. Sexually exhausted rats had no APO-induced erections, a significant depression relative to the other groups. Copulation did not affect yawning in either study. Stretching was rarely seen and may not be a reliable component of the syndrome. We infer that copulation has a biphasic effect on the erectile component of the "PE/SYS," mediated in part by changes in neurochemical activity that are specific to brain systems involved in erection, or at least do not include systems that regulate yawning. [Supported by HD-08933.]

447.10

RESERPINE-INDUCED ORAL DYSKINESIA IN RATS: EFFECT OF DOSE AND COMPARISON TO TETRABENAZINE. J. L. Neisewander*, E. Castañeda, and D. A. Davis. Department of Psychology, Arizona State University, Tempe, AZ 85287-1104.

Repeated administration of reserpine (1 mg/kg) in rats produces a severe depletion of monoamines, and induces the development of oral dyskinesia that reaches a maximal level within 3 days. To examine whether lower doses of reserpine would result in a slower development of oral dyskinesia, rats were injected SC with either 0, 0.01, 0.1, or 1.0 mg/kg reserpine every other day for 60 days. Oral dyskinesia was measured 24 hr after injections by recording the incidence of tongue protrusions (TPs) for 30 min. Rats treated with 1 mg/kg developed TPs within 3 days, whereas rats treated with the 0.1 mg/kg developed TPs after 10-20 days, and rats treated with 0.01 mg/kg had not developed TPs by 60 days of treatment. These findings suggest that less severe depletion of monoamines may result in a slower development of TPs, similar to the protracted development of tardive dyskinesia in humans. Furthermore, we suggest that a severe depletion of monoamines may induce neurochemical changes similar to those that underly tardive dyskinesia, but at an accelerated rate. We also examined whether tetrabenazine, another monoamine depleting agent, would induce oral dyskinesia. Rats were injected with either vehicle, reserpine (1 mg/kg, SC, every other day), or tetrabenazine (3 mg/kg, SC, either once or twice daily) for 21 days. TPs, grooming, and locomotor activity were measured for 30 min 12-24 hr after the last injection. Both reserpine and tetrabenazine treatment produced an increase in TPs. Tetrabenazine, however, also produced an increase in grooming and locomotor activity relative to the other groups, suggesting the increase in TPs produced by tetrabenazine may be due to an increase in overall activity rather than a dyskinesia. Reserpine produced a more severe depletion of monoamines (88-94%) relative to tetrabenazine (28-58%). Reserpine also produced a 450-900% increase in dopamine and serotonin turnover as measured by neurotransmitter to metabolite ratios, whereas tetrabenazine did not alter turnover. Relationships between changes in brain monoamine levels and behavior will be discussed.

447.11

SCHEDULE-INDUCED POLYDIPSIA INCREASED BOTH MESOLIMBIC-DOPAMINERGIC AND PONTINE-NORADRENERGIC ACTIVITIES IN THE RAT BRAIN. T. H. Yin, C.C. Lu, Y.P. Liu and C.S. Tung. Department of Physiology, National Defense Medical Center, PO Box 90048, Taiwan, R.O.C.

We have demonstrated previously that the activity of schedule-induced polydipsia (SIP) was persistently depressed after bilateral symmetrical locus coeruleus lesions and ventral tegmental area lesions in succession. The hypothesis that central catecholaminergic neurons mediate animal behaviors in arousal or coping processes, e.g., SIP, was tested by the demonstration of concomitant changes in neurotransmission in regions of appropriate nerve terminals. By using HPLC-ECD techniques, the biochemical derivatives including DA, NE, 5-HT, DOPAC, DOPA, MHPG-SO_4 and 5-HIAA were measured to assess the regional turnover of monoamines in SIP and control rats. It was found in the SIP rat that DA levels and DA synthesis and utilization in the limbic area were increased and that NE synthesis in several pontine coeruleo-NE projections, e.g., hippocampus, cortex and pons, were also increased. Conversely, the NE-metabolism in the hippocampus and cortex and 5-HT metabolism in the same areas were decreased in the SIP rat. We therefore concluded that enhanced actions of both DA-limbic system and NE-coeruleo system are important in the process of SIP establishment.

447.12

DOPAMINERGIC CONTROL OF MALE COPULATORY BEHAVIOR IN QUAIL: RECEPTOR AUTORADIOGRAPHY AND PHARMACOLOGICAL STUDIES. P. Absil*, S. Das, G.F. Ball, J.M. Casto and J. Balthazart Laboratory of General and Comparative Biochemistry, University of Liège, Belgium, Dept of Psychology, Johns Hopkins Univ., Baltimore, MD 21218, USA.

In quail, dopamine (DA) turnover in the medial preoptic nucleus (POM) is higher in males than in females. In that POM is a key site mediating the action of testosterone (T) on sexual behavior, this suggests that DA may be involved in the control of this behavior. Also, tyrosine hydroxylase-immunoreactive fibers innervate aromatase containing cells suggesting that DA may modulate the aromatization of T into estrogens, a limiting step in the behavioral activation. We now demonstrate the presence of D1 and D2 dopaminergic receptors in the quail brain as identified by autoradiography using [^{3}H]SCH23390 and [^{3}H]raclopride as ligands. Highest levels of binding were found in the basal ganglia. In addition, lower densities of receptor were identified in areas directly implicated in the control of reproduction such as the preoptic area and hypothalamus. The behavioral effects of DA were analyzed by in vivo pharmacological manipulations. In a first experiment, the DAergic agonist, apomorphine (APO; 1µg to 10 mg/kg) inhibited male sexual behavior induced by T in castrated (CX) males. Inhibition was statistically significant for doses higher than 10µg/kg and complete at 1 mg/kg. In a second experiment, repeated injections of APO (0, 10 or 1000 µg/kg for 9 days) did not change the sensitivity of the DA receptors in connection with behavior activation. The T-induced copulatory behavior measured in CX males 24 hours after the last APO injection was not affected but the dose of 1 mg inhibited the behavior in tests performed 15 min after the injection. Several reports have indicated that in rat, APO stimulates certain aspects of male behavior. The inhibitory effects observed here may reflect a higher density or higher sensitivity of the D2 dopamine autoreceptors in quail resulting in a decreased DAergic transmission after APO administration. This hypothesis was tested by measuring behavioral effects of APO in quail pretreated with spiperone, a specific D2 antagonist. Spiperone (0.5 mg/kg) alone enhanced the T-induced copulatory behavior and completely blocked the inhibitory effects of APO. These data support our interpretation but additional studies are required to elucidate the precise role of D1 and D2 receptors in the control of sexual behavior in male quail.

448.1

HIGH-PEAK BLOOD ETHANOL CONCENTRATIONS RESULTING FROM POSTNATAL EXPOSURE TO ETHANOL AFFECT MEMORY-BASED BUT NOT CUED DISCRIMINATIONS IN THE INFANT RAT.

J. L. Diaz-Granados*, P. L. Greene, G. Y. Espinoza, & A. Amsel. Department of Psychology & Institute for Neuroscience, University of Texas, Austin, TX, 78712.

We have previously shown that postnatal exposure to ethanol that produces high-peak blood ethanol concentrations in infant rats, results in significant deficits in hippocampal neuroanatomy and discrimination learning based on the memory of singly alternated rewarded (R) and nonrewarded (N) trials (Greene, et al., *Behav. Neurosci.*, 106:51, 1992). In the present experiment, we tested infant rats, artificially reared and exposed to ethanol from postnatal day 4 to 9, in a straight alley on a discrimination using rough and smooth floor textures as cues. On day 4 postpartum, pups were fitted with gastric cannulas and a nutritionally adequate diet was infused intragastrically for 15 minutes once every hour. In 4 consecutive morning feedings one group of pups received an ethanol-adulterated diet while postnatal controls received an isocaloric diet. (A third group was raised normally in a litter.) The remaining 20 feedings for all pups were with unadulterated diet. On days 17-18, animals were tested on one of three discrimination schedules: single alternating R and N, random R and N, or random R and N followed by a discrimination reversal. The ethanol-exposed animals were not different from the normals or the artificially-reared controls in either alternating, or the random or reversed discriminations. These results indicate that the defect in discrimination reported earlier in ethanol-exposed, hippocampally damaged animals is a memorial deficit and not a general deficit in discrimination, since pups discriminate normally when external cues are made available. Supported by NIAAA grant AA07052.

448.2

DIFFERENTIAL PERINATAL EFFECTS OF HIGH-PEAK BLOOD ETHANOL CONCENTRATION ON LEARNED PERSISTENCE IN THE WEANLING RAT.

Abram Amsel*, Paul Greene, & Jaime L. Diaz-Granados. Department of Psychology and Institute for Neuroscience, University of Texas, Austin, Texas, USA.

The partial reinforcement extinction effect (PREE), an indicant of relative learned persistence, was tested in a straight alley for a food reward in 21-day-old rats after prenatal, postnatal or combined exposure to high-peak blood ethanol concentration (BEC). Pregnant dams were intubated once per day from gestational day 12 to 18 with either an ethanol-adulterated (5g/kg) or an isocaloric control diet. On day 4 postpartum, pups were fitted with gastric cannulas and were reared artificially from days 4 to 10: A nutritionally-adequate diet was infused intragastrically for 15 minutes once every hour. Pups in the postnatal EtOH condition received 4 consecutive feedings every morning with ethanol-adulterated diet while postnatal controls received an isocaloric diet. The remaining 20 feedings for all pups were with unadulterated diet. Pups with either prenatal or postnatal exposure to ethanol showed an attenuation of the PREE. The PREE was spared in the combined-exposure condition. This seemingly paradoxical result confirms an earlier one involving a significantly lower BEC (Wigal & Amsel, *Behav. Neurosci.*, 104:116, 1990). Brain weights were significantly reduced as a function of postnatal exposure to ethanol. Supported by NIAAA grant AA07052.

448.3

SEX-DEPENDENT EFFECTS OF PRENATAL ALCOHOL EXPOSURE ON CIRCADIAN RHYTHMS OF TEMPERATURE AND ACTIVITY IN RATS.

B. Zimmerberg*, K.M. Broadhurst and R.C. True. Williams College, Williamstown, MA 01267

Fetal Alcohol Syndrome is associated with altered sleep and feeding patterns in newborns. This experiment investigated persistent effects of in utero alcohol exposure on circadian rhythms in adult offspring. Adult male and female rats born to either control, pair-fed or alcohol-exposed dams were monitored for body temperature and activity using a telemetry system under various LD schedule conditions. Baseline (12:12 LD) or 14:10 LD measures were unaffected by prenatal treatment, but females were more active, had higher mean body temperature, and had higher maximal temperatures that peaked earlier than males. Under a free-running schedule (LL), alcohol-exposed males were less active than controls; females did not differ by treatment. Mean and maximum temperatures were higher in alcohol-exposed males and lower in alcohol-exposed females than same sex controls, thus they no longer displayed the typical sex-dependent pattern. Although all subjects demonstrated phase advancement, alcohol-exposed subjects lagged behind controls. Morphometric analysis of the volume of the SCN was inconclusive. These results suggest that the stress of a free-running schedule revealed underlying thermoregulatory dysfunction caused by prenatal alcohol exposure. (Supported by NIAAA AA08605)

448.4

PARALLEL SLEEP AND MEMORY DEFICITS IN ADULT RATS AFTER PRENATAL EXPOSURE TO ALCOHOL.

W.S. Stone*, H.J. Altman, P. Parekh and P.E. Gold. Dept. Psychology, U. of Virginia, Charlottesville, VA 22903, and Dept. of Beh. Animal Res., Lafayette Clinic, Detroit, MI 48207 .

Learning and memory deficits after prenatal alcohol exposure occur both during development and in adulthood. Sleep deficits are also prominent in neonatal humans and rats. Our previous findings demonstrate that paradoxical sleep and memory are correlated in several amnesic populations, suggesting the possibility that deficits in paradoxical sleep will accompany deficits in memory in adult rats prenatally exposed to alcohol.

To address this issue, pregnant Sprague-Dawley rats were fed a liquid diet including 35% ethanol-derived calories, or were pair-fed an isocaloric diet, from gestation days 8-19. Sleep (3 hr records via cortical electrodes), 8-arm radial maze (1 trial/day for a maximum of 20 trials or a criterion of 3 consecutive days of 1 error or less, followed by 1 trial with a 1 h delay interposed between choices 4 and 5), and inhibitory avoidance (1 ma, 1 s FS, 24 h train-test interval) performance were assessed in 5-month-old female offspring of alcohol-exposed and pair-fed control groups (Ns = 9).

The alcohol-exposed group learned the radial maze more slowly than did the pair-fed control group, but did not differ on other behavioral measures. In addition, measures of paradoxical - but not nonparadoxical - sleep were significantly reduced in the alcohol-exposed group. These findings provide the first evidence, in any species, that prenatal exposure to alcohol results in selective and persistent deficits in sleep. They also show more generally that measures of paradoxical sleep may be useful biological markers of memory deficits. (Supported by NSF BNS-9012239, ONR N0001489-J-1216, and NIA AG 07648. We gratefully acknowledge the Fetal Alcohol Res. Ctr. at Wayne State U. for providing the alcohol-exposed rats).

448.5

ETHANOL CONSUMPTION SUPPRESSES IMMUNE RESPONSE IN FETAL ETHANOL-EXPOSED RATS.

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Ethanol consumption during pregnancy may alter immune function of offspring. Our previous work found that fetal ethanol-exposed (E) animals had decreased thymocyte number, decreased splenic lymphocyte response to concanavalin A (ConA), and a functional defect in T-cell response to a secondary stimulation by IL-2, as compared with pair-fed (PF) and control (C) animals. Importantly, deficits occurred in E males but not females. In the present study we examined the vulnerability of E offspring to the immunosuppressive effects of ethanol exposure in adulthood.

Adult Sprague-Dawley males and females from prenatal E, PF and C conditions were assigned to E or PF adult treatment groups. Six groups were thus formed, with Prenatal--Adult treatments as follows: E--E, E--PF; PF--E, PF--PF; C--E, C--PF. Adult E or PF treatment was continued for 4 wk.

Sex differences in treatment effects were found. For females, C--PF animals had higher thymus cell counts than animals in all other groups. There were no differences among females from the 3 prenatal groups in splenic lymphocyte response to ConA, although adult ethanol consumption had some suppressive effects on response in E and C animals. For males, thymus cell counts were lower in E--E than in E--PF animals; no other treatment groups showed this differential response. In addition, both E--E and E--PF males exhibited a suppressed lymphocyte response to ConA; males from prenatal PF and C conditions showed no suppression unless exposed to ethanol in adulthood. Supported by AA07789 (JW) and AA07731 (TRJ).

448.6

ACUTE ETHANOL EXPOSURE DEPRESSES STIMULATED GLUTAMATE RELEASE IN THE HIPPOCAMPUS OF THE DEVELOPING GUINEA PIG.

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It has been proposed that glutamate (GLU) mediates ethanol (E) teratogenesis in the hippocampus. Our objective was to elucidate the effect of acute E exposure on spontaneous and potassium ion (K^+)-stimulated release of GLU in the hippocampus of the near-term fetal and adult guinea pig. GLU release was measured as efflux in 300-μm thick, transverse, hippocampal slices. Acute *in vivo* E exposure involved oral intubation of 4 g E/kg body weight in near-term pregnant and adult male and female guinea pigs, which were studied 1 hr after dosing. Acute *in vitro* E exposure involved incubation of tissue slices with 48 mM E, the apparent maximal blood [E] at 1 hr after 4 g E/kg body weight. E did not affect spontaneous GLU efflux. *In vitro* E decreased K^+-stimulated GLU efflux in the fetus and adult. *In vivo* E and *in vivo* plus *in vitro* E decreased K^+-stimulated efflux of GLU in the fetus, but not in the adult. These data indicate that: (a) in the near-term fetal guinea pig, acute E exposure depresses stimulated GLU release in the hippocampus, and (b) in the adult guinea pig, tolerance develops to the depressant effect of E on stimulated hippocampal GLU release after a single E dose. (Supported by the MRC of Canada)

448.7

THIAMINE-DEPENDENT ENZYME CHANGES IN THE BRAINS OF ALCOHOLICS: RELATIONSHIP TO THE WERNICKE-KORSAKOFF SYNDROME.
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Previous studies in experimental animal models suggest that the cerebral dysfunction and cell death in the Wernicke-Korsakoff Syndrome (WKS) result from reductions of thiamine-dependent enzyme activities in brain (Butterworth, Alcohol & Alcoholism, 24, 271-279, 1989). As part of a series of studies of the role of thiamine deficiency in the pathogenesis of the WKS in humans, activities of the thiamine-dependent enzymes pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase (αKGDH) and transketolase (TK) were measured by standard spectrophotometric procedures in samples of frontal cortex and cerebellum of 10 alcoholic patients who died in hepatic coma with no clinical or pathological evidence of WKS, 2 alcoholic patients with neuropathologically confirmed WKS and 10 control subjects free from neurological disease. Reductions of TK (by 75%), PDHC (by 82%) and αKGDH (by 90%) were observed in the brains of WKS patients. Activities of the non-thiamine-dependent enzyme glutamate dehydrogenase were within normal limits in brain tissue in all alcoholic patients ± WKS. Activities of all three thiamine dependent enzymes were unchanged in the brains of alcoholic patients dying in hepatic coma. These findings demonstrate, for the first time, a direct link between reductions in activities of thiamine-dependent enzymes and WKS in humans (Funded by The Medical Research Council of Canada).

448.8

NEURONAL AND ASTROCYTIC RECEPTOR CHANGES IN AUTOPSIED BRAIN TISSUE FROM ALCOHOLIC PATIENTS WITH HEPATIC ENCEPHALOPATHY.
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As part of a study of neurochemical mechanisms in alcohol-related brain damage, neuronal and astrocytic binding sites for specific radioligands were measured in autopsied brain tissue from 9 cirrhotic alcoholic patients and an equal number of control subjects free from neurological disease. Neuropathological examination revealed no evidence of Wernicke's Encephalopathy. Binding densities (Bmax) and affinities (Kd) were obtained by Scatchard analysis using standard filtration assays and GFB filters. No changes of Kd or Bmax for the GABA-A/Benzodiazepine receptor complex were observed using ^{3}H-muscimol, ^{3}H-flunitrazepam or ^{3}H-Ro 15-1788. Binding parameters for the glutamate receptor ligands ^{3}H-kainate or ^{3}H-MK801 were likewise unaltered in the brains of alcoholics. Densities of muscarinic cholinergic receptors, evaluated using ^{3}H-quinuclidinyl benzilate (^{3}H-QNB) were reduced in frontal cortex (by 23%) and temporal cortex (by 27%) and densities of astrocytic mitochondrial ("peripheral-type") benzodiazepine receptors (PTBR's) were increased by 48% in frontal cortex of alcoholics. Similar changes in PTBR's were observed in rat brain following portacaval anastomosis (Giguère et al., Brain Res. 1992) suggesting that these changes result from chronic liver disease rather than alcohol per se. These findings suggest that (i) neuronal cell loss in alcoholics dying in hepatic coma is restricted to a modest loss of cortical cholinergic neurons and (ii) chronic liver disease results in increased densities of mitochondrial benzodiazepine receptors. (Funded by The Medical Research Council of Canada)

448.9

CHRONIC INTRAGASTRIC INFUSION OF ETHANOL RESULTS IN THE PRODUCTION OF TOLERANCE TO ETHANOL IN MICE.
W. Cao*, T.K. Landon, A.C. Collins. Institute for Behavioral Genetics, Univ. of Colorado, Boulder, CO 80309

The present study characterized chronic intragastric infusion as a method for developing tolerance to ethanol in mice. The selectivity bred long sleep (LS) and short sleep (SS) mice were used. Male mice were anesthetized and were surgically prepared with a catheter in the stomach. The mice were infused every 6 hours with physiological saline or ethanol (3.0 or 4.0 g/kg). Potential tolerance to ethanol was determined by measuring the effects of challenge doses of ethanol on body temperature, open field and Y-maze activities. In addition, the mice were given an anesthetic dose of ethanol (6.0 g/kg for SS, 3.5g/kg for LS) and the duration of sleep time was scored. The mice were tested 6 hours after termination of infusion. Chronic infusion resulted in tolerance to virtually all of the effects of ethanol in both mouse lines. However, the LS developed tolerance more readily (after shorter periods of infusion) and to a greater degree. Chronic infusion did not result in changes in the rate of ethanol metabolism in either mouse line which suggests that this procedure results in pharmacodynamic tolerance to ethanol's effects. Thus, the intragastric infusion method, which allows for very precise regulation of dose and dose interval, is a useful technique for producing tolerance to ethanol in mice. Supported by AA-06391 and DA-00116.

448.10

FURTHER CHARACTERIZATION OF AN ANIMAL MODEL OF ETHANOL WITHDRAWAL "KINDLING".
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We have previously demonstrated that animals exposed to ethanol (EtOH) for a total of 48 hrs exhibit more severe withdrawal seizures if exposure is divided into three 16 hr intoxication/8 hr abstinence cycles than when the 48 hrs of exposure occurs in a single bout. This pattern of results supports the "kindling" hypothesis of EtOH withdrawal in that repeated episodes of EtOH withdrawal result in a progressive intensification of withdrawal seizures. The present study was designed to further characterize this animal model of EtOH withdrawal "kindling" and examine whether such a "kindled" response is still evident when withdrawal testing is conducted following an additional intoxication bout. Adult male C3H mice were chronically exposed to EtOH vapor in inhalation chambers for 40 hrs prior to withdrawal testing. Prior to this 40 hr bout of intoxication, one group (Multiple Withdrawal; MW) received 3 cycles of 16 hrs EtOH vapor separated by 8 hr periods of abstinence; a second group (Continuous Exposure; CE) received the same total EtOH exposure (48 hrs) without interruption; and a third group (Single Withdrawal, SW) did not receive any EtOH exposure prior to the 40 hr test cycle. A control (C) group was included that did not receive any EtOH exposure throughout the experiment. Blood EtOH concentrations (BEC) following the 40 hr period of EtOH exposure were 100-140 mg/dl for all EtOH-exposed groups. Mean areas under the 24 hr curve for handling-induced convulsions after the 40 hr EtOH exposure period for MW, CE, SW, and C groups were 33±4, 18±4, 3±1, and 0±0, respectively. These results indicate that differences in the severity of EtOH withdrawal seizures due to differences in prior withdrawal experience can be demonstrated even when later EtOH exposure patterns are equated. Supported by the VA Medical Research Service and NIAAA.

448.11

EFFECT OF ETHANOL ON ADENOSINE RECEPTORS CONTRIBUTES TO THE DEVELOPMENT OF THE WITHDRAWAL SYNDROME.
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It has been hypothesized that stimulation of adenosine receptors is involved in ethanol (ETOH) intoxication and the development of tolerance. We investigated an ethanol withdrawal symptom ("anxiety-like" behaviors) using the elevated plus maze (EPM) paradigm (Lal et al., Alcohol 8:467, 1991). Long-Evans hooded rats were given a balanced liquid diet containing 4.5% ETOH for 10 d (Lal et al., JPET 247:508, 1988). Twelve h after a final ETOH dose (3g/Kg, po), rats were tested in the EPM. We observed a significant reduction in the open-arm activity and the number of total arm entries indicative of ETOH withdrawal. Pre-treatment with an A1 adenosine agonist, R(-)-N6-(2-phenylisopropyl)-adenosine (PIA, 0.08-0.32 mg/Kg, ip, 15 min), had little effect on performance in the EPM during ethanol withdrawal. Acute treatment with an A1 adenosine antagonist, 8-cyclopentyl-1,3-dimethylxanthine (CPT, 0.02-0.16 mg/Kg, ip, 60 min), exacerbated ETOH withdrawal with a further reduction in open-arm activity. Chronic treatment with CPT (0.04-0.16 mg/Kg, ip, 2X/d) during the last 6 days of ETOH diet administration resulted in a dose related increase in the amount of time spent in the open-arms of the EPM, but had little effect on the number of total arm entries. These data support the hypothesis that an ethanol stimulated increase in adenosine receptor activity may be associated with the development of dependence and that blockade of adenosine receptors during ethanol treatment reduces the display of "anxiety-like" behaviors during ethanol withdrawal. Supported by NIAAA Grant AA06890.

448.12

EFFECT OF POST-TRAINING ETHANOL AND HYPERBARIC EXPOSURE ON APPETITIVE TASK MEMORY IN MICE.
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Studies examining post-training ethanol's effects on memory have found conflicting task-dependent results. Recent work suggests that this apparent contradiction may be due to an inherent aversive component of ethanol. According to this hypothesis, treatment with ethanol results in the addition of aversive information to the pretreatment stimulus complex, altering retention performance. Low level hyperbaric exposure using a helium-oxygen (heliox) gas mixture has been shown to antagonize a number of ethanol's acute and chronic behavioral effects. The present study investigated whether hyperbaric exposure antagonized ethanol's aversive effects on appetitive task memory. Male C57BL/6J mice were individually trained to find a cheese pellet. Mice were immediately injected i.p. following training with saline or 2.0 g/kg ethanol (20% w/v) They were then returned to their home cage and exposed to 1 ATA air, 1 ATA heliox, or 12 ATA heliox for 2 hours. Retention performance was measured 24 hours after training in the non-drug state. Saline-air control mice ate the pellet significantly quicker after training, while all other mice exhibited a non-significant increase in latency, suggesting amnesia. However, habituation of locomotor activity and pellet approaches in all groups indicates that memory of the environment remains intact, while grooming data suggest that the amnesic response in the helium and ethanol groups reflects a subtle aversive effect of these treatments. Collectively, these results suggest that post-training exposure to heliox, like ethanol, is aversive and does not impair memory storage processes. Further studies are necessary to determine the mechanism of helium's aversive effect and whether pressure can antagonize the aversive effect of ethanol in this task. (Supported by NIAAA grants AA03972 & AA05234).

448.13

ANTAGONISM OF ETHANOL-INDUCED CONDITIONED PLACE PREFERENCE BY THEOPHYLLINE. C. L. Cunningham*, D. H. Malott, F. O. Risinger. Oregon Health Sciences University, Portland, OR 97201-3098

Acute exposure to ethanol (EtOH) elevates adenosine levels in brain, and it appears that adenosine may be involved in the central depressant actions of EtOH. The present study examined the role of adenosine in mediating EtOH's rewarding effects by assessing the impact of a nonselective adenosine antagonist, theophylline, on ethanol-induced conditioned place preference. Inbred mice (DBA/2J) were exposed to a Pavlovian discriminative conditioning procedure in which a distinctive floor (tactile) stimulus (CS+) was paired four times with either EtOH (2 g/kg, IP), theophylline (30 mg/kg), or the combination of theophylline and EtOH. A different floor stimulus (CS-) was paired only with saline. Conditioning trial duration was 30 min and the theophylline pre-treatment interval was 30 min. Preference testing was conducted in the absence of either drug. Both EtOH and theophylline increased general activity on conditioning trials and their combination produced greater activity than either drug alone. As expected, EtOH alone resulted in conditioned place preference. However, theophylline alone produced conditioned place aversion. The group receiving the combination of theophylline and EtOH showed no evidence of place conditioning, suggesting these drugs cancelled each other's hedonic effects. This outcome does not appear to represent pharmacological antagonism, but seems more readily interpreted as an instance of behavioral or "functional antagonism."

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DRUGS OF ABUSE: STIMULANTS I

449.1

FUNCTIONAL EFFECTS OF ACUTE ETHANOL IN ALCOHOL PREFERRING AND NON-PREFERRING RATS. LJ Porrino*[1], LJ Vogt[1], L Lumeng[2], T-K Li[2] and MJ Lewis[3]. [1]Bowman Gray School of Medicine, Winston Salem, NC, [2]Indiana University, Indianapolis, IN, [3]Howard University, Washington DC.

To study genetic influences on responses to ethanol, selective breeding has been used to develop the alcohol preferring (P) and alcohol non-preferring (NP) rat lines. We have shown that P rats have higher baseline rates of cerebral metabolism than NP rats in olfactory tubercle, while NP rats have significantly higher rates in hippocampus (Lewis, M.J., Neurosci. Abstr, 1991). In the present study, we have examined the functional effects of an acute dose of ethanol in P and NP rats using the quantitative autoradiographic 2-[14C]deoxyglucose method. Rates of glucose utilization were measured 10 minutes after the ip administration of 0.5 g/kg ethanol or vehicle. In NP rats ethanol administration significantly increased rates of glucose utilization in the olfactory tubercle, nucleus accumbens, ventral pallidum, and basolateral amygdala, and decreased rates in the lateral habenula. This pattern is quite similar to the distribution of changes observed in outbred Sprague-Dawley rats administered ethanol. In contrast, the same dose of ethanol had more modest effects in P rats. Only the posterior olfactory tubercle, ventral pallidum, and lateral habenula appeared affected by ethanol and these changes in rates of glucose utilization were much less pronounced than in NP rats. These data suggest that NP rats are more sensitive than P rats to the acute effects of a low dose of ethanol on brain functional activity. The metabolic response to ethanol, however, appears qualitatively similar in the 2 lines, although quantitatively different, in that the same neural circuits are activated in both lines, but to a different degree.

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449.2

EFFECT OF ETHANOL AND GLUTATHIONE ON REACTION TIME IN RATS. Z.M. Post*, P.K. Randall, S.W. Leslie and C.K. Erickson. Div. of Pharmacol., Coll. of Pharm., Univ. of TX, Austin, TX 78712.

Glutathione (GSH) and NMDA similarly stimulate calcium uptake into dissociated brain cells. However, ethanol (EtOH) inhibits only GSH-stimulated uptake, perhaps because GSH blocks its purported site of action, the glycine site on the NMDA receptor complex. The interaction between GSH and EtOH has proven to be behaviorally significant in that rats given a hypnotic dose of EtOH regained the righting reflex sooner when pretreated with intracerebroventricular (ICV) GSH. The purpose of this study was to determine whether GSH pretreatment would also lessen EtOH-induced impairment of a reaction time (RT) task in rats.

On week 1 of a simple crossover design, behaviorally tolerant rats (n=12) were administered EtOH (i.g., 2.8 g/kg, 20% w/v in water) 15 min after ICV GSH (10 µl, 20 mM) or vehicle (10 µl artificial cerebrospinal fluid); on week 2, EtOH followed the alternate ICV agent. Although EtOH by itself impaired normal success and speed of response (85 v. 98% and 312 ± 24 v. 204 ± 11 msec at 30 min, respectively), performance after GSH was no different from vehicle. In a second experiment, EtOH-naive rats (n=10) were given 2.4 g/kg EtOH after ICV GSH or vehicle. Again, EtOH alone impaired response (63% success and 453 ± 29 msec) but GSH-treated rats were not protected. This lack of effect is probably due, at least in part, to the fact that the animals are highly practiced by the time drugs are administered. Also, there is great incentive to perform successfully, and thus, compensatory behavior is developed rapidly. It would be appropriate to test a behavior of intermediate demand which measures motor control, but to which less behavioral tolerance to EtOH develops. (Supported by funds from the Univ. of TX.)

449.3

EFFECTS OF NICOTINE ON CEREBRAL METABOLISM AND SUBJECTIVE RESPONSES IN HUMAN VOLUNTEERS. J.M. Stapleton*, J.E. Henningfield, D.F. Wong, R.L. Phillips, S.F. Gilson, R.F. Grayson, R.F. Dannals, and E.D. London, NIDA Addiction Research Center, and the Johns Hopkins Medical Institutions, Baltimore, MD.

Effects of nicotine (1.5 mg, i.v.) on cerebral metabolic rates for glucose (CMR_{glc}) are being studied in human volunteers, including cigarette smokers and nonsmokers. CMR_{glc} is measured by positron emission tomography using the [18-F] fluorodeoxyglucose method. Subjective and cardiovascular responses also are recorded.

Preliminary results indicated that smokers showed globally higher CMR_{glc} than nonsmokers in the placebo condition, and that both groups showed a widespread decrease of about 10% in response to nicotine. Smokers gave higher subjective responses than nonsmokers to questions about drug liking (e.g., "Did the drug have good effects?" or "How much did you like the drug?"). There were no differences between smokers and nonsmokers on ratings of drug strength (e.g., "How strong was the drug effect?" or "How much did you feel the drug?"). Smokers showed less nicotine-induced tachycardia than nonsmokers, presumably due to tolerance. The findings suggest that nicotine resembles other drugs of abuse in that it reduces CMR_{glc}. Relationships between CMR_{glc} and effects on mood are under investigation.

449.4

BEHAVIORAL EFFECTS OF NICOTINE CONSUMED BY RATS C. Ksir* and G. Mellor. Department of Psychology, University of Wyoming, Laramie WY 82071

Rats that had not consumed water for 24 hr were placed into 41 x 41 x 30 cm clear acrylic chambers with a 10-ml drinking tube in one corner. Each rat remained in the chamber for one hour, and activity was recorded using an Omnitech monitor. Water was given in the home cages for 2 hr after each session. By the 7th day all rats drank all 10 ml of water in the test chamber within 5 min. On the 8th -14th days the drinking tube contained 10% sucrose with 10 µg/ml nicotine. At first there was no apparent effect on locomotor activity, but over the 7 days of nicotine testing the total distance moved during the hour increased more than threefold. Subsequent test days alternated between presentation of 10% sucrose alone or sucrose plus varied nicotine concentrations. A significant dose-effect relationship was found with locomotor activity increasing in response to 5, 10 and 20 µg/ml nicotine solutions. Pretreatment with 1.0 mg/kg mecamylamine 20 min prior to tests with 10 µg/ml nicotine had no effect on volume consumed or time to drink, but did reduce the locomotor reponse.

Rats will drink 75 ml/day of 10 ug/ml nicotine in 10% sucrose in 24-hr, 2-bottle drinking tests with plain water in the 2nd bottle. Those rats quickly stop drinking significant amounts of nicotine either when the sucrose is removed from the mixture or when a third bottle is presented containing 10% sucrose without nicotine. Thus, we have been unable to demonstrate that rats develop a dependence on nicotine after consuming this sucrose-nicotine mixture. These findings call into question theories of drug dependence that tie reinforcing properties of drugs to their locomotor stimulant effects.

449.5

THE EFFECT OF MDL 28,133, A MIXED D2/5-HT2 ANTAGONIST, ON NICOTINE-INDUCED FACILITATION OF BRAIN STIMULATION REWARD. P.Z. Manderscheid*, R.A. Frank, J.H. Kehne, C.J. Schmidt & S.M. Sorensen. Dept. Of Psychology, Univ. of Cincinnati, Cincinnati, OH 45221 and Marion Merrell Dow Research Inst., Cincinnati, OH 45215.

There is evidence to suggest that increases in dopamine in the nucleus accumbens mediates the rewarding effects of psychomotor stimulants and that serotonin and dopamine antagonists can reverse stimulant-induced changes in mesolimbic dopamine activity. Previous work from our laboratory has shown that MDL 28,133 (MDL) can attenuate the reinforcing effect of both d-amphetamine and cocaine. The present study assessed the ability of MDL 28,133 to attenuate or block nicotine-induced facilitation of brain stimulation reward.

Rats implanted with bipolar ventral tegmental area electrodes were administered 1.0, 3.0, or 6.0 mg/kg MDL (IP) 1 hr prior to testing. MDL alone did not produce significant shifts in reward thresholds. However, when co-administered with nicotine (0.4 & 0.8 mg/kg IP, 45 min post-MDL), MDL produced a dose-dependent reversal of the threshold lowering effect of nicotine. Analysis of response rates indicated that MDL did not interfere with the animal's performance, as do other compounds with dopamine antagonist properties (i.e., haloperidol). This suggests that compounds with a serotonergic component may be effective pharmacotherapies in the treatment of psychomotor stimulant abuse. This research was supported by NIDA NRSA grant DA05482 to P.Z. Manderscheid and NIDA grant DA04483 to R.A. Frank.

449.6

PURKINJE CELL ELECTROPHYSIOLOGICAL SENSITIVITY TO NICOTINE IN TWO MICE STRAINS. R. DE LA GARZA*, D. ZENISEK, D. DURCHER, J. SAMS, A.C. COLLINS, AND M.J. MARKS. VAMC, Univ. of Colo., Denver, and Inst. for Behav. Gen. Univ. of Colo., Boulder, CO 80217.

A succesful strategy of pharmacogenetics is to compare the relative drug sensitivity of different strains of mice to the actions of psychoactive agents in order to investigate behavioral and physiological mechanisms of drug action. In the present studies, two mice strains (C57/BL and BUB) that differ profoundly in their behavioral sensitivity to nicotine (Marks et al., Pharmacol. Biochem. Behav. 33: 667-678, 1989) were used to study the electrophysiological sensitivity to nicotine in cerebellar cortex Purkinje cells. The Purkinje cell sensitivity of the C57/BL mice was considerably greater than the same cells of BUB mice. The ED50 values for nicotine actions were 39 psi x sec for the C57/BL mice and 91 psi x sec for the BUB mice ($p < .05$). These differences are consistent with (both in magnitude and direction) the relative sensitivites of these two mouse strains to several behavioral and physiological responses to nicotine. These two strains also differ in the development of tolerance following chronic i.v. infusion with nicotine (Marks et al., JPET 259: 392-402, 1991); the C57/BL mice developed maximal tolerance following infusion with a 4 mg/kg/hr nicotine dose whereas the BUB mice do not develop much tolerance at this dose. Consequently, mice of both strains were infused with 4 mg/kg/hr nicotine for 10 days and Purkinje cell sensitivity to nicotine was determined within 24 hours after withdrawal. Infusion with this dose of nicotine resulted in significant increases in cerebellar [3H]-nicotine binding, but α-[125I]-bungarotoxin (BTX) binding was unchanged by this treatment. Chronic nicotine infusion also did not evoke changes in Purkinje cell sensitivity in either mouse strain. The absence of an effect of chronic treatment on electrophysiological response to nicotine is consistent with the observations that infusion does not change cerebellar BTX binding.

449.7

CAFFEINE AND NICOTINE DO NOT MAINTAIN SELF-ADMINISTRATION IN RATS. J. Robinson*, S.L. Vrana, J. Broadbent, and S.I. Dworkin. Dept. of Physiology & Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

Although caffeine (CAF) and nicotine (NIC) are widely used, their reinforcing characteristics remain obscure. Attempts were made to assess the reinforcing efficacy of these compounds in rats. Six male Fischer 344 rats were implanted with indwelling jugular catheters and then trained on a fixed ratio (FR) schedule of food presentation. Animals were then allowed access to CAF (0.25 or 0.5 mg/infusion) or NIC (10 μg/inf) on a FR 1 schedule. In a different paradigm, one rat was given access to food, water, and CAF (0.25 mg/inf) 24 hr/day on a concurrent chained FR 1, FR 9 schedule. Responding maintained by CAF in the single-lever chambers resulted in a relatively high rate of responding following food-training, which substantially declined with time. CAF (0.25 mg/inf) infusions declined by approx. 75% in the second month as compared with the first. Increasing the dose (0.5 mg/inf) did not change response rates. Under the concurrent chained schedule only food and water maintained responding. These data indicate that CAF does not maintain reliable self-administration (SA) in rats, even when the animals were trained with food. It was found previously (Dworkin *et al.*, Soc. Neurosci. Abs. 17, 425, 1991) that NIC did not engender and maintain SA when it was delivered over a 5.6 sec time period. We examined if infusion duration could affect NIC SA. Rats allowed access to NIC did not attain reliable SA when infusions were delivered over 0.9 sec. These data confirm that NIC and CAF are not potent reinforcers in Fischer rats. (Supported by a contract from the R.J. Reynolds Tobacco Co.)

449.8

NRTP-DAMAGED RATS DO NOT DEVELOP NORMAL CAFFEINE TOLERANCE. M.C. Fratzke*B.E. Digman, and R.M Chesire, Department of Psychology, University of Hawaii, Honolulu, Hawaii 96822.

Bilateral electrolytic lesions of the nucleus reticularis tegmenti pontis (NRTP) can release uninhibited, rapid forward locomotion (1-4). NRTP-damaged rats show atypical responses to a number of substances with abuse liability, including morphine or ethanol, which fail to block their movement (3,5-6) and amphetamine or caffeine, which partly reintegrate their movement (7-11). In this study, we examined the development of tolerance to caffeine in NRTP-damaged rats. 12 Long-Evans hooded rats were subjected to bilateral electrolytic lesions of the NRTP, and 5 were used as unoperated controls (we include 8 animals from a previous study (9)). Rats were injected either once per day for 7 days or 4 times total over 44 days with 20 mg/kg caffeine in normal saline (data were pooled). Normal rats showed increases in locomotion and movement typical of multiple exposures to caffeine (12-16), developed tolerance and returned to baseline at about the same rate reported elsewhere (12-16). NRTP-damaged animals also increased, then decreased their locomotion, but increases were over four times that of their baseline, and remained at levels at least three times that of their baseline. Thus, although NRTP-damaged rats did decline somewhat in activity on repeated exposure to caffeine, they did not develop typical tolerance. The results suggest that NRTP damage significantly delays and perhaps can prevent the development of tolerance to caffeine. The data support self-medication theories of substance use/abuse.

449.9

THE EFFECTS OF CAFFEINE ON CORTICAL AROUSAL. D.Velikonja, S.J. Segalowitz and S. Reinis*. Dept. of Psychology, University of Waterloo, Waterloo, ONT, N2L 3G1 and Brock University, St. Catharines, ONT, L2S 3A1.

Individual differences in reactivity and tolerance to caffeine are predicted to play a critical role in cortical arousal effects as measured by electrophysiological variables. Furthermore, it is believed that caffeine will have time-based effects on cortical arousal varying with daily patterns of activity and caffeine consumption. This hypothesis was tested in 20 first year university undergraduate students using a within-subjects design. Each subject participated in two test sessions, in which they consumed one half cup of naturally decaffeinated coffee or one containing powdered caffeine in doses based on the subjects body weight (3mgs/kg). Subjects were blind to experimental conditions. They proceeded to complete questionnaires related to their reactivity and tolerance to caffeine, and whether they were morning or evening types. One half hour elapsed between their consumption of the beverage and EEG testing. Subject's reaction time performance, auditory P300s and visual CNVs were compared on the two occasions. Prior to their date of testing subjects were required to monitor their caffeine consumption over a two-week period in order to determine their habituation to caffeine. A general increase in the amplitude of both N_1 and P_3 are expected, as well as a decrease in reaction time variability, which we expect will be related to individual differences in the CNV. We also predict that an increase in the CNV will also be related to decreased P_3 amplitude jitter in habitual caffeine consumers who are expected to have a higher tolerance and lower reactivity to the caffeine, and to an increase in P_3 amplitude jitter in non-habitual caffeine consumers who are expected to have lower tolerance and higher reactivity to the caffeine.

449.10

EFFECTS OF STEREOISOMERS OF ALANINE AND SERINE ON PHENCYCLIDINE-INDUCED HYPERACTIVITY IN THE RAT. Y.Tanii*, T.Nishikawa, A.Hashimoto and K.Takahashi, Natl. Inst. of Neurosci., NCNP, Tokyo, Japan and Dept. Neuropsychiatry, Toyama Med. & Pharm. Univ., Toyama, Japan.

Phencyclidine(PCP) has been considered to induce a schizophreniform psychosis in humans and behavioral disturbance in experimental animals by blocking the N-methyl-D-aspartate(NMDA) receptor. This study was performed to elucidate the effect of intracerebroventricular(i.c.v.) infusion of allosteric agonists for NMDA receptor on PCP-induced hyperactivity in the rat. Drugs were infused 10min before PCP administration (10mg/Kg,intraperitoneally). The locomotor activity was evaluated based on the method of Sturgeon et al. (1979) with minor modifications. In some experiments, the locomotor activity was also quantitated automatically with Animex-Auto (Muromachi-kikai Co., Japan). D-Alanine and D-serine significantly reduced the PCP-induced hyperactivity in a dose dependent manner. In contrast, the L-isomers of these amino acids were much less effective in antagonizing the hyperactivity induced by PCP. This stereoselectivity suggests that the antagonism of behavioral effects of PCP may be due to activation of the strychnine-insensitive glycine binding site, since the D-forms are more potent than the L-forms as agonists for the allosteric regulation site of NMDA receptor. In support of this view, 7-chlorokynurenic acid (i.c.v.) (a selective inhibitor of glycine allosteric site) reversed the diminution by D-Ser of hyperactivity elicited by PCP. The present findings suggest that stimulation of the allosteric regulation site of the NMDA receptor might possess a therapeutic efficacy in psychosis attributed to PCP and schizophrenia.

449.11

PHENCYCLIDINE, (+) MK-801 AND TCP-INDUCED CIRCLING PREFERENCE: CORRELATION WITH MONOAMINE LEVELS IN SELECTED REGIONS OF THE RAT BRAIN. S.F. Ali[1*], G.D. Newport[1], and H.S. Bracha[2]. [1]Div. of Neurotoxicology, National Center for Toxicological Research,FDA, Jefferson, AR. 72079, [2]Neuropsych. Lab., Dept. of Psychiatry, UAMS, Little Rock, AR 72205.

Phencyclidine (PCP; angel dust) is a drug of abuse known to produce a behavioral state in humans resembling schizophrenia/psychosis. PCP is a noncompetitive NMDA receptor antagonist, and produces a variety of behaviors in rats including circling, however, the behavioral effects of other noncompetitive NMDA receptor antagonists such as (+) MK-801 or TCP are still being elucidated. Here, adult female rats were dosed with PCP (10 mg/kg,ip), (+) MK-801 (0.1 mg/kg,ip) or TCP (5 mg/kg,ip) and circling preference was recorded for two hours before sacrifice to determine monoamine levels by HPLC/EC. Animals injected with PCP, (+) MK-801 or TCP showed a preference to turn to the left (65%, 72%, 62% respectively). PCP and (+) MK-801 also produced a significant increase of DOPAC and HVA in whole caudate nucleus (CN), nucleus accumbens and olfactory tubercles in both sides of the brain. Further dissection of the CN into medioventral and dorsolateral regions revealed that HVA was increased bilaterally except in globus pallidus where we found significant increases in dopamine (DA), DOPAC and HVA only on the left side after PCP and (+) MK-801 administration. However, after TCP treatment the effects were similar but did not reach statistically significant levels. These data suggest that PCP, (+) MK-801 and TCP produce a greater preference to turn left than right, a finding similar to that found in human psychosis. Furthermore, it is possible that this preference is due to the higher concentrations of DA, DOPAC and HVA found in the left side of the globus pallidus after drug administration.

449.12

Sigma-induced inhibition of adrenomedullary [^{3}H]NA reuptake is not mediated by the antidepressant site bound by [^{3}H]desipramine. C. Rogers* and S. Lemaire, University of Ottawa, Ottawa, Ontario Canada K1H 8M5.

The ability of several σ ligands to inhibit uptake of noradrenaline has been examined previously (Massamiri, 1991; Rogers, 1991). At present, the mechanism(s) involved in this inhibition are unknown. Evidence has been presented to suggest that σ ligands block noradrenaline reuptake by interaction with [^{3}H]desipramine ([^{3}H]DMI) receptors. The purpose of this investigation was to determine the degree of interaction of σ ligands with [^{3}H]DMI binding sites in bovine adrenal medulla. [^{3}H]DMI binding was dependent upon Na^+, protein concentration, time, temperature, and was saturable (k_D=2.87 nM B_{max}=216 pmol/g protein). [^{3}H]DMI binding was displaced by σ ligands, although the rank order of potency of these ligands did not parallel that found in [^{3}H]NA reuptake assays in this tissue. This data suggests that σ ligands and desipramine inhibit [^{3}H]NA reuptake through distinct receptors in bovine adrenal medulla. This work was supported by the HSFC, C.R. is a HSFC scholar.

449.13

INDIVIDUAL DIFFERENCES IN THE SENSITIVITY TO CORTICOSTERONE'S REINFORCING EFFECTS AND IN CORTICOSTERONE-INDUCED DOPAMINE RELEASE MAY BE A BIOLOGICAL BASIS FOR SENSATION-SEEKING. P.V. Piazza*, V. Deroche, F. Rougé-Pont, J.M. Deminière, S. Maccari, M. Le Moal and H. Simon. Laboratoire de Psychobiologie des Comportements Adaptatifs INSERM. U259-Univ. Bordeaux II. Domaine de Carreire 33077 Bordeaux Cedex-France.

Sensation-seeking is a human personality trait associated with drug use, compulsive sexual practices, and more generally with the seeking of situations commonly considered stressful. A common feature of such situations is activation of the hypothalamo-pituitary-adrenal axis leading to corticosterone secretion. Since, glucocorticoids have euphoric effects in certain individuals and increase the reinforcing properties of drugs in animals, a higher sensitivity to the reinforcing effects of glucocorticoids might be a biological basis for sensation-seeking. In order to answer this question, we have studied the reinforcing properties of corticosterone, by means of intraveous self-administration, and the effects of this hormone on the mesolimbic dopaminergic (DA) system, by means of in vivo microdialysis. The DA system has been studied because it is considered the main substrate of drug reinforcement. The experiments show that: i) corticosterone, in the range of stress levels, induces intravenous self-administration and increases DA release in the nucleus accumbens; ii) animals that, similar to high sensation-seekers, are more responsive to novelty and more prone to self-administer drugs, are also more sensitive to the reinforcing effect of corticosterone and have a higher corticosterone-induced dopamine release in the n. accumbens. These results suggest that different sensitivity to corticosterone's reinforcing effects may be a biological basis for the individual differences associated with sensation-seeking. Furthermore, individual differences in corticosterone-induced-dopamine release may be the neural substrate of different sensitivity to corticosterone's reinforcing properties. These findings provide new insights on the physiology of glucocorticoids and, since these hormones influence drug self-administration as well as immune functions, these results may also be relevant to clinical practice.

DRUGS OF ABUSE: COCAINE AND DOPAMINE NEURONAL SYSTEMS

450.1

INCREASE IN AN ELECTROCHEMICAL SIGNAL OF DOPAMINE IN THE NUCLEUS ACCUMBENS DURING SELF-ADMINISTRATION OF COCAINE BY RATS. R. Depoortere,*[1] C. D. Blaha,[2] F. G. Le Piane,[2] J. D. Lane,[1] M. W. Emmett-Oglesby[1] and A. G. Philips[2]. [1] Dept. of Pharmacology, TCOM, Fort Worth, TX 76107 and [2] Dept. of Psychology, UBC, Vancouver, B.C., Canada, V6T 1Y7.

The reinforcing properties of cocaine are thought to be mediated by an increased release of dopamine (DA) in the nucleus accumbens (n. acc.). We used the chronoamperometry technique, which has been successfully applied to monitor DA levels in discrete brain structures (Blaha and Philips, *J. Neurosci. Meth.*, **34**:125-133, 1990), to investigate if an electrochemical signal of DA in the n. acc. would increase during self-administration of cocaine by rats. Rats were bilaterally implanted with a stearate-modified carbon paste recording electrode in each n. acc., as well as with a catheter in the right jugular vein. DA levels in the n. acc. were assessed by recording the DA oxidation currents every 30 sec for each recording electrode while rats were self-administering cocaine (0.25, 0.50 or 1.0 mg/0.1 ml) under a FR 2 schedule. Self-administration of cocaine was found to increase DA levels in a dose-dependent manner. Furthermore, increases in DA levels were found to be, in part, time-related to the pattern of self-administration. This set of data suggests that increases in DA levels in discrete brain stuctures can be monitored with a short time resolution during self-administration of cocaine by rats. Supported by grants DA RO1-4137 (M. E-O), TX-ATP 3711 & 9768031 (J. D. L.), MRC-PG-23 (A. G. P.).

450.2

CHRONOAMPEROMETRIC MEASUREMENTS OF DOPAMINE LEVELS IN RAT NUCLEUS ACCUMBENS DURING COCAINE SELF-ADMINISTRATION. A. Gratton*, R.A. Wise and E. Kiyatkin. Douglas Hosp. Res. Ctr, McGill Univ. & Ctr Studies Behav. Neurobiol., Concordia Univ., Montréal, Canada.

Several lines of behavioral evidence have linked the positive reinforcing properties of cocaine with its ability to elevate extracellular levels of dopamine (DA) in nucleus accumbens (NAcc). Few investigators have measured changes in DA levels during cocaine self-administration, and most that have used microdialysis which does not provide sufficient temporal resolution to monitor moment-to-moment changes in DA levels associated with operant responding. We used high-speed chronoamperometry to monitor, during 5-6 consecutive daily recording sessions, DA-dependent electrochemical signals in NAcc of rats allowed to lever-press for intravenous cocaine (0.8mg/kg/injection). The changes in the electrochemical signal associated with lever-pressing for cocaine depended on the day recordings were performed. Typically, responses for cocaine during the first session were each followed by an abrupt and long-lasting decrease of the electrochemical signal which gradually returned to baseline levels at the end of the session when cocaine was no longer available. In contrast, the first cocaine injection of the second and subsequent recording sessions was followed by a pronounced and gradual increase in the signal. This pattern changed, however, with responses that followed in the session. Subsequent responses for cocaine were each preceeded by a reliable increase and followed by a comparable decrease in the electrochemical signal. A visual cue paired with each response for cocaine was, alone, sufficient to increase electrochemical signals in experienced animals. Increases in the signals were also observed when access to the lever was blocked or when the infusion pump was inactivated. The present experiment indicates that the effects of self-administered cocaine on extracellular DA levels in NAcc change as they become conditioned to environmental stimuli; elevated DA levels during cocaine self-administration occured only on the second and subsequent testing sessions. Further increases in extracellular DA were linked to operant responding but appeared to be more closely associated with events other than response reinforcement per se.

450.3

BASAL DOPAMINE LEVELS IN THE NUCLEUS ACCUMBENS ARE DECREASED DURING COCAINE WITHDRAWAL AFTER UNLIMITED-ACCESS COCAINE SELF-ADMINISTRATION. F. Weiss,* A. Markou and G.F. Koob. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Clinical observations indicate that chronic cocaine abuse is followed by a withdrawal syndrome resembling an episode of major depression. An analogous syndrome of "post-cocaine anhedonia" characterized by significant elevations in brain stimulation reward thresholds has recently been reported in rats after extended periods of intravenous cocaine self-administration (Markou & Koob, *Neuropsychopharmacology*, 1991). This wthdrawal-associated reward deficit may involve a specific impairment in dopamine (DA) neurotransmission in the region of the nucleus accumbens (NAC). To test this hypothesis we have monitored extracellular DA levels in the NAC by intracranial microdialysis in awake rats during withdrawal from cocaine after periods of unlimited-access, intravenous self-administration (9.5 to 21.75 hours). Cocaine withdrawal was associated with significant reductions in basal DA overflow that persisted up to 10 hours. Maximal inhibition of DA release (Mean ± S.E.M.: 66.15 ± 3.3 percent of basal levels) was observed at 4-6 hours after termination of cocaine self-administration and was positively correlated ($r = 0.93$) with the duration of the preceding self-administration episode. The results suggest that suppression of basal DA release in the NAC is a direct adaptive consequence of chronic cocaine exposure and may constitute an important neurochemical correlate of the cocaine withdrawal syndrome as measured by brain stimulation reward thresholds.

450.4

REVERSE TOLERANCE OF SEROTONIN AND DOPAMINE IN THE NUCLEUS ACCUMBENS AND DORSAL RAPHE NUCLEUS INDUCED BY CHRONIC COCAINE TREATMENT. L.H. Parsons* and J.B. Justice, Jr. Dept. of Chemistry, Emory University, Atlanta, GA 30322.

The effect of chronic cocaine administration on the response to a cocaine challenge was examined for serotonin (5-HT) and dopamine (DA) in the nucleus accumbens (N ACC) and dorsal raphé nucleus (DRN) of the rat using a dual probe in vivo microdialysis procedure. Rats were treated for ten days with cocaine (20 mg/kg, i.p.; n = 6) or saline (0.9%, 0.05 ml/kg, i.p.; n = 6), and all animals received a 10 mg/kg i.p. injection of cocaine on the test day (day 11). Based on maximum percent increases from cocaine challenge, DA was significantly sensitized in chronically treated animals in both the N ACC and DRN, though there was no significant interaction between region and degree of sensitization. 5-HT was also significantly sensitized in both regions of chronically treated animals, and there was a significant interaction between region and degree of sensitization, indicating that the cell body region produced greater 5-HT sensitization than the N ACC terminal field. Because both DA and 5-HT were obtained from the same probe in a given region for each animal, DA/5-HT area under the curve (AUC) ratios were used to compare the sensitization of DA relative to 5-HT. In both regions there were reduced DA/5-HT AUC ratios in chronic as compared with acute animals, though this reduction reached significance only in the DRN. This suggests that the 5-HT system is relatively more sensitized to chronic cocaine administration than the DA system.

Additionally, the interaction between 5-HT and DA was assessed by adding various concentrations of 5-HT to the perfusate of a microdialysis probe in the N ACC and observing the concurrent change in dialysate DA. 5-HT was found to concentration-dependently increase dialysate DA, with perfusate 5-HT concentrations between 100 and 400 nM. This effect was antagonized with differing effectiveness by the relatively non-specific 5-HT_1 antagonist pindolol, the specific 5-HT_2 antagonist LY 53,857, and the relatively specific 5-HT_3 antagonist MDL 7222. These results indicate that 5-HT stimulates extracellular DA levels in the N ACC.

450.5

INCREASES IN EXTRACELLULAR BIOGENIC AMINE CONCENTRATIONS FOLLOWING COCAINE OR HEROIN SELF-ADMINISTRATION S.E. Hemby*, T.J. Martin, C. Co, S.I. Dworkin, & J.E. Smith. Center for Neurobiology of Drug Abuse, Dept. of Phys./Pharm., Bowman Gray School of Medicine, Wake Forest Univ., Winston-Salem, NC 27157.

Nucleus accumbens dopamine is believed to mediate the reinforcing effects of psychotropic compounds. Cocaine self-administration enhances extracellular NACC dopamine concentrations in a dose dependent manner (Pettit and Justice, 1989, *PBB*, 34, 899-904). Response-independent administration of opiates enhances extracellular dopamine concentrations although this effect has not been demonstrated during response-dependent administration. The present study was initiated to determine whether extracellular catecholamine concentrations in the NACC are elevated during heroin self-administration in a similar manner to that observed during cocaine self-administration. Responding was maintained by either intravenous infusions of heroin (60 or 100 μg/kg/infusion) or cocaine (330 μg/kg/infusion). Following a minimum of twenty days stable responding, microdialysis probes were inserted through previously implanted cannulae. The following day, dialysate samples were collected in five minute intervals throughout the two-hour self-administration session. HPLC coupled to electrochemical or ultraviolet detection was used to assay the dialysate samples for catecholamines and cocaine, respectively. Cocaine self-administration increased extracellular NACC dopamine and serotonin although metabolite concentrations did not significantly differ from baseline. In addition, preliminary results suggest that heroin self-administration produced significant elevations in dopamine comparable to the relative increase observed with cocaine. (Supported in part by USPHS Research Grants DA-01999, DA-03628, and DA-06634)

450.6

60-DAY CHRONIC COCAINE TREATMENT DESENSITIZES EXTRACELLULAR DOPAMINE OVERFLOW IN CAUDATE-PUTAMEN BUT NOT IN NUCLEUS ACCUMBENS. J. Chen*, R. Marmur, E. Rosenbaum, W. Paredes and E.L. Gardner, Departments of Psychiatry and Neuroscience, Albert Einstein College of Medicine, New York, NY 10461

Rats with *in vivo* brain microdialysis probes in the nucleus accumbens (Acc) and caudate-putamen (CPu) were treated with cocaine (10 mg/kg/day, i.p.) for 5, 30 and 60 days. 24 hrs after the last cocaine injection, rats of each group were challenged with the same dose of cocaine and extracellular dopamine (DA) overflow in Acc and CPu measured by *in vivo* microdialysis. In both Acc and CPu, biochemical sensitization of extracellular DA overflow was not seen in rats given repeated cocaine for 5 days. In contrast, 30 days of chronic cocaine *did* robustly augment the enhanced extracellular DA overflow produced by the challenge dose of cocaine. In animals given 60 days of chronic cocaine, striking differences between Acc and CPu manifested themselves. In Acc, the augmented extracellular DA overflow to cocaine challenge seen after 60 days of cocaine was identical to that seen after 30 days of cocaine. In CPu, after 60 days of chronic cocaine, the enhanced extracellular DA overflow produced by cocaine challenge was identical to that seen with *no* prior cocaine treatment. Thus, in Acc neurochemical sensitization to repeated cocaine occurs, and the sensitization maintains itself, while in CPu cocaine sensitization occurs, but then some compensatory mechanism comes into play to produce a neurochemical *de*sensitization to cocaine. This suggests that cellular mechanisms underlying cocaine sensitization differ between the mesolimbic and mesostriatal DA systems. (Supported by a research grant from the Aaron Diamond Foundation).

450.7

EFFECTS OF DAILY MULTIPLE INJECTIONS OF COCAINE ON THE DOPAMINERGIC SYSTEMS IN RATS: AN *IN VIVO* MICRODIALYSIS STUDY. I.M. Maisonneuve* and M.J. Kreek, The Rockefeller University, New York, NY 10021.

The aim of this study was to investigate the response of the dopaminergic systems to cocaine using a pattern of drug administration that mimics cocaine users' binges. Rats received three i.p. injections of cocaine (3 x 10 mg/kg) or saline (3 x 1 ml/kg) over a period of two hours for 13 days. On day 14 each rat received the three cocaine injections and the time course of dopamine (DA) and its metabolites were monitored in the nucleus accumbens and in the striatum using *in vivo* microdialysis. In both regions extracellular DA basal levels were not significantly different in saline versus cocaine treated rats (3.69 ± 0.69 nM versus 2.64 ± 0.53 nM in the nucleus accumbens, $p<0.26$; 9.32 ± 2.4 nM versus 7.11 ± 1.42 nM in the striatum, $p<0.12$; n=5). Acute cocaine administration led to an increase in extracellular DA levels that plateaued after the second cocaine injection (3.5 times greater than basal levels in the nucleus accumbens, 3 times in the striatum). Chronic administration altered this pattern. The peak in DA levels was achieved only after the third injection, and in the striatum, in contrast to the nucleus accumbens, the extracellular DA concentrations did not reach the maximum levels observed after an acute challenge. The present results differ from the conclusions of studies involving a single daily cocaine injection where DA levels were reported to be markedly enhanced by chronic exposure. Therefore the pattern of cocaine administration appears to be a major factor in the response of the dopaminergic systems. (Supported by the Aaron Diamond Foundation and DA-P50-05130).

450.8

IMPORTANCE OF THE DOPAMINE TRANSPORTER IN DETERMINING CLEARANCE OF EXOGENOUS DOPAMINE IN RAT BRAIN: IN VIVO ELECTROCHEMICAL STUDIES N.R. Zahniser*, W.A. Cass and G.A. Gerhardt, Depts. of Pharmacology and Psychiatry, Univ. of Colorado Hlth. Sci. Ctr., Denver, CO 80262.

In vivo electrochemistry was used to investigate the mechanisms contributing to the clearance of locally-applied dopamine (DA) in dorsal striatum and nucleus accumbens of urethane-anesthetized rats. Chronoamperometric recordings were continuously made at 5 Hz using Nafion-coated carbon fiber electrodes. When a finite amount of DA (12-100 nl, 200 μM barrel concentration) was pressure-ejected at 5-min intervals from a micropipette positioned 280 ± 30 μm from the electrode, transient and reproducible increases in DA (0.5-4 μM) were detected. Substitution of α-methyl-DA, which is a substrate for the DA transporter but not for monoamine oxidase, for DA in the micropipette did not substantially alter the time course of the resulting signals. These results indicate that metabolism of locally-applied DA to dihydroxyphenylacetic acid is not responsible for the decline in the DA signal. Similarly, changing the applied oxidation potential from 0.45V to 0.80V, which allows detection of 3-methoxytyramine formed from DA via catechol-O-methyltransferase, had little effect on the signal amplitude and time course. In contrast, local application of the DA uptake inhibitors cocaine or nomifensine (800 μM barrel concentration, applied 30-60 sec before DA) significantly increased the amplitude and time course of the DA signals in both brain regions, a result similar to that observed with systemic administration of cocaine. Taken together, these data indicate that uptake of DA by the neuronal DA transporter, rather than DA metabolism, is the major mechanism for clearing locally-applied DA from the extracellular space of striatum and accumbens. (Supported by USPHS DA04216, DA00174 & NS09199 and NSF BNS-9110308)

450.9

REPEATED COCAINE PERSISTENTLY REDUCES THE CLEARANCE RATE OF EXOGENOUS DOPAMINE IN RAT NUCLEUS ACCUMBENS. W.A. Cass*, G.A. Gerhardt, K. Gillespie, P. Curella and N.R. Zahniser. Depts. of Pharmacology and Psychiatry, Univ. of Colorado Hlth. Sci. Ctr., Denver, CO 80262.

We investigated whether differential changes in the dopamine (DA) transporter in the nucleus accumbens (NAc) or dorsal striatum could be involved with cocaine-induced behavioral sensitization by monitoring the clearance of locally applied DA in anesthetized rats using *in vivo* electrochemistry. Rats were given daily injections of cocaine (10 mg/kg i.p.) or saline for seven days, withdrawn for seven days, and prepared for electrochemical recordings. When a finite amount of DA (2.5-40 pmoles) was pressure-ejected at 5-min intervals from a micropipette positioned 270 $\pm$ 30 μm from the recording electrode, transient and reproducible increases in extracellular DA were detected. In response to a challenge injection of cocaine (10 mg/kg i.p.), the signals in the NAc of the cocaine-treated rats became prolonged and the clearance rate of DA decreased, indicating significant inhibition of the DA transporter. In contrast, in the striatum there was a transient increase in the DA clearance rate. In saline-treated animals the signals from both regions were similar to signals from untreated animals given an acute injection of cocaine (10 mg/kg i.p.) or saline. Quantitative autoradiography with ^{3}H-mazindol revealed that the affinity of the DA transporter for cocaine and the density of binding sites were similar in cocaine- and saline-treated rats. Behaviorally, 50% of the cocaine-treated animals were sensitized; however, both sensitized and unsensitized animals displayed similar changes in DA clearance rate. Nonetheless, the observed decrease in DA clearance rate in the NAc of the cocaine-treated rats is consistent with increased DA transmission in response to cocaine challenge. Supported by USPHS DA04216, NS09199 & NSF BNS-9110308.

450.10

EFFECTS OF SPECIFIC BRAIN AMINE DEPLETING LESIONS ON COCAINE-INDUCED CONDITIONED INCREASES IN LOCOMOTOR OUTPUT. A.Pert*, D.N. Thomas and R.M. Post. Biological Psychiatry Branch, NIMH, Bld. 10, Rm. 3N212, Bethesda, MD. 20892

Environmental stimuli paired with cocaine acquire the ability to elicit increases in locomotor behaviour and potentiate the actions of cocaine during subsequent presentations. We utilized a simple and efficient design to evaluate the neuronal substrates which underlie the conditioned effects of cocaine. On DAY 1 Rats were injected with either saline (UNPAIRED) or 40mg/kg cocaine (PAIRED) before placement in locomotor chambers for 30mins. 1hr following return to the home cage animals that received saline were injected with 40mg/kg cocaine, whilst those that received cocaine initially were injected with saline. On DAY 2, all rats were injected with 10mg/kg of cocaine prior to placement in the locomotor chamber for 30mins. Conditioned locomotor activity is revealed by significant increases in locomotor output of the PAIRED versus the UNPAIRED group on DAY 2. We have previously found that 6-OHDA lesions of the nucleus accumbens and amygdala prevent the development of such conditioning with cocaine. The purpose of these studies was to evaluate the role of other brain amines systems as well as frontal cortex and striatal DA in cocaine-induced conditioning. Animals in each treatment group were lesioned bilaterally in the frontal cortex, striatum and locus coeruleus with 6-OHDA or in the median and dorsal raphe with 5,7-DHT. Controls groups were sham lesioned. One week following surgery, the animals were conditoned and tested as described above. Neither frontal cortex or striatal lesions had an effect on the acquisition of conditioned locomotor activity, although the striatal lesions did seem to attenuate the conditioned stereotypy. Lesions of the locus coeruleus or raphe had no effect on cocaine-induced conditioning. These findings suggest that the critical dopaminergic neural substrates for cocaine-induced conditioned locomotor behaviour are the amygdala and the nucleus accumbens. Serotonergic and noradrenergic pathways do not appear to play a role in cocaine-induced conditioning in our paradigm.

450.11

EFFECTS OF INTRA-ACCUMBENS AND INTRA-AMYGDALOID SCH23390 ON INTRAVENOUS COCAINE SELF-ADMINISTRATION IN THE RAT. A. McGregor and D.C.S. Roberts*. Life Sciences Research Centre, Carleton University, Ottawa, K1S 5B6, Canada.

The nucleus accumbens (NAcc) and its heavy dopaminergic (DA) innervation is generally considered to be a neural substrate pivotal in mediating the reinforcing effects of psychostimulant drugs. However, the amygdaloid complex is another neural site receiving a substantial DA innervation that has been relatively overlooked with respect to mechanisms of drug abuse. This work investigated the possible involvement of the amygdaloid complex (AMY) in cocaine self-administration (0.6mg/injection) under a fixed ratio schedule (FR1) of reinforcement and compared its contribution to that of the NAcc. Bilateral injections of the D1 antagonist, SCH23390, were made directly into the NAcc or AMY.

In both sites a significant dose dependent (0.1µg - 2.0µg/side/0.5µl) increase in the rate of self-administration was produced, demonstrating that both sites make a contribution to cocaine reinforcement mechanisms. In addition however, blockade of the D1 receptor within the AMY produced a two-fold increase in the rate of cocaine intake with respect to that produced within the NAcc. These results suggest that in addition to the NAcc, the AMY also has a contribution to make to cocaine reinforcement mechanisms. (Supported by the MRC of Canada).

450.12

INDIVIDUAL DIFFERENCES IN VULNERABILITY TO DRUG-ADDICTION: BEHAVIORAL AND BIOCHEMICAL CORRELATES. **M.J.D. Miserendino*, T.A. Kosten, X. Guitart, S. Chi, and E.J. Nestler. Depts. of Psychiatry and Pharmacology, Yale Univ. Sch. of Med., New Haven, CT. 06508**

One model used to examine individual differences in drug preference uses the inbred Fischer 344 and Lewis rat strains, which show differential oral self-administration of opiates, cocaine, and alcohol. We have shown inherent biochemical differences in reward-implicated sites between these strains, with an upregulated cAMP system in the nucleus accumbens (NAc) and altered levels of tyrosine hydroxylase (TH) and neurofilaments in the ventral tegmental area (VTA) of the drug-preferring Lewis rat (Beitner-Johnson et al., Brain Res. 561,147 1991; Guitart et al., Synapse, in press). We have also demonstrated strain differences in other drug-related behaviors: compared to Fischer rats, Lewis rats show greater locomotor sensitization and conditioned place preference, and more readily acquire intravenous cocaine self-administration.

We have also begun to examine similar individual differences in biochemistry and behavior within a single rat strain (Sprague Dawleys). Rats were assessed for locomotor activity in a novel environment, and the highest (HR) and lowest (LR) responders were selected for biochemical analysis. Compared to HR rats, LR rats showed higher levels of cAMP dependent protein kinase in the NAc, and of TH in the VTA. We are currently testing HR and LR rats for the acquistion of cocaine self-administration.

Such integrated biochemical and behavioral assessments should enable the identification of some of the biochemical factors that contribute to individual genetic vulnerability to drug addiction.

450.13

DIFFERENTIAL REGULATION OF THE COCAINE ACCEPTOR AND DOPAMINE UPTAKE SITES IN RAT BRAIN FOLLOWING COCAINE SELF-ADMINISTRATION AND WITHDRAWAL. J.M. Wilson*, M. Carroll, J.N. Nobrega, H.B. Niznik and S.J. Kish. Univ. Toronto, Canada and Univ. Minnesota, Minneapolis.

Using quantitative autoradiography, the influence of unlimited cocaine self-administration (6 weeks) on binding to the dopamine (DA) uptake (0.25 nM [^{3}H]GBR 12,935 displaced with 1 μM mazindol) and cocaine acceptor (10 nM [^{3}H]WIN 35,428 displaced with 30 μM cocaine) sites in rat brain was examined. In rats sacrificed while still on cocaine (n = 10) specific binding of [^{3}H]GBR 12,935 to the DA transporter in striatum, n. accumbens (nacs), substantia nigra (SN) and ventral tegmental area (VTA) was unaltered relative to controls (n = 5). However, specific binding of [^{3}H]WIN 35,428 to the cocaine acceptor sites was significantly *elevated* ($p < 0.05$) in striatum (+63%) and nacs (+73%). Following 3 weeks withdrawal from unlimited cocaine self-administration specific binding of [^{3}H]GBR 12,935 was unaltered whereas binding of [^{3}H]WIN 35,428 was significantly *reduced* ($p < 0.05$, n = 5) in striatum (-33%), nacs (-46%), SN (-51%) and VTA (-62%). The observation of unaltered binding of [^{3}H]GBR 12,935 three weeks after the last administration of cocaine, suggests that there is no permanent degeneration of rat brain dopaminergic nerve terminals. However, the elevated binding of [^{3}H]WIN 35,428 to the cocaine acceptor site during chronic cocaine self-administration, but reduced binding during withdrawal, suggest that the cocaine acceptor might be sensitive either to the extracellular concentration of DA or to the presence and subsequent absence of cocaine, which acts like an antagonist at the DA transporter. Altered affinity/density of the cocaine acceptor could modulate DA reuptake, and it will be important to establish the functional significance of such changes following chronic cocaine exposure and during the course of withdrawal. (Supported by NIDA grant DA07182.)

450.14

Withdrawal From Continous or Intermittent Cocaine: Dopamine Autoreceptor Sensitivity.

KING, G.R., KUHN, C., AND ELLINWOOD, E.H., JR.*

Previous research in this laboratory inidicates that daily intermittent injections of cocaine result in an enhanced dopamine (DA) response in caudate-putamen brain slices, to different cocaine concentrations. In contrast, the continuous infusion of an equivalent daily dose of cocaine results in an attenuated DA response in caudate-putamen brain slices, to different cocaine concentrations. One possible mechansim mediating these effects is changes in DA autoreceptor sensitivity. Daily, intermittent cocaine injections may result in DA autoreceptor subsensitivity, while the continuous infusion of cocaine may result in DA autoreceptor supersensitivity. The present experiment examined this possibility. Rats were pre-treated with 40 mg/kg/day of cocaine for 14 days by either subcutaneous injections or continous infusion by osmotic minipumps. The rats were then withdrawn from the pretreatment regime for 7 days. On Day 7, the rats were sacrificed, and caudate-putamen slices rapidly obtained. The slices were then placed in glass perfusion chamber, and superfused with artifical CSF for sixty minutes. At the end of this period, the slices were electrically stimulated with a train of supramaximal, unipolar, rectangular waves (20 mA, 2 msec) at 3 Hz for 90 pulses (S_1). Ten, 2 min samples were collected. This electrical stimulation was repeated 60 minutes later (S_2). However, 30 min prior to the S_2 period, the brain slices were perfused with either 0, 1, 2, or 4 μM sulpiride, a D_2 receptor antagonist. The data were analyzed by determining the [S_2/S_1] ratio for different sulpride concentrations. This research was supported by NIDA grant DA05303, and NIH grant T32- MH15177.

450.15

DOSE-DEPENDENT CHANGES IN THE RAT DOPAMINERGIC RECEPTOR SYSTEM AFTER CHRONIC ADMINISTRATION OF COCAINE. M.E. Alburges*†, N. Narang†, C. Johnson and J.K. Wamsley†. *Neuropsychiatric Research Institute, Fargo, ND 58103; †Medical School, University of Zulia, Maracaibo, Venezuela.

We have previously reported time-dependent alterations in the rat dopaminergic receptor system after fixed doses of cocaine (15.0 mg/kg, i.p.). In the present study, the dose-dependent effects of cocaine on the dopaminergic system were determined. Rats were injected with cocaine (5.0,10.0,15.0,20.0 and 25.0 mg/kg, i.p., b.i.d.) or saline for a 21 day period. [^{3}H]Cocaine and [^{3}H]SCH23390 binding in cortices and striatum from animals injected with 10.0 and 15.0 mg/kg were significantly higher than the control animals. [^{3}H]BTCP binding in striatal tissue was significantly increased in animals injected with 10.0 and 15.0 mg/kg of cocaine; however, in cortices of these animals, significant changes were seen with only 15.0 mg/kg of cocaine. Changes in [^{3}H]raclopride binding in these tissues were not present. These results indicate chronic exposure to cocaine produces a dose-dependent upregulation in cortical and striatal D_1 and DA-uptake sites.

450.16

COCAINE AND AMPHETAMINE: A COMPARISON OF THEIR EFFECTS ON DOPAMINE OVERFLOW IN THE STRIATUM AND AMYGDALA. E. Museo,* A. Zocchi, and A. Pert. Biological Psychiatry Branch, NIMH, Bethesda, MD, 20892.

Experiments were carried out to determine whether cocaine and d-amphetamine have similar effects on dopamine (DA) overflow in the striatum and amygdala. Male Sprague-Dawley rats were anaesthetized prior to and following the implantation of a microdialysis probe into either the striatum or amygdala. These structures were perfused (0.5 µL/min) with either cerebrospinal fluid (CSF) or CSF containing varying amounts of either cocaine or amphetamine. Dialysate samples were collected every 20 min and analyzed for DA content using a microbore HPLC/EC system. Whereas the application of cocaine (0.01, 0.1, and 1 mM) into the striatum increased DA overflow in a concentration-related manner, cocaine produced either weak or inconsistent effects in the amygdala. (A difference in sensitivity between the striatum and amygdala was also found, albeit much less marked, when cocaine was administered systemically (40 mg/kg, i.p.).) In the case of amphetamine (0.1, 1, and 10 µM), its application into the striatum also increased DA overflow in a concentration-related manner; unlike cocaine, however, amphetamine produced consistent concentration-related increases when administered into the amygdala (even though the striatum was apparently more sensitive). These findings suggest that the effects of amphetamine and especially cocaine differ depending on whether the drugs are administered into the striatum or amygdala. The possibility is raised, then, that cocaine binding sites or DA transporters located in the striatum differ from those located in the amygdala.

450.17

THE EFFECT OF CONTINUOUS COCAINE ADMINISTRATION ON ALTERATIONS IN STRIATAL EXTRACELLULAR DOPAMINE METABOLISM. J.W. Lipton*[1] & R.E. See[2]. Depts. of Psychology, UCLA[1], Los Angeles, CA 90024-1563 & Washington State University [2]

It is well established that intermittent schedules of psychostimulants produce behavioral sensitization while continuous regimens tend to cause tolerance. *In-vivo* microdialysis was utilized to examine extracellular striatal dopamine and monoamine metabolite (DOPAC, HVA, 5-HIAA) levels at different times during and after continuous cocaine administration. Male Sprague-Dawley albinos were implanted with subcutaneous cocaine pellets which release approximately 105mg of cocaine freebase over 5 days. Rats were sampled before the beginning cocaine administration, on day 5 of cocaine, 24 hours post-cocaine or 7 days post-cocaine. Dose-response relationships were established for infusions of quinpirole (QP) through the dialysis probe (10^{-7},10^{-6}, 10^{-5} M). DA levels were decreased in dose response fashion with no group differences among animals on day 5 of continuous cocaine, 24h or 7 days post-cocaine as compared to drug-naive controls. At 24h after cocaine HVA was significantly decreased vs drug-naive animals (75% vs 91% respectively). There was a significant difference in QP induced metabolite levels between animals 7 days post-cocaine vs drug-naive controls DOPAC (60% vs 91%), HVA (75% vs 115%) and 5-HIAA (85% vs 60%). Amphetamine (AMPH) mediated DA release in striatum indicated that 24 hours after the cessation of continuous cocaine there was a non-significant decrease in the amount of DA release as compared to drug-naive controls (880% increase vs 1342% increase, respectively). Immediately after the local AMPH infusion there was a significant difference between cocaine exposed animals and controls in the disposition of DOPAC (110% vs. 88%), HVA (116% vs 102%) and 5-HIAA (99% vs. 118%). While group differences in DA were non-significant due to high variability, it is evident that there are significant time-dependent alterations DA metabolism when the nigrostriatal system is pharmacologically challenged.

450.18

CHRONIC COCAINE PRODUCES "TOLERANCE" OF METABOLIC ACTIVITY IN RAT BRAIN REWARD REGIONS, R. P. Hammer, Jr.*, A.R. Mateo, W.S. Pires, E.S. Cooke and B.B. Young, Lab. of Cellular & Molecular Neuropharmacology, Dept. of Anatomy & Reprod. Biology, Univ. Hawaii School of Medicine, Honolulu, HI 96822.

Repeated daily cocaine administration produces various sustained neurochemical effects. We have examined the effect of daily treatment for a total of 14 days with saline vehicle or 10-20 mg/kg cocaine HCl administered for 1, 3, 7, 10 or 14 days on regional cerebral metabolic rate (rCMR) measured using the quantitative method of Sokoloff. A pulse of [^{14}C]2-deoxyglucose was administered i.v. 10 min after i.p. drug or vehicle injection in freely moving animals, after which timed arterial blood samples were collected for measurement of radioactivity and plasma glucose values across a 45 min exposure period. Regional autoradiographic analysis was used to determine rCMR values in 61 regions; the results were compared by ANOVA followed by Newman-Keuls post-hoc tests. Significant main effects of treatment occurred only in the nucleus accumbens (NAc) core and shell, olfactory tubercle, piriform and anterior cingulate cortices, basolateral amygdala, and ventromedial striatum. In all cases, acute treatment produced slight increases of rCMR which was unchanged from control values after 3 or 7 days of treatment but was significantly reduced after 10 or 14 days of treatment. This biphasic effect was greater in the rostral NAc than in the caudal NAc. Although repeated daily cocaine administration is reinforcing (lowers the threshold of rewarding intracranial self-stimulation), metabolic response in mesolimbic terminal regions shifts from initial activation to sustained reduction. Such chronic drug-induced changes could represent a metabolic manifestation of tolerance in critical brain regions. Supported by USPHS awards DA06645 and HD01161.

450.19

QUANTITATIVE MICRODIALYSIS UNDER TRANSIENT CONDITIONS. R.J. Olson* and J.B. Justice Jr., Dept of Chemistry, Emory University, Atlanta, GA 30322

A method based on the point of no net flux (Lönnroth et al., Am. J. Physiol. 256:E250-E255, 1989) has been developed for quantitative microdialysis under transient conditions. Four groups of rats were perfused with either 0,10,20 or 40 nM dopamine (DA) at 0.6 ul/min. Dialysate DA was collected and measured for time intervals prior to and during drug administration. Data from the four groups were combined at each time point and used with linear regression to determine the in vivo recovery and the extracellular concentration of DA as a function of time. It was hypothesized that changes in uptake and release would change probe recovery. No change in recovery was observed in the striatum with 4mm probes during haloperidol induced DA release (1 mg/kg, I.P., n = 6 per group). However, it is possible that the already high baseline recovery (78%) masked otherwise evident changes. In order to examine the effect of uptake inhibition on recovery, cocaine (20 mg/kg, I.P.) was administered and dialysate was collected using 2mm probes in the nucleus accumbens. Recovery decreased from 48% at baseline to 25% twenty minutes following drug administration and returned to 48% seventy minutes following drug (n=3 per group). This corresponded in time to the increase and return to baseline of DA. Recovery was used at each time point to calculate extracellular DA from dialysate DA. Extracellular DA was 5.08 +/- 0.25 nM at baseline and 44 nM 20 minutes following drug administration. A higher percent increase was observed in extracellular DA than was evident in dialysate DA. These results support previous work which suggests that probe recovery is dependent on active neurotransmitter properties such as uptake and release (Parsons et al., J. Neurosci. Meth., 40:131-137, 1991). The present method appears useful for quantitatively characterizing the pharmacology of neurotransmitter systems.

450.20

THE EFFECTS OF DISCRETE LESIONS OF THE SUB-COMMISSURAL VENTRAL PALLIDUM ON COCAINE SELF-ADMINISTRATION IN THE RAT. P. Robledo* and G. F. Koob. The Scripps Research Institute, La Jolla, CA. 92037.

The involvement of the nucleus accumbens in mediating cocaine reinforcement has been largely established. Previous work from our laboratory has demonstrated that Ibotenic acid lesions of one of the output regions or the nucleus accumbens, the sub-lenticular substantia innominata, produced significant decreases in the highest ratio obtained in rats self-administering (SA) cocaine. In this study, we investigated the importance of another accumbal output, the sub-commissural ventral pallidum, in mediating the reinforcement properties of cocaine in the rat. Animals were trained to self administer cocaine (0.75 mg/kg/inj) via an intravenous catheter on a FR5 schedule of reinforcement. Subsequently, rats were either given bilateral i.c. injections (0.5 µl per side) of Ibotenic acid (10 µg/µl lesion group) or vehicle (sham group) into the sub-commissural ventral pallidum. Five days post-lesion, cocaine SA on a FR5 schedule was resumed for three days. Next, a dose effect function was determined in one 3 hour session. A progressive ratio schedule in which the ratio requirement was increased after each reinforcement was also used. The lesion group showed a significant decrease in FR5 responding for cocaine five days after the lesion as compared to the sham group. While the lesion produced decreases in responding for cocaine at all doses, the rate of responding was inversely proportional to the dose. However, compared to sham animals, in the progressive ratio task, no effect was found in the total number of rewards nor in the highest ratio obtained in lesioned rats. These results, taken together with our previous data, support the hypothesis that the region commonly called the ventral pallidum is a functionally heterogeneous structure which may have an equally heterogeneous relationship with the nucleus accumbens. Further, these results suggest that within the ventral pallidum there are some areas that are more critically involved in cocaine reinforcement than others. Supported by NIDA grant DA04398.

451.1

NEUROTOXIC EFFECTS OF PRENATAL MPTP ON MONOAMINE AND PEPTIDE SYSTEMS OF THE MARMOSET BRAIN. J. Del Río*[1], I. Pérez-Otaño[1], M.T. Herrero[2], M.R. Luquin[2], C. Oset[1], and J.A. Obeso[2]. Depts. of Pharmacology(1) & Neurology (2), Univ. of Navarra Medical School, Pamplona, Spain.

Chronic administration of MPTP to adult marmosets induces alterations in brain dopamine, serotonine and peptide systems. It is not known if MPTP can also produce neurotoxic effects on the developing brain of primates. In this study, MPTP was incidentally administered to two female marmosets who became pregnant. The monkeys received MPTP (1.25-2.5 mg/kg s.c., twice a week) during the whole gestational period, except for the last 15 days before term, when pregnancy was noticed. Baby marmosets (n=3) were sacrificed 5 months after birth and levels of dopamine (DA), serotonin (5-HT) and its metabolites, DOPAC, HVA and 5-HIAA were determined in several brain areas by HPLC and compared to age-matched controls(n=6). Substance P (SP) content was measured in the basal ganglia by RIA. A significant reduction of DA and its metabolites, DOPAC and HVA, was found in the caudate nucleus, putamen and n. accumbens of marmosets exposed "in utero" to MPTP, but not in other areas studied. In contrast to the extensive and severe 5-HT loss induced by chronic MPTP treatment of adult animals, no change in striatal 5-HT content was found in baby marmosets and only extrastriatal 5-HT systems were affected. A significant decrease in SP levels was observed in the s. nigra of baby marmosets. The results demonstrate that MPTP can cross the placenta and exert its neurotoxic effect on monoamine and peptide systems in the fetal brain of primates. These findings show the possibility of "in utero" lesion of several neurotransmitter systems in the marmoset brain and indicate that non-melanized neurons (as is the case in the fetus) are also susceptible to MPTP. (Supported by EEC and JALS Fdn.).

451.2

PARTIAL NIGROSTRIATAL 6-OHDA LESION AS A MODEL OF PRECLINICAL PARKINSON'S DISEASE. J. Fornaguera-Trias, R.K.W. Schwarting*, F. Boix and J.P. Huston. Inst. of Physiol. Psychol. I, Univ. of Düsseldorf, Germany

The study of moderate dopamine (DA) lesions in animals could serve as a model of the preclinical stage of Parkinson's disease, both in the search for possible behavioral predictors and concurrent neuronal changes. Therefore, we studied asymmetries in turning and thigmotactic scanning in rats with unilateral injections of 6-OHDA into the substantia nigra and compared them with the degree of striatal DA depletion. Animals with severe lesions (<20% residual DA) showed spontaneous ipsiversive turning and more scanning with the side of the face ipsilateral to the lesion. These asymmetries were reversed by apomorphine. Animals with moderate depletions (20-65% DA) did not show spontaneous asymmetries in turning, but an ipsilateral asymmetry in scanning. Most important is that out of this group, animals with 45-65% residual DA levels showed ipsiversive turning and ipsilateral scanning under apomorphine. These results suggest that scanning is a more sensitive measure than turning for the detection of functional asymmetries after moderate striatal DA lesions. The ipsiversive asymmetries observed after apomorphine in animals with moderate depletions might be due to ipsilateral changes in self-regulatory mechanisms of the nigrostriatal DA system, such as in DA autoreceptors, which in turn might be related to compensatory mechanisms in the preclinical stage of Parkinson's disease.

451.3

EFFECTS OF CHRONIC SINEMET TREATMENT ON RATS WITH 6-OHDA INDUCED PARTIAL NIGROSTRIATAL LESION. F.S.Junn (Kim)*, K.Steece-Collier, & T.J.Collier. Dept. of Neurobiology & Anatomy, University of Rochester, Rochester, NY, 14642.

The short term symptomatic improvement in Parkinson's patients with Sinemet can be very dramatic. However, progressive increase in disability of such patients coupled with toxic byproducts from autooxidation of L-dopa and dopamine suggested the possibility of L-dopa causing further deterioration of the disease. We tested the effects L-dopa injected as Sinemet-25/250 b.i.d., i.p. (50mg L-dopa equivalent/kg) for eight weeks into 12 Fisher 344 rats whose nigrostriatal system was partially lesioned with unilateral intra-nigral injection of 4 ug of 6-hydroxydopamine. Such partially lesioned rats demonstrated rotational response to amphetamine (5mg/kg), but not to apomorphine (0.25mg/kg). Saline injections were given b.i.d. to 11 control rats matched for the amphetamine induced rotation. The L-dopa group showed 30% reduction in amphetamine-induced rotation, whereas the control group showed no change from the baseline (p=0.13). There were no signifcant differences in either the total high affinity DA uptake sites or D1-receptors as measured by GBR and SCH23390 autoradiography. Spiperone D2-receptor showed a decreasing trend in the L-dopa treated group (p=0.15). The trunkal and limb dystonia following L-dopa injection were also measured. No further progression of dystonia was observed throughout the duration of the experiment. Our results thus far suggest that this time course of L-dopa treatment does not appear to cause further decline in the nigrostriatal dopamine system in partially lesioned rats. Supprted by grants from the UPF & NIH NS24032.

451.4

SUBTHALAMIC NUCLEUS ABLATION AS A METHOD FOR REDUCING APOMORPHINE-INDUCED ROTATIONAL BEHAVIOR IN 6-OHDA LESIONED RODENTS B.H. Hallas*, R.M. Cebelenski, P. Jacovina, M.F. Zanakis, E. Fazzini. N.Y. College of Osteopathic Medicine, N.Y. , N.Y. University, N.Y.

A rodent model for Parkinson's Disease is the chemical lesion of the Substantia Nigra (SN) with 6-hydroxydopamine (6-OHDA), which, following apomorphine administration, results in quantifiable rotational behavior. This study investigated the influence of the Subthalamic Nucleus (STN) in animals previously lesioned with 6-OHDA and induced to rotate with apomorphine. Adult rats received stereotaxic injections of 6-OHDA into the left SN. After 21 days, apormorphine was injected subcutaneously once a day for 14 additional days. Ten minutes after the injection, the number of rotations were counted for each animal over a period of 15 minutes. After establishment of the rotational baseline (at 14 days), the left STN was ablated by stereotaxic electrolytic lesions. Control animals received no STN ablation. All animals were then retested for rotational behavior as above after a recovery period of 14 days. It was found that the majority of the experimental animals demonstrated a significant reduction of rotational behavior compared to controls.

However, four animals exhibited ipsilateral rotation following, STN ablation (i.e. to the left). These animals were tested for 14 additional days and then received an electrolytic lesion to the contralateral (right) STN. After a sufficient recuperation time these animals were retested for rotational behavior following apomorphine injection. It was found that these animals then demonstrated a significant decrease in rotational behavior. It can therefore be concluded that ablation of the STN ameliorates apomorphine-induced rotational behavior following 6-OHDA SN lesions possibly by reducing abnormally increased medial globus pallidus output.

451.5

AKINESIA DURING DOPAMINE AGONIST INDUCED CIRCLING BEHAVIOR AFTER SEVERE UNILATERAL NEOSTRIATAL DOPAMINE DEPLETION IN RATS. D. Norton, T. Schallert*, and T.A. Jones. Neuroscience Inst. and Dept. of Psychology, Univ. of Texas, Austin, TX 78712.

Circling behavior after unilateral 6-hydroxydopamine (6-OHDA)-induced neostriatal dopamine (DA) depletion has been widely used as a model for screening anti-parkinsonian agents. However, the unilateral 6-OHDA model, as presently used, fails to mirror one of the most debilitating symptoms of Parkinson's disease: akinesia. During circling, all limbs make stepping movements, including the limbs contralateral to the denervated neostriatum. In this study a novel behavioral test was used to isolate and compare stepping movements of the ipsilateral and contralateral limbs of rats with unilateral neostriatal DA depletion. We found that in the undrugged condition and under amphetamine, the ipsilateral (non-impaired) forelimb shifts the weight of the animal to a new location in space (DA-dependent stepping), whereas the contralateral forelimb makes only catch-up steps designed to re-establish support of the body's weight (DA-independent stepping). When the contralateral forelimb is isolated so that it alone bears the weight of the animal (by raising the hindquarters and ipsilateral forelimb off the ground), it is akinetic. Preliminary results indicate that when the depletion is severe, the DA agonist apomorphine causes contraversive hyperkinesia in the ipsilateral limb, but does not reverse akinesia in the contralateral forelimb. The role of DA in movement initiation will be discussed in view of these data. (Supported by NS 23964.)

451.6

REMACEMIDE AND L-DOPA SYNERGIZE TO REVERSE AKINESIA IN MONOAMINE-DEPLETED RATS. RV Eller, A Howell and J T Greenamyre,* Dept. of Neurology, University of Rochester, and Fisons Pharmaceuticals, Rochester, NY, 14642.

In rodent and primate models of Parkinson's disease, antagonists of the NMDA and AMPA subtypes of glutamate receptors have been shown to synergize with L-DOPA to reverse parkinsonian motor deficits. In this study, we have used monoamine-depleted rats to examine the antiparkinsonian potential of remacemide (Fisons), an anticonvulsant with activity at the NMDA receptor ion channel. Male Sprague-Dawley rats (125-150 g) were rendered akinetic by administration of reserpine (5 mg/kg, i.p.) 24 h prior to testing. Motor activity was quantified using cages equipped with infrared sensor beams. In reserpinized rats, L-DOPA increased horizontal locomotor activity in a dose-dependent fashion. When a subthreshold dose of L-DOPA (75 mg/kg, i.p.) was co-administered with remacemide (5 - 40 mg/kg, p.o.) there was a dose-dependent increase in horizontal locomotor activity. Unlike MK-801, remacemide does not produce locomotor stimulation in normal rats. Moreover, remacemide potentiates the effects of L-DOPA over a broad dose range, in contrast to MK-801 and CPP.

(This work was supported, in part, by the Hope Geoghegan Fund and the Davenport-Hatch Foundation.)

451.7

RECOVERY OF VOLITIONAL MOTOR FUNCTION BUT NOT ROTATIONAL ASYMMETRY IN HEMI-PARKINSONIAN MONKEYS. W.W. McLaughlin*, I.S. Schneider and D.P. Roeltgen, Dept. of Neurology, Hahnemann University, Philadelphia, PA. 19102.

The extent and status of a unilateral lesion of the nigrostriatal dopamine (DA) system has typically been assessed by quantifying rotational asymmetry in response to DA agonist administration. Since agonist-induced rotation, whose mechanisms are still obscure, may not be a good behavioral index of parkinsonism, particularly in monkeys and humans, we examined the extent to which the status of volitional motor function, perhaps a better index of parkinsonism in monkeys, and rotational asymmetry coincide. In particular, we examined whether rotational asymmetry, a methodologically simple behavioral index, could accurately reflect the functional status of hemi-parkinsonian monkeys. Four male rhesus monkeys (approx. 4 yrs. old) were trained to perform tasks which measured bar press rate, reaction/movement times, and lateralized attention (i.e., response to double simultaneous stimulation, response to lateralized moving stimuli, and lateralized reward retrieval). Animals were then made hemi-parkinsonian by intracarotid infusion of MPTP-HCL (2.5 mg/kg) and re-tested on the above-mentioned tasks over the next year. Within the first 6 months after lesioning, animals showed dose-dependent rotational asymmetry to apomorphine or D2 agonist N-0923 stimulation and significant unilateral impairment in motor function (with the use of the limb contralateral to their lesion) and attentional abilities (assessed with the limb ipsilateral to their lesion). Six to 12 months after lesioning, 3 animals showed significant improvement in performance of volitional motor tasks and all monkeys showed distinct reversal of at least some of their initial lateralized attentional deficits. Despite this behavioral improvement, rotational asymmetry in response to dopamine agonists was still intact and in fact, more robust than when the animals were behaviorally impaired. These results suggest that over time, functional improvement can occur in hemi-parkinsonian monkeys and that rotational asymmetry may not be a good index of the functional status of the dopamine-denervated striatum in the monkey. Supported by Whitby Research, Inc. and MH 46531.

451.8

PRETREATMENT WITH A GANGLIOSIDE MIXTURE DOES NOT PREVENT MPTP TOXICITY IN AGING MICE. X. L. Chen*, F. Roisen and M. Gupta, Department of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, KY 40292.

MPTP has been shown to produce a loss of dopaminergic neurons in the substantia nigra (SN) as well as decreased dopamine levels in the striatum. Furthermore, pretreatment of young adult mice with a mixture of bovine brain gangliosides can reduce MPTP toxicity on the dopaminergic neurons of the SN (Gupta et al., Brain Res. 527, 1990). The present studies were undertaken to investigate if aging mice, that are more sensitive to MPTP treatment, respond differently to the ganglioside pretreatment. Aging (12 months old) male C57BL/6 mice were anesthetized and stereotaxically injected with either the ganglioside mixture (Fidia, 200mg/ml in PO_4) or PO_4 alone in each lateral ventricle (2µl each). 16-18 hr later, one half of the ganglioside and vehicle-injected mice received multiple injections of MPTP (total dose 90mg/kg i.p.) over a two day period. Three days later, half of the animals from each group were anesthetized, perfused intracardially with fixative and brain sections processed for tyrosine hydroxylase (TH) immunocytochemistry. The remaining mice were decapitated, fresh brains removed, striata dissected and processed for HPLC to detect the levels of dopamine and its metabolites. The results show that, while MPTP produced a decreased number of TH-positive neurons in the SN and marked depletion of dopamine levels in the striatum, pretreatment with the ganglioside mixture did not prevent the MPTP-induced loss of TH-positive neurons in the SN nor reduction of dopamine levels in the striatum. These data, therefore, demonstrate that aging mice respond differently to pretreatment with gangliosides than young adults. Supported by USPHS grant NS24291 to MG.

451.9

FUNCTIONAL ASSIMETRIES IN MANUAL TESTS IN NORMAL AND LONG TERM MPTP TREATED MONKEYS.EFFECTS OF GM1 GANGLIOSIDE. M.E.Emborg*,S.Lipina,J.Goland,C.Abel, J.A.Colombo PRUNA(CEMIC-CONICET) Av.Galvan 4102, (1431) Bs.As.,Argentina.

GM1 has been reported to protect pigmented neurons of substantia nigra from MPTP lesions.We evaluated behavioral effects of GM1 treatment in 3 *Cebus apella* with a persistent hemiparkinsonian syndrome(Emborg et al 1991) after 20-22 months of an intracarotid infusion of MPTP-HCL(1.2mg//kg). MPTP treated and 3 normal monkeys(2-4kg,13-16 years)were trained in a primate chair to solve motor-cognitive tests.Normal animals learned in 2-3 sesions the 3 levels,with variable manual preference between tests.MPTP monkeys could not resolve level 2 or 3.The hemiparkinsonian monkeys received 20 daily saline injections or GM1(FIDIA, 20mg/kg) in saline for 29 days,being evaluated (C8-9)in a primate chair weekly.After 3 weeks of saline or GM1, circling activity was tested before and after apomorphine administration(0.4mg /kg).During saline or GM1 treatment the animals did not improve their performance and the apo tests did not reveal changes in circling behavior.At present time we are conducting a follow up post GM1 treatment. Supported by: CONICET,CIRHE,CEMIC, Petrolera Argentina S.Jorge.

451.10

U-91356A: A NOVEL DOPAMINE AGONIST WITH POTENTIAL ANTIPARKINSONIAN ACTIVITY. P.J.K.D.Schreur*, N.F.Nichols, and M.P.Stone. CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

U-91356A is a novel dopamine agonist, (*R*)-5-(propylamino)-5,6-dihydro-4*H*-imidazo[4,5,1-*ij*]-quinolin-2(1*H*)-one hydrochloride.

In reserpinized NSA (Harlan CF-1) mice, U-91356A caused locomotor stimulation at 1 and 10 mg/kg i.p. Apomorphine HCl (apo), lisuride H-maleate (lis), (-)-quinpirole HCl (quin), and pergolide mesylate (perg) were also stimulants in reserpinized mice, but bromocriptine mesylate (bromo) was not. In the same reserpinized mice, U-91356A and lis antagonized apo-induced locomotor stimulation; bromo was slightly active while apo and perg were inactive as antagonists. In normal, nonreserpinized B6C3F1 mice (Harlan) all of the above compounds antagonized amphetamine. U-91356A was active orally and s.c. as well as i.p.

In Sprague-Dawley (Charles River) rats with unilateral 6-OH-dopamine lesions of the substantia nigra, U-91356A caused contralateral turning at 0.3 and 3 mg/kg s.c. Quin and perg also caused contralateral turning, but bromo did not cause any turning at doses up to 3 mg/kg.

U-91356A's maximal locomotor stimulant activity was less than that seen for apo, quin, and perg. Indeed, U-91356A actually reversed some of the stimulation caused by apo or amphetamine. This suggests that U-91356A may be a useful antiparkinsonian agent with less propensity to produce dyskinesias than other dopamine agonist agents may have.

451.11

U-91356A, A POTENT, EFFECTIVE, ORALLY ACTIVE D2 AGONIST POTENTIALLY USEFUL IN TREATING PARKINSON'S DISEASE. M.F.Piercey,* M.W.Moon, W.E.Hoffmann, R. Walters, P. Blanchette, and P. Bedard. The Upjohn Co., Kalamazoo, MI. 49001 and Leval University, Quebec, Canada.

U-91356A, (U-91, or (*R*)-5-(propylamino)-5,6- dihydro-4*H*-imidazo[4,5,1-*ij*]-quinolin-2(1*H*)-one hydrochloride) is a novel dopamine (DA) agonist that binds to both D2 (Ki = 1.3 nM) and 5-HT1A (Ki = 58 nM) receptors. U-91 did not bind at 1 uM to a variety of additional adrenergic, 5-HT, cholinergic, DA, benzodiazepine or opiate receptors. Bromocriptine, lisuride, and pergolide all had high binding affinities for several non-DA receptors. U-91 (ED50 = 32 ug/kg i.v.), bromocriptine (ED50 = 1804 ug/kg), pergolide (ED50 = 23 ug/kg) and lisuride (ED50 = 74 ug/kg) all depressed substantia nigra DA neuron firing, but this was complete only with U-91356A. Higher U-91 doses depressed dorsal raphe 5-HT neurons (ED50 = 204 ug/kg). U-91 was orally available in rats (po/iv = 60%) and monkeys (po/iv = 28%). In MPTP-treated monkeys, U-91 successfully reversed parkinsonian symptoms both subcutaneously (30 ug/kg) and orally (1-2 mg/kg, duration 7-9 hrs). The data suggests that U-91's greater selectivity, efficacy and oral availability could provide a novel improved treatment for Parkinson's Disease.

451.12

THE EFFECT OF MPTP ± L-DOPA UPON SOCIAL MEMORY/ RECOGNITION IN MICE. D.E. Dluzen* and J. Kreutzberg, Department of Anatomy, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Retired breeder male CD-1 mice were treated with either MPTP (10 mg/kg ip, q.i.d., x1d, N=10) or saline (N=10) and at 5 days post-treatment tested in a habituation-dishabituation paradigm. Three 2-minute presentations of the same stimulus (ovariectomized) female (habituation) was followed by three 2-minute presentations using a different stimulus female (dishabituation) with an inter-trial interval of 20 minutes. Investigation times ($\bar{x}$ ± SEM in seconds) of saline animals decreased over the habituation trials (103 ± 10, 63 ± 10, 37 ± 9) while those of MPTP animals did not decrease until the third trial (85 ± 11, 85 ± 12, 43 ± 4). A clear dishabituation response from trial 3 to 4 was shown by saline (37 ± 9 to 72 ± 11) but not MPTP (43 ± 4 to 58 ± 9) animals. Treatment of MPTP animals with L-DOPA one hour prior to test (50 mg/kg ip, N=9) resulted in habituation (94 ± 8, 45 ± 8, 28 ± 4) and dishabituation (93 ± 10) scores which were similar to saline-treated animals. These results demonstrate that MPTP treatment disrupted initial habituation and dishabituation responses in male mice while treatment of MPTP animals with L-DOPA restored their responses to that observed in saline animals. Such findings suggest that administration of the dopaminergic neurotoxin MPTP impairs social memory/recognition processes, an effect which can be rectified with exogenous L-DOPA treatment.

451.13

THE TRAINING METHOD AFFECTS PERFORMANCE IN THE "STAIRCASE TEST" FORELIMB REACHING TASK: POSSIBLE IMPLICATIONS FOR SEVERITY OF 6-OHDA LESION EFFECTS. R.J. Mandel* and B.K. Kaspar Dept. of Psych., U. of Illinois, 603 E. Daniel St., Champaign, IL 61820

A new method for assessing the forelimb reaching capabilities of individual forepaws in the rat has recently been described (Montoya et al., *J. Neurosci. Meth.* 36:219-228, 1991). Using this test, Montoya et al., (*Prog. Brain Res.* 82:459-466, 1990) have reported reaching deficits in the forelimb opposite to a unilateral nigrostriatal 6-hydroxydopamine (6-OHDA) lesion. Another group, Gray et al., (*Soc. Neurosci. Abst.* 17:501,1991), using 10 food pellets/well reported milder 6-OHDA-induced deficits than those reported by Montoya et al. who use 2 pellets/well. The present study was undertaken to determine whether the amount of food provided during training is a significant factor in reach training. Two groups of normal rats (n=10) were trained for 5 consecutive days (15 min/day) with either 10 pellets or 2 pellets per well (80 total pellets vs. 16 total pellets) and then the conditions were crossed-over for training on days 6-10. Prior to training, both groups were reduced to >85% of pre-training body weight and maintained at this level by supplemental feeding when necessary. Rats given the opportunity to eat 80 pellets per session learned well and continued to improve performance when switched to 2 pellets/well, eventually consuming 75% of the pellets available. In contrast, the rats initially trained with 2 pellets/well ate few pellets and never consumed more than 5% of the pellets even though they had access to 80 pellets on days 6-10. In addition, the group which originally had access to 80 pellets was able to maintain >85% body weight with little supplementation on days 1-5. This was not the case for the rats trained with 2 pellets/well: they received constant dietary supplements in order to remain above 85% body weight. Thus, the rats initially trained with access to 80 pellets could work to maintain their body weight while the other group could not. Thus, it appears that the *a priori* motivational level of rats affects the degree of post-6-OHDA lesion deficit as indicated by the different levels of post-lesion performance reported by Gray et al., as compared with Montoya et al., (1990).

451.14

HIGH RESOLUTION PET STUDIES OF GLUCOSE METABOLISM IN PARKINSON'S DISEASE. J.L. Eberling*, B.C. Richardson, B.R. Reed, W.J. Jagust. Center for Functional Imaging, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720, and University of California, Davis, CA 95616.

There have been several reports of decreased regional cerebral metabolic rates for glucose (rCMRglc) in Parkinson's disease (PD), although others find no differences between PD patients and controls. Differences in the cognitive status of the PD patients may account for some of these inconsistencies. We report the results of a PET study using ^{18}F-fluorodeoxyglucose (FDG) to measure rCMRglc in 8 nondemented PD patients and 8 age-matched control subjects. Three contiguous tomographic levels through the temporal lobes were scanned, along with one tomographic level through the basal ganglia that included frontal and parietal cortex. We used previously determined rate constants and an operational equation to determine rCMRglc, and these values were averaged over the three tomographic levels for temporal lobe structures.

Repeated measure analyses of variance revealed significant ($p<0.05$) main effects for both tomographic levels, with rCMRglc values averaging 23% below control values for all regions studied. For the temporal lobe level, there was also a significant ($p=0.0001$) group x region interaction, indicating that the pattern of rCMRglc differed between the two groups. The group x region interaction was not significant ($p=0.5$) for the basal ganglia level. A series of Bonferroni corrected t-tests revealed that the PD patients had significantly lower rCMRglc than controls in right and left visual association areas, right primary visual cortex, and right and left parietal cortex. While these differences could reflect motor impairment, the posterior location of these deficits has often been associated with cognitive dysfunction and may predict future cognitive impairment.

451.15

VISION IN PARKINSON'S DISEASE: SEVERAL BASIC CAPACITIES ARE UNIMPAIRED. D.K. Kurylo*, S. Corkin, and J.H. Growdon. Dept. of Brain and Cognitive Sciences and the Clinical Research Center, MIT, Cambridge, MA 02139.

Parkinson's disease (PD) is characterized by a loss of dopaminergic (DA) neurons in the nigrostriatal pathway. PD patients also have decreased retinal DA levels (Harnois and DiPaolo, 1990), which may interfere with early visual processing. Consistent with this idea, the pattern electroretinogram and spatial and temporal contrast sensitivity are abnormal in PD. In order to explore the generality of these findings, we administered a series of visual psychophysical tests to 10 nondemented PD patients (mean age: 64.9 years) and 29 age-matched control subjects (mean age: 67.9 years). Based upon the Hoehn and Yahr stages of disability, 1 patient was classified as Stage I, 8 as Stage II, and 1 as Stage III. All PD patients were receiving DA medications. Psychophysical measurements were made of fine pattern discrimination, color discrimination along the red-green and blue-red primary color axes, and contrast sensitivity to flickering (3, 7.5, and 15 Hz) and moving (3.15, 4.72, and 9.44 °/s) stimuli. In all cases, stimuli were presented within 1.0 X 1.0° squares at a retinal eccentricity of 7.5°. Subjects responded in a four-position forced-choice procedure, and thresholds were determined with the method of constant stimuli. Under these conditions, the performance of PD patients did not differ significantly from age-matched control subjects on any of the visual capacities tested. It appears that some visual capacities are spared in PD, at least in patients who are taking DA medication.

451.16

THE EFFECTS OF GM_1 GANGLIOSIDE, EGF, AND bFGF ON THE NIGROSTRIATAL DOPAMINE SYSTEM IN THE MPTP-TREATED MOUSE. L. DiStefano*, M.G. Smith and J.S. Schneider. Department of Neurology, Hahnemann University, Philadelphia, PA 19102.

GM_1 ganglioside, epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) have all been suggested to exert trophic influences on the nigrostriatal dopamine (DA) system and to stimulate at least partial restoration of striatal DA levels following injury to the nigrostriatal system. The present study has compared the ability of these substances, administered alone or in combination, to facilitate recovery in the DA system after an MPTP-induced lesion. Young (8-10 wk. old) male C57/Bl6J mice received MPTP injections (30mg/kg, i.p.) daily for 5 days. Three days later, they were implanted with Alzet osmotic minipumps to infuse EGF, bFGF, NGF or cytochrome C into the lateral ventricle at a rate of 2-5µg/wk over a 2 wk. period. In addition to EGF or bFGF infusions, some animals also received daily GM_1 injections (30mg/kg, i.p., daily) for 2 wks. Other animals received MPTP for 5 days and either daily GM_1 (30mg/kg, i.p.) or saline injections for 2 wks. GM_1 and EGF alone increased striatal DA levels approximately 25% after 2 wks. of treatment, while bFGF increased striatal DA levels by 18% over the same period. Cytochrome C and NGF had no effect on striatal DA levels. EGF and bFGF, when combined with GM_1, increased striatal DA levels by 38% and 30% respectively, suggesting a synergistic effect of GM_1 and these factors on striatal neurochemistry. MPTP treatment alone caused a mean 38% loss of tyrosine hydroxylase-positive substantia nigra pars compacta (SNc) neurons. MPTP-treated animals that received GM_1, EGF, or GM_1 plus EGF all had SNc cell counts significantly higher than MPTP control animals although there was no synergistic effect of GM_1 plus EGF on this parameter. Cell counts in GM_1-treated animals were higher than in animals that only received EGF. These results suggest that GM_1 ganglioside probably works in concert with other trophic factors to both enhance dopamine neurochemistry and neuronal survival following damage to the dopamine system. Supported by Fidia Pharmaceutical Corp.

NEUROMUSCULAR DISEASES I

452.1

SODIUM CHANNEL MUTATIONS IN NEUROMUSCULAR DISEASE. A.I.McClatchey, R.H.Brown J.F.Gusella* and collaborators. Neurogenetics Lab. and Day Neuromuscular Research, Mass. Gen. Hosp. and Harvard Med. Sch., Charlestown, MA 02129.

The human skeletal muscle sodium channel gene (SCN4A) has been linked to both paramyotonia congenita (PMC) and hyperkalemic periodic paralysis (HPP), two neuromuscular disorders for which an abnormal, tetrodotoxin-sensitive sodium current can be detected in affected muscle. As a prelude toward understanding sodium channel malfunction in these diseases, we have examined the SCN4A locus in more detail. By completing the entire genomic structure of the SCN4A gene and obtaining knowledge of intron sequences flanking each exon, we have developed PCR-based assays for the detection of SCN4A mutations. We initially detected two point mutations in affected members of PMC families. Both are predicted to affect amino acid alterations in the cytoplasmic loop connecting domains III and IV of the sodium channel, a region thought to be crucial for channel inactivation.

We have now detected two new mutations in different regions of the sodium channel. One is present in affected members of a separate PMC family, and the other in affected members of a family whose clinical features resemble both PMC and HPP. With the disclosure of these and other mutations in the SCN4A gene, we may be able to catalogue disease phenotypes and correlate them with different affected regions of the sodium channel.

452.2

SEVERE ADULT-ONSET NEUROMUSCULAR DISORDER IN A TRANSGENIC LINE OF MICE. B. Popko[1], D. Kelly[1], A. Miletovich[3], U. Francke[3,4], and K. Suzuki*[2]. [1]Dept. of Biochemistry, [2]Dept. of Pathology, Brain and Development Research Center, U. of North Carolina, Chapel Hill, NC 27599. [3]Dept. of Genetics and [4]Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA 94305

We have generated a transgenic line of mice in which the transgene has disrupted the function of an endogenous gene essential for normal muscle function. Mice homozygous for the BPFD transgene in our transgenic line BPFD#36 develop a postural abnormality at around three months of age. These mice walk oddly with stiffened and/or splayed hind legs, adopting a hunched posture with some exhibiting frank kyphosis of the thoracic spine. These symptoms progress gradually to severe motor dysfunction. Heterozygous animals from this line, as well as heterozygous and homozygous animals from all other BPFD transgenic lines, do not express this abnormal phenotype. This suggests that the BPFD#36 transgene has disrupted the function of an endogenous gene. The transgene integration site was mapped by fluorescent in situ hybridization to chromosome 11 bands B4-B5. Pathology was found in skeletal muscle and peripheral nerve of the mutant animals. The muscles from both upper and lower extremities show necrosis and regeneration with muscle fiber splitting, internally-located nuclei and variable fiber size. Schwann cell - axon interactions also appear disrupted. Although many axons are well myelinated in these mice, there are also bundles of bare axons that are partially encircled by a single Schwann cell. Within these bundles there are axons large enough to be myelinated. These results suggest that the muscle degeneration that occurs in BPFD#36 animals may be secondary to a defect in Schwann cell - axon interactions.

452.3

TERMINAL MOTOR AXON MORPHOLOGY IN HOMOZYGOUS HEREDITARY CANINE SPINAL MUSCULAR ATROPHY. K. Alderson and L.C. Cork*, Department of Neurology, University of Utah, Salt Lake City, Utah and Division of Comparative Medicine, Johns Hopkins University, Baltimore, Maryland

Dogs homozygous for hereditary canine spinal muscular atrophy develop rapid progressive muscle weakness. We evaluated the morphology and myelination of distal motor nerves in the tail base paraspinal muscle of homozygous pups at three stages, early, middle, and late, to determine if muscle weakness correlated with abnormalities of distal motor axons. Compared to age-matched normal dogs, early affected pups had increased unmyelinated axonal collaterals and increased terminal innervation ratio. Mid-stage pups had thin, unmyelinated axonal collaterals, and evidence of Wallerian degeneration in intramuscular nerves. End stage pups (9 weeks of age) had a paucity of motor axons, an irregular distribution of neurofilaments within the axon, and aberrant growth cones. We conclude that as hereditary canine spinal muscular atrophy progresses, intramuscular motor axons are lost, thin unmyelinated axonal collaterals develop, and the organization of neurofilaments within the axon is altered. Both the loss of motor axons and the lack of myelin may have clinical and electrophysiological consequences.

452.4

MOTOR UNIT PROPERTIES IN HEREDITARY CANINE SPINAL MUSCULAR ATROPHY. M.J. Pinter*, L.C. Cork and N. Wallace. Dept. of Anatomy and Neurobiology, Medical College of Pennsylvania and Div. of Comparative Medicine, Johns Hopkins University School of Medicine.

Hereditary canine spinal muscular atrophy (HCSMA) is an autosomal dominant disease which is characterized by progressive weakness and eventual loss of lower motoneurons. Using intracellular recording and stimulation of single motoneurons, we have studied medial gastrocnemius (MG) motor unit properties in a litter of HCSMA pups aged 10-13 weeks. In units investigated thusfar, motoneurons were found to respond normally to low frequency (1 Hz.) antidromic stimulation of peripheral axons and to possess electrical properties similar to adult cat motoneurons. In heterozygous pups (4), the motor unit population was composed of types FR and S units with no evidence for the existence of type FF units. We have not yet confirmed this motor unit composition in normal animals, but the predominance of types FR and S units is consistent with available enzyme histochemistry evidence for normal adult canine MG muscle. In 2 homozygous pups studied thusfar, two populations of units were detected. In the first, intracellular stimulation of motoneurons failed to produce detectable motor unit force despite the presence of antidromic motoneuron activation following peripheral MG nerve stimulation. This failure occurred in 30-50% of the tested motoneuron population and was associated with an inability to detect EMG responses linked to the intracellular stimulation. In the other population, stimulation produced measurable motor unit mechanical responses best characterized as types FR and S but with force outputs lower than in the heterozygous group.

452.5

ALTERATIONS IN NEUROFILAMENT mRNA IN HEREDITARY CANINE SPINAL MUSCULAR ATROPHY. N.A. Muma* and L.C. Cork. The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Hereditary canine spinal muscular atrophy (HCSMA) is a dominantly inherited motor neuron disease in which distal axonal caliber is reduced in lower motor neurons. Because several animal models show that neurofilament protein gene expression is a major determinant of axonal caliber, we examined neurofilament gene expression in HCSMA early in the clinical disease. Using quantitative in situ hybridization we found that the levels of mRNA encoding the low molecular weight neurofilament protein subunit (NF-L) were significantly different from levels of mRNA encoding the high molecular weight neurofilament protein subunit (NF-H) and poly-A+ mRNA in cervical spinal cord enlargement of dogs with HCSMA compared to control dogs. The levels of poly-A+ mRNA in neurons in the same region were comparable in dogs with HCSMA and controls. If neurofilament protein subunit levels are found to follow the mRNA levels in this animal model, our results would suggest that decreased expression of the NF-L gene is sufficient to inhibit neurofilament function, i.e., maintenance of axonal caliber, probably by disrupting normal neurofilament assembly.

452.6

LOCALIZATION OF DYSTROPHIN IN HUMAN AND MONKEY BRAIN. Johnny Huard, Pierre-Yves Côté, Jean-Pierre Bouchard, Carol L. Richards* and Jacques P. Tremblay. Neurobiology Laboratory, Enfant-Jésus Hospital, 1401, 18e Street, Québec (Qc), G1J 1Z4

Duchenne muscular dystrophy (DMD) has been characterized by a lack of dystrophin expression. This protein (420 kDa) is localized in the sarcolemma of normal muscle fibers and is absent of DMD and mdx skeletal muscle. Dystrophin would be involved in maintaining muscle membrane integrity during repeated cycles of contraction and relaxation. Molecular biology techniques have also permitted to detect dystrophin transcript in the central nervous system (CNS). In fact 50% of DMD patients suffer also a mental retardation probably due to learning and memory problems. Dystrophin was localized in several brain regions of human and monkey brains using antibodies against the rod domain and C terminus region. A clear visualisation of dystrophin was permitted by an indirect immunoperoxidase method using an amplification with biotin and avidin. Dystrophin-like immunoreactivity was observed in brain region involved in learning and memory such as hippocampal formation, cerebral cortex and the dentate nucleus of cerebellum. A positive reaction was also present in brain regions implicated in motor function (spinal cord, cerebellum, thalamus and substantia nigra). The Western blot analysis confirmed the presence of a 420 kDa polypeptide in these brain regions with both antidystrophin antibodies. However the rod domain antibody also detected a dystrophin-like protein. These results raise the possibility that the lack of dystrophin is involved in the cognitive impairment observed in several DMD patients. However it will be important to analyze in more details the clinical symptoms of DMD patient to understand the dystrophin role in brain.

452.7

DYSTROPHIN DISTRIBUTION IN MUSCLE AND NEURONS OF DUCHENNE MUSCULAR DYSTROPHY HUMAN FETUSES. **I.Mussini, S. Torelli, M.A. Marzilli, A. Jaranowska and F. Gremo*. C.N.R. Unit for Muscle Biol.& Physiopath., Padova; Dept. of Cytomorphol., Med.School, Cagliari (Italy).**

Duchenne Muscular Dystrophy (DMD) is the result of the deficiency of a specific cytoskeletal protein called dystrophin, which binds to the inner plasma membrane. We have previously shown that dystrophin can be present in DMD human fetal neurons in culture. Therefore, we analysed the distribution of dystrophin and spectrins in muscle and neurons of two DMD human fetuses (12-14 week-old) with immunocytochemistry. Different muscles were frozen and serial sections (10 μm thick) were incubated with monoclonal antibodies against β-spectrin, the C-terminal portion of dystrophin and different isoforms of myosin heavy chains (MHC). Neurons were grown in culture as previously described (Torelli et al., in "Plasticity and Regeneration of the Nervous System", P.S. Timiras et al. Eds, Plenum Press, New York, pp. 121-134, 1991), fixed with formaldehyde and incubated with the two first antibodies. Results showed that all myotubes and the few immature myofibers were positive for β-spectrin, but negative for dystrophin. On the contrary, some neurons were positive for dystrophin and all showed an abnormal distribution of spectrin. Noteworthy was the presence of adult slow MCH in some young myofibers and in myotubes. These results help in understanding the mechanism of expression of different genes, including the DMD gene, during human development. (Supported by Telethon-Italy, 1991).

452.8

A 120 K HIGH AFFINITY LAMININ BINDING PROTEIN IN BRAIN IS CLOSELY RELATED TO THE DYSTROPHIN-ASSOCIATED GLYCOPROTEIN DYSTROGLYCAN. S.Gee[1], R. Blacher[1], P. Douville[1], P. Yurchenco[2] and S.Carbonetto[1]*, [1]Centre for Research in Neurosciences, McGill University, Montreal General Hospital Research Institute, Montreal, Canada; Department of Biochemistry, [2]Athena Neurosciences, San Francisco, California; [3]Robert Wood Johnson Medical School, Piscataway, New Jersey.

Laminin is a large extracellular matrix glycoprotein that mediates its effects on cells through integrin and non-integrin receptors. When brain proteins separated by SDS-PAGE and transferred to nitrocellulose are probed with ^{125}I-labelled laminin, a single prominent band of ~120 kDa binds laminin specifically (Smalheiser et al., 1987, PNAS, 84:6457-61; Douville et al., 1988, JBC, 263:14964-9). This band consists of two distinct laminin binding proteins. One of these, LBP120, is closely related to dystroglycan, a muscle laminin receptor of 156 kDa that is associated with dystrophin through a cell surface glycoprotein complex. The glycosylation, extracellular localization, and ability to extract LBP120 with cation chelators or high salt are consistent with it being a peripheral membrane protein. Laminin binding to LBP120 is of high affinity (Kd= 40 nM), is Ca^{++} dependent, and is mediated primarily by the heparin binding domain E3. Dystroglycan is reduced or absent in dystrophic muscle; alterations in brain LBP120/dystroglycan may be involved in the neural deficits associated with Duchenne's muscular dystrophy.

452.9

RETARDATION OF FAST AXONAL TRANSPORT IN WOBBLER MICE. H. Mitsumoto, J.M. Jacob, K. Kurahashi, and I.G. McQuarrie*. Cleveland VA Med. Ctr., and Cleveland Clinic Foundation., Cleve., Oh. 44106.

To investigate axonal function in a subclinical model of motor neuron disease, we have examined anterograde axonal transport in hindlimb motor neurons of wobbler mice. For fast transport, tritiated amino acids were injected into the spinal cord. Transport distances were determined from the distribution of labeling after 2 or 3 hr; fast transport was 18% slower in wobbler mice than in unaffected littermates ($p<0.01$). For slow transport, ^{35}[S]-methionine was injected. Transport rates were determined from distances to the peaks of labeling for structural proteins separated by SDS-PAGE after 2 or 3 wks. Rates for slow components a (SCa) and b (SCb) were unchanged by wobbler disease. Because the rate of fast retrograde transport is also unchanged (Mitsumoto et al., 1990), we conclude that subclinical disease is associated with an impairment of fast anterograde transport. Because disruption of the Golgi apparatus by the neuronopathy is unlikely to affect the fast transport rate, we suggest that an axonopathy may be affecting the fast transport motor (kinesin).

Supported by an NINDS Young Investigator Award to HM (NS-21724) and a VA Career Development Award to IGM.

452.10

QUANTITATIVE EVALUATIONS OF MUSCLE STRENGTH IN WOBBLER MICE: IN VIVO AND IN VITRO STUDIES. **K. Ikeda, H. Mitsumoto*, Dept. of Neurology, Cleveland Clinic Foundation, Cleveland, OH 44195**

This study is to establish the techniques to quantitatively measure muscle strength in wobbler mice, since objective techniques are limited in the animal model. Seven to 9 week-old wobbler mice and their healthy littermates were studied. 1) In vivo electric nerve stimulation test (ENST): Under halothane anesthesia, the right radial, medial and ular nerves were cut at the level of the humerus head and musculocutaneous nerve was stimulated. Maximum muscle contractions of the biceps were measured by a force displacement transducer. 2) In vitro muscle twitching test (MTT) : The left biceps and triceps muscles were removed with both end of the humerus. The one end was fixed to the tissue bath, while the other was attached to the transducer. The first 5 muscle twitches at the supramaximal electric stimulations were recorded. 1) ENST (mean ± SD g) was 3.55 ± 0.53 and 10.24 ± 0.92 in wobbler mice (n=9) and controls (n=9) respectively ($p<0.001$). Muscle-weight adjusted ENST responses were also significantly different between the two groups ($p<0.01$). 2) MTT (mean ± SD) was 1.53 ± 0.25 and 4.92 ± 0.36 in wobbler mice (n=6) and controls (n=8) respectively ($p<0.001$). MTT responses normalized for muscle weight were also significantly different between the both groups ($p<0.001$). These two techniques we have established appear to be useful to evaluate the clinical effects of novel neurotrophic factors which be tested first in the animal model.

452.11

Excitatory Amino Acid Receptors in the Cervical Spinal Cord of the Wobbler Mouse: A Quantitative Autoradiographic Analysis. **C. Krieger*[1], R. Lai[1], H. Mitsumoto[2] and C. Shaw[3], [1]Dept. of Medicine and [3]Opthalmology, University of British Columbia, Vancouver, B.C. and [2]Cleveland Clinic Foundation, Cleveland, OH.**

Previous studies have suggested that motoneuron death might develop as a consequence of an excitatory neurotoxic compound with agonist action at certain excitatory amino acid (EAA) receptor subtypes on motoneurons. To investigate the possibility that a selective reduction of EAA receptor subtypes might be observed as a result of motoneuron death subsequent to EAA receptor activation, we measured the distribution and density of EAA receptors in the cervical spinal cord of the wobbler mouse using quantitative receptor autoradiography. Homozygous wobbler mice develop progressive motoneuron loss, especially in the cervical spinal cord and brain stem. NMDA receptors labelled with 25 nM [^{3}H] MK801 were located in both the ventral and dorsal horns and displayed little regional variation in binding. AMPA binding sites, evaluated with 20nM [^{3}H] CNQX, were more concentrated in the dorsal horn than the ventral horn. Kainate binding sites were labelled with 30 nM [^{3}H] kainate, and receptor binding was located largely in the dorsal horn. [^{3}H] Glutamate binding sites (35nM) were most abundant in the dorsal horn of the spinal cord. NMDA, kainate, glutamate and AMPA receptor binding densities were not significantly different in the ventral horns of wobbler mice compared to those of control litter mates. These results suggest that motoneuron loss in the wobbler mouse is not mediated by excitotoxins acting on EAA receptors.

452.12

EARLY AND SELECTIVE IMPAIRMENT IN THE FAST ANTEROGRADE TRANSPORT (FAT) RATE OF GLYCOPROTEINS IN ACRYLAMIDE (AC) NEUROPATHY. T. Storm-Dickerson, P.S. Spencer*^ and B.G. Gold#. Center Res. Occup. and Environ. Toxicol. and #Depts. of Cell Biol. & Anat. and ^Neurol., Oregon Health Sci. Univ., Portland, OR 97201

Distal axonal degeneration (dying back) is the most common pattern of axonal pathology observed in a variety of human disorders. We examined FAT of proteins and glycoproteins in AC neuropathy, a prototypical model of progressive axonal degeneration. Six-week-old male rats given a single injection of AC (5-100 mg/kg, i.p.) 20 minutes before or after injection of ^{3}H-leucine or ^{3}H-fucose, respectively, into the L5 dorsal root ganglion (DRG) demonstrated no alteration in the maximal transport rate (front of the curve) for radiolabeled proteins, whereas the glycoprotein FAT rate was reduced (by 18%) compared to age-matched controls; data was normalized to the radioactivity present in the DRG. A defect in FAT of glycoproteins was noted up to 49 days following a single 100 mg/kg dose, but was reversible (beginning as early as 7 days) following a lower dose (50 mg/kg). Repeated injections of AC (30 mg/kg/day, for 7 days) did not exacerbate the degree of glycoprotein transport impairment. No alteration in the amount of transported materials was found. A correletion was observed between the degree of FAT deficit and axonal degeneration; following pretreatment with β,β'-iminodipropionitrile (which exacerbates axonal loss by AC), AC (75 mg/kg) elicited a marked impairment in both protein and glycoprotein FAT rate. We suggest that repeated, low dose AC exposure leads to an irreversible impairment in the delivery of FAT glycoproteins to the distal axon resulting, over time, in axonal degeneration. Supported by NS19611.

452.13

A STUDY OF THE ADVERSE EFFECTS OF 5-HT PRECURSOR-TREATMENTS RELATED TO EMS. Moto Sakuma,* Moto R.R.C. Co.,Detroit Mi 48224.

A comparison was made of the adverse effects of seven drug treatments which used 5-HT precursors. The adverse effects were collected from others' publications. The underlying cause of Eosinophillia-Myalgia Syndrome (EMS) was sought. Neuroleptic Malignant Syndrome(NMS) was added to these clusters for comparison between EMS and NMS. All clusters were subclassified into eight signs(general, skin, muscle, visceral, neurolgical, psychiatric, blood physiological, and chemical analysis). The appearances of these signs were expressed numerically, and histgram were drawn to compare the pattern. The similarity of those patterns resulted in two separate group: 1. TRP(tryptophan)group -(TRP alone treatment, TRP plus Monoamine Oxydase, TRP plus tricycle antidepressant), 2. 5-HTP (5-hydroxy tryptophan) group-(5-HTP alone treatment,and TRP plus co-enzyme). The TRP alone treatment's effects showed little resemblance of pattern to that of EMS. In conclusion, EMS may be caused by combined influences involving by thr TRP toxicity with conditions of genetic background and/or underlying metabolic abnormality as the predisposition.

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SYMPOSIUM. MOLECULAR ANALYSES OF NEURONAL PHYSIOLOGY AND POTENTIAL FOR GENE THERAPY USING HERPES SIMPLEX VIRUS VECTORS. A.I. Geller, Children's Hospital (Chairperson); R.L. Neve, McLean Hospital; M.J. During, Yale University School of Medicine; K. O'Malley, Washington University School of Medicine; and H.J. Federoff, Albert Einstein College of Medicine.

The development of a defective Herpes Simplex Virus (HSV-1) Vector System that allows gene transfer directly into neurons and glia, in culture or in the adult mammalian brain, may facilitate a molecular genetic analysis of neuronal physiology and brain function, and may provide the means to gene therapy of neurological diseases. The symposium will i) describe the properties of the HSV-1 vector system, ii) describe a molecular genetic approach to neuronal physiology and brain function that is based on the introduction of unregulated signal transduction enzymes into neurons, and iii) propose gene therapy approaches to both Parkinson's and Alzheimer's Diseases that involve in vivo expression of tyrosine hydroxylase and nerve growth factor, respectively. The presentations will be: (1) Dr. Geller will detail the properties of the HSV-1 vector system, and will describe experimental strategies using the vector system. (2) Dr. Neve will report on insights gained into the role of signal transduction pathways in controlling neuronal physiology, and neurotransmitter release in particular, utilizing a genetic intervention strategy in which unregulated signal transduction enzymes (eg. adenylate cyclase, protein kinase C) are introduced into cultured neurons. (3) Dr. During will report on introducing these same unregulated signal transduction enzymes into the adult brain and their effects on animal behavior. For example, introduction of an unregulated PKC, under the control of the tyrosine hydroxylase promoter, into the substantia nigra of unlesioned rats causes a long-term asymmetrical rotational behavior. (4) Dr. O'Malley will describe efforts to develop gene therapy for Parkinson's Disease. Recombinant tyrosine hydroxylase can be functionally expressed in cultured striatal neurons; and introduction of this gene into the striatum of 6-OH dopamine lesioned rats results in behavioral recovery. (5) Dr. Federoff will describe efforts towards gene therapy by expression of neurotrophic factors. Expression of NGF from a HSV-1 vector supports the survival of cultured sympathetic neurons and induces choline acetyltransferase activity in cultured CNS neurons. Introduction of recombinant NGF into the superior cervical ganglia of adult rats can prevent specific effects of axotomy; and introduction of NGF into basal forebrain cells is under study.

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SYMPOSIUM: THALAMIC MECHANISMS OF NOCICEPTION.
F.A.Lenz*, Johns Hopkins (Chairman); E.G.Jones, UC Irvine; A.V.Apkarian, SUNY Syracuse, M.C.Bushnell, Univ. Montreal

The principal sensory nucleus and adjacent nuclei of primate thalamus comprise a region that is involved in pain-signalling pathways. Evidence will be presented that there are two separate anatomical domains within this region (rod and matrix domains), and that the spinothalamic tract (STT) terminates selectively within the matrix domain. Inputs from STT may explain the activity of neurons in this region which respond to both innocuous and noxious stimuli (WDR neurons). These neurons may mediate pain discrimination since: 1) they encode painful stimuli well enough to explain the psychophysics of pain discrimination 2) stimulation at recording sites for these neurons in man may evoke somatic or visceral pain, as well as innocuous thermal sensations and 3) injection of local anesthetic in this region blocks discrimination of both innocuous and noxious thermal stimuli. These findings suggest that WDR neurons in the region signal acute pain.

Following injuries to the nervous system there is evidence of anatomic reorganization of the rod and matrix domains. Patients with pain following nervous system injury exhibit physiologic reorganization and increased calcium spike associated bursting activity in the region. These changes in neuronal activity may be related to NMDA associated channels that are activated by noxious stimuli. The convergence of innocuous and noxious inputs in the region may be related to the pain evoked by innocuous stimuli which occurs in many patients following nervous system injury. Therefore synaptic and intrinsic cellular mechanisms governing thalamic neuronal activity may mediate acute somatic and visceral pain as well as symptoms of pain syndromes observed following injuries to the nervous system.

NEUROENDOCRINE REGULATION II

456.1

DISTRIBUTION OF ESTROGEN RECEPTOR-CONTAINING NEURONS IN PREPUBERTAL FERRETS IN THE PRESENCE AND ABSENCE OF GONADAL STEROIDS. C.L. Sisk* and L.L. DonCarlos. Neuroscience Prog./Dept. Psychol., Michigan State Univ., E. Lansing MI 48824, & Dept. Cell Biol., Neurobiol. & Anatomy, Loyola Univ. Sch. Medicine, Maywood IL 60153.

Puberty in male ferrets is marked by profound increases in gonadotropin secretion and the onset of copulatory behavior. Responsiveness to negative feedback effects of testosterone (T) on gonadotropin release decreases during puberty, while responsiveness to behavioral effects of T increases. The neuroendocrine and behavioral effects of T are in part mediated by aromatization of T to estradiol (E). One way in which pubertal shifts in brain responsiveness to T might occur is via alterations in steroid receptor content. As a first step in examining this question, we asked 1) What is the distribution of estrogen receptors in the prepubertal male ferret? 2) Is estrogen receptor content altered by E or T in these animals? The H222 antibody (gift of Abbott Laboratories) was used to detect estrogen receptor immunoreactivity (ER-IR) in the brains of prepubertal male ferrets that were castrated and treated with vehicle, E, or T. ER-IR cells were found in the preoptic area, amygdala, and in the ventromedial (VMH) and arcuate nuclei of the hypothalamus, all areas known to be important in reproductive function. Overall, the staining intensity and number of ER-IR cells was decreased by E and by T, compared with controls. However, a dorsal-ventral column of cells in the lateral VMH was consistently more darkly stained and contained more cells in steroid-treated ferrets compared with controls. Thus, steroid hormones may differentially regulate steroid receptor content in specific cell groups. This suggests a mechanism by which responsiveness to T changes in opposite directions during puberty in neural substrates underlying neuroendocrine versus behavioral functions. Supported by HD26483, HD00950, and the Potts Foundation.

456.2

ESTROGEN RECEPTOR mRNA IN THE BRAIN OF FEMALE RATS DURING PREGNANCY. C.K. Wagner* and J.I. Morrell. Institute of Animal Behavior, Rutgers University, Newark, NJ 07102

In vitro estrogen receptor (ER) binding studies have demonstrated regionally- and temporally-specific changes in ER levels in the brain over the course of pregnancy (Physiol. Behav. 50:1263, 1991). In this study, in situ hybridization was used to examine steady state levels of ER mRNA in the brain of pregnant female rats. Females were time-mated and sacrificed on day 8, 16 or 22 of pregnancy or on postpartum day 1. ER mRNA was detected by hybridization to an 850 base ^{35}S-labelled cRNA probe, complementary to the mRNA encoding the steroid binding domain of the 3' untranslated region (rat cDNA - M. Muramatsu; 1 x 10^6 cpm/slide). Hybridization took place at 60°C for 20hrs; autoradiographic exposure: 2-6 wks. Many heavily labelled cells were found in the periventricular and medial preoptic area. Labelled cells were also found in the septum, bed nucleus of the stria terminalis, arcuate, ventromedial nucleus, and medial and cortical nuclei of the amygdala. The regulation of ER during pregnancy may alter neural sensitivity to estrogen in a regionally-specific and time-dependent manner to induce behaviors and endocrine events that are unique to this reproductive state. (HD 22983 to J.I.M.)

456.3

ESTROGEN RECEPTOR mRNA IN THE BRAIN OF DOMINANT AND SUBORDINATE MALE MICE FOLLOWING SOCIAL CONFLICT. C.A. Lisciotto*. Institute of Animal Behavior, Rutgers University, Newark, NJ 07102.

Aggressive encounters produce behavioral, neurochemical, and endocrinological changes in both dominant and subordinate animals. In situ hybridization was used to determine the location and relative amount of estrogen receptor (ER) mRNA in the brains of adult male CFW mice following aggressive experience. Aggressive encounters took place in the home cage, using a resident-intruder paradigm. Mice were given 5 bouts of fighting experience and were sacrificed 4 hours after the last fight. ER mRNA was detected with an 850 base ^{35}S-labeled cRNA probe. Hybridization (1 X 10^6 cpm/slide) took place at 60°C for 20 hours; autoradiograms were exposed for 4 weeks. ER mRNA-containing cells were abundant in the medial preoptic area (MPOA), bed nucleus of the stria terminalis, medial amygdala (MA), and ventromedial and arcuate nuclei. Dominant males had fewer ER mRNA-containing cells in the MA compared to subordinate males. Moreover, in the MPOA of dominant males there was a significant positive correlation between the relative amount of ER mRNA per cell and attack frequency ($r=0.89$). Aggressive behavior of CFW mice is mediated by estrogenic metabolites of testosterone. By altering ER mRNA content, aggressive experience may alter sensitivity to estrogen within brain regions important for aggressive behavior.

456.4

HORMONAL REGULATION OF TYROSINE HYDROXYLASE AND OPIOID PEPTIDE GENE EXPRESSION IN THE HYPOTHALAMUS. R. B. Simerly*. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

The anteroventral periventricular nucleus of the hypothalamus (AVPv) is thought to play a critical role in mediating hormonal feedback on gonadotropin secretion. Recently we reported that the AVPv contains sexually dimorphic populations of neurons that express tyrosine hydroxylase (TH), proenkephalin (PENK) or prodynorphin (PDYN), and each displays unique patterns of mRNA regulation in response to sex steroids (see Simerly, Mol Brain Res. 6:297; Mol. Cell. Neurosci. 2:473). In the present study, in situ hybridization was used to evaluate TH, PENK and PDYN gene expression in the AVPv during the estrous cycle, and in response to progesterone treatment. Levels of TH mRNA within the AVPv are inversely correlated with circulating levels of estradiol with the greatest number of cells detected in female rats sacrificed on the morning of diestrus and the fewest in animals killed on the morning of proestrus. Treatment of estrogen-primed ovariectomized female rats with progesterone for either 3 or 24 hours produced no detectable changes in the number of TH mRNA-containing neurons in the AVPv relative to controls. In contrast, PENK expression remains relatively constant across the estrous cycle and, like TH, levels of PENK mRNA are not altered by treatment with progesterone. Finally, levels of PDYN mRNA were reduced slightly on the afternoon of proestrus, but increased on the day of estrus suggesting that the high levels of progesterone that occur during this period may induce PDYN gene expression. Double in situ hybridization studies are underway to determine if these different patterns of hormonal regulation correlate with the differential expression of estrogen and progesterone receptors within TH, PENK and PDYN mRNA-containing neurons in the AVPv. Supported by NIH grant NS-26723.

456.5

OVARIAN STEROID HORMONES REGULATE GLUTAMIC ACID DECARBOXYLASE (GAD) MESSENGER RNA LEVELS IN THE HIPPOCAMPUS OF THE RAT. N.G. Weiland*, Laboratory for Neuroendocrinology, Rockefeller University, New York, NY 10021.

Estradiol and progesterone influence learning, memory and epileptic seizure activity, functions mediated in part by the hippocampus. Normal hippocampal function is dependent on precise interactions between the excitatory glutamatergic and inhibitory GABAergic systems. To determine whether or not ovarian steroid hormones interact with GABAergic neurons of the hippocampus, the levels of mRNA for GAD, the rate limiting enzyme for GABA synthesis, were measured using *in situ* hybridization histochemistry with ^{35}S-labelled antisense or sense (control) riboprobes transcribed from the feline GAD cDNA (generously provided by A. Tobin). The levels of mRNA for GAD were analyzed in selected regions of dorsal hippocampus and medial basal hypothalamus in three groups of rats: 1) 2-week ovariectomized (OVX), 2) OVX plus 2 days of estradiol (E), and 3) E plus 6 h of progesterone (EP). Rats were killed at 1500 h. GAD mRNA levels in E rats increased in the GABAergic neurons associated with the pyramidal cell layer in CA1 but not in any other region of the hippocampus. EP reversed the E-induced increase in GAD mRNA in CA1 and induced a small decrease in GAD mRNA in the hilus. No effect of E or EP on GAD mRNA levels was observed at this time of day in any region of the medial basal hypothalamus measured. In conclusion, estradiol and progesterone may alter learning and seizure activity by altering the function of GABA neurons in the hippocampus.

456.6

EFFECTS OF CASTRATION ON GABA TURNOVER IN MICRODISSECTED BRAIN REGIONS IN THE MALE RAT. D.R. Grattan, C.A. Sagrillo, M. Selmanoff and D.S. Ruchkin*, Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201-1559.

There is considerable evidence that neuroendocrine regulation involves the inhibitory neurotransmitter GABA. This study determined whether changes in the turnover rates of GABA in discrete brain regions could be detected following castration. GABA concentrations were measured in 13 microdissected brain regions by HPLC with electrochemical detection following precolumn derivatization with o-phthalaldehyde and β-mercaptoethanol. Two days after castration or sham operation, male rats were decapitated either before or 30 minutes after inhibition of the GABA degrading enzyme GABA transaminase by injection of aminooxyacetic acid (AOAA, 100 mg/kg, i.p.). GABA accumulation following injection of AOAA was used as an index of GABA turnover. Non-specific postmortem GABA elevation was prevented by injection of 3-mercaptopropionic acid (120 mg/kg i.p.) 2.5 minutes prior to decapitation. GABA accumulation decreased following castration in the diagonal band of Broca at the level of the organum vasculosum of the lamina terminalis (DBBovlt), lateral to the DBBovlt, dorsomedial preoptic nucleus (MPNdm), arcuate nucleus and median eminence, increased in the lateral and medial septal nuclei, and was unaffected in the cingulate cortex, striatum, ventrolateral MPN, locus coeruleus and medullary A1 and C1 regions. These results are consistant with the hypothesis that the negative feedback of testosterone on LH release involves steroid-sensitive GABAergic neurons in the rostral and medial basal hypothalamus. (Supported by NIH Grant HD21351).

456.7

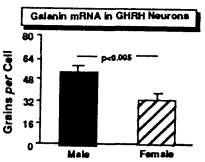
LOCALIZATION OF ANDROGEN AND ESTROGEN RECEPTORS WITH GRF AND SRIF NEURONS IN RAT HYPOTHALAMUS. J.T. French, W.E. Stumpf, R.A. King*, E.M. Wilson and M. Sar. Dept. of Cell Biol. & Anat., Labs. for Reprod. Biol., Univ. of North Carolina, Chapel Hill, NC 27599.

This study was conducted to determine whether GRF or SRIF neurons contain androgen receptor (AR) or estrogen receptor (ER). A dual immunocytochemistry method was applied to visualize AR and ER in GRF and SRIF neurons. Adult male rats with or without testosterone propionate treatment (100μg/100 g BW) each received intraventricularly colchicine (50μg/rat). 18-20 hr later rats were perfused with Zamboni's fixative, followed by a rinse with 10% sucrose in 0.1M phosphate buffer (pH 7.4). Brains were frozen and 10μm serial sections were processed for dual immunostaining. Sections were initially stained for AR and ER by the ABC method with antipeptide antibodies AR32 and ER715 respectively using DAB. After receptor staining, the sections were immunostained with GRF and SRIF antibodies using 4-chloro-1-naphthol. AR and ER localized in nuclei of neurons of the arcuate, periventricular and ventromedial nucleus where GRF and SRIF cell bodies exist. Nuclear localization of AR was found in the majority of SRIF neurons but not in GRF neurons. However, ER localization was observed in GRF but not in SRIF neurons. These data suggest that estrogen directly affects GRF neurons, while testosterone directly affects SRIF neurons. Since testosterone is converted to estradiol in certain brain regions an indirect effect of testosterone on GRF neurons cannot be ruled out. (Supported by NIH Grant 17479 and T32-HD07201.)

456.8

SEXUAL DIMORPHISM OF GALANIN GENE EXPRESSION IN GROWTH HORMONE-RELEASING HORMONE NEURONS. H.A. Delemarre-van de Waal, K.A. Burton, E.B. Kabigting, R.A. Steiner, and D.K. Clifton*, Ob/Gyn & Physiol/Biophys, U. Wash., Seattle, WA 98195 and Pediatrics, Free U. Hosp., Amsterdam.

Sexually dimorphic growth hormone (GH) secretion in the rat is likely due, at least in part, to differences in the activity of hypothalamic growth hormone-releasing hormone (GHRH) neurons. Galanin is colocalized in GHRH neurons by immunocytochemistry and stimulates GH secretion when administered exogenously. Based on these observations, we hypothesized that galanin mRNA is coexpressed in GHRH neurons and that this coexpression is sexually dimorphic. To test this hypothesis, we performed double-label *in situ* hybridization on coronal hypothalamic sections obtained from male (n=5) and female (n=5) rats. GHRH mRNA was hybridized with a cRNA probe labeled with digoxigenin-UTP, and galanin mRNA was hybridized with a cRNA probe labeled with ^{35}S-UTP. GHRH mRNA-positive cells were visualized by an alkaline phosphatase color reaction. Galanin mRNA levels were measured by counting autoradiographic grains over individual GHRH mRNA-positive cells with a computerized image analysis system. We confirmed that GHRH neurons in both male and female rats coexpress galanin mRNA. In addition, we observed a pronounced sexual dimorphism in levels of galanin mRNA in GHRH neurons, with males exhibiting values ~60% greater than females. This sex difference was statistically significant in GHRH neurons of the arcuate ($p<0.02$) and periarcuate ($p<0.001$) nuclei and tended toward significance in the ventromedial hypothalamic nucleus ($p<0.07$). However, in neither sex did GHRH neurons in the paraventricular nucleus express detectable levels of galanin mRNA. We conclude that galanin gene expression by GHRH neurons is sexually dimorphic, and we infer that differential secretion of galanin by GHRH neurons helps to orchestrate the different patterns of the GH secretion between male and female rats.



456.9

THE EXPRESSION OF GALANIN IN LHRH NEURONS IS INHIBITED IN PREGNANT AND LACTATING RATS. I. Merchenthaler* Functional Morphology Section, LMIN, NIEHS, NIH, Research Triangle Park, NC 277709.

Galanin (GAL) coexists with LHRH in a subset of preoptic neurons and functions as a potent LHRH secretagogue. The incidence of colocalization is estrogen-dependent, i.e., the number of cells containing both LHRH and GAL is four-times higher in female than in male rats. In the present studies, a very low incidence of colocalization is reported in pregnant and lactating rats. Cycling female, pregnant and lactating female rats were treated with colchicine. One day later, their brains were perfusion-fixed and 30 μm sections were prepared with a vibratome. Sections were immunostained for GAL using anti-rat GAL serum and the ABC technique. The presence of fusiform, GAL-immunoreactive, "LHRH-like" neurons, was used as indication for colocalization of the two peptides. While in cycling female rats 75% of the LHRH cells contained GAL, in pregnant or lactating rats, the incidence of colocalization was almost undetectable.

Progesterone has been shown to blunt the effect of estrogen at the level of the brain and pituitary. The high levels of progesterone present in both pregnant and lactating animals may be responsible for abolishing estradiol-induced increase in GAL gene expression within LHRH-containing neurons. In addition, prolactin levels are also high in both animal models; therefore, a role of prolactin in this phenomenon cannot be ruled out. Among other mechanisms, the high levels of progesterone and/or PRL, may also prevent ovulation during pregnancy and lactation by inhibiting estradiol-induced GAL gene expression. Therefore, progesterone and/or prolactin could be responsible for the low incidence of GAL/LHRH colocalization during pregnancy and lactation.

456.10

Estrogen Induction of Galanin and Immediate Early Gene Expression in the Rat Anterior Pituitary Gland. T. Csikos, J.K. McDonald+, and J.I. Koenig*. Dept. of Physiology, Georgetown Univ. Sch. Med., Washington, D.C. 20007, +Dept. of Anatomy & Cell Biology, Emory Univ. Sch. Med., Atlanta, GA 30322.

Estrogen (E) induces galanin gene expression in the rat anterior pituitary gland (rAP) by unresolved mechanisms. A solution hybridization/RNase protection assay was developed to simultaneously quantify multiple mRNA species in total cellular RNA samples. Time- and dose-dependent effects of E on the expression of *c-fos*, *c-jun*, two immediate early genes, and galanin were studied in the rAP *in vivo*. Ovariectomized rats received E implants and were sacrificed 12, 24, 48 and 72 hrs later or 72 hrs after receiving implants containing increasing doses of E. rAP RNA was isolated and 10μg was incubated with antisense ^{32}P-labeled cRNA probes for *c-fos*, *c-jun* and galanin. Protected fragments were produced by S1 nuclease digestion followed by denaturing polyacrylamide gel electrophoresis. Fragments were visualized by autoradiography and quantified by densitometry or liquid scintillation counting. Standard curves were prepared for each probe by synthesizing sense strand cRNAs. E-induced galanin gene expression as early as 12 hrs after capsule implantation which increased 500 fold by 72 hrs. *c-fos* expression parallels galanin expression but is induced only 50-fold. However, *c-jun* expression is only weakly induced by E. While both galanin and *c-jun* expression are positively correlated with E dose, prolonged exposure to high doses of E appears to depress *c-fos* expression. These results suggest that E induced the transient expression of the *c-fos* gene in the rAP. Since an AP-1 site is present in the upstream region of the galanin gene, *c-fos* may participate in regulating galanin expression. The expression of *c-jun* is only weakly induced by E, these findings are consistent with a role for AP-1 in E-induced changes in rAP gene expression.

456.11

PREGNENOLONE SYNTHESIS IN RAT BRAIN: REGULATION BY MITOCHONDRIAL DBI RECEPTOR (MDR) AGONISTS. A. Korneyev*, B.S. Pan, A. Guidotti, E. Costa. Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ. Sch. of Med., Washington, DC 20007, USA

Evidence that neurosteroids are potent modulators of the action of GABA at $GABA_A$ receptors has prompted the investigation of the mechanisms that control brain glial mitochondrial steroidogenesis. A crucial step in this regulation is the interaction of DBI - a polypeptide abundant in steroidogenic cells - with the glial MDRs (P.N.A.S., in press). MDRs recognize with high affinity 4'-chlorodiazepam (4'-Cl-DZ) - a full agonist - FGIN-1-27 [N-N-di-n-hexyl-2-(4-fluorophenyl)indol-3-acetamide)] - a new highly specific and potent agonist - and the isoquinoline carboxamide PK 11195 - a partial agonist. To test *in vivo* whether stimulation of MDRs by 4'Cl-DZ and FGIN-1-27 increases pregnenolone (P) production, we determined by HPLC-RIA the P content in brain of male rats acutely adrenalectomized/castrated (A/C) (to eliminate peripheral sources of P) and pretreated with 200 mg/kg i.p. of trilostane (an inhibitor of P metabolism). The P content in microwaved brains of A/C rats (4-8 ng/g) was slightly increased by pretreatment with trilostane, reflecting a low P synthesis rate in these animals. ACTH (5 IU/kg) and electroconvulsive shock did not affect the brain P content. The content of P, but not that of 17-α-hydroxypregnenolone and dehydroepiandrosterone, in frontal cortex and cerebellum increased after treatment with 4'Cl-DZ and FGIN-1-27. The action of both drugs on P brain content was dose and time dependent. The effect of 4'-Cl-DZ was maximal (70-150% increase) with 15-20 μmol/kg, 5 to 10 min after the i.v. injection. The effect of oral administration of FGIN-1-27 was maximal (200 to 300% increase) with 400-800 μmol/kg and peaked at approximately 1 hr. To prove a direct involvement of MDRs in the action of FGIN-1-27, animals were pretreated with the partial agonist PK 11195 (100 μmol/kg i.p.). PK 11195 did not modify the P brain content, but prevented the accumulation of P induced by FGIN-1-27. The data suggest that MDRs, presumably located in glial cells, play a role in *in vivo* pregnenolone biosynthesis.

456.12

DIAZEPAM BINDING INHIBITOR (DBI) AND MITOCHONDRIAL DBI RECEPTOR (MDR) IN ACTH INDUCED STEROIDOGENESIS. S. Cavallaro, A. Korneyev, K. E. Krueger*, E. Costa, and A. Guidotti. Fidia-Georgetown Institute for the Neuroscience, Georgetown University School of Medicine, Washington DC, 20007 USA

The MDR (previously known as peripheral-benzodiazepine receptor) is a binding site located on the outer mitochondrial membrane, which is highly enriched in steroidogenic cells such as adrenocortical, Leydig and glial cells. In these cells MDR stimulation has been shown to facilitate the intramitochondrial cholesterol transfer, a rate-limiting step in steroid biosynthesis. The stimulation of this receptor in hypophysectomized rats, by the administration of Ro5-4864 (15 μmoles/kg, iv), a selective MDR ligand, increased adrenal steroid biosynthesis. Seven minutes after the injection, the levels of corticosterone were maximally increased in adrenal (400%) as well as in plasma (300%). This effect was preceded by the increase in adrenal pregnenolone content (140%), when this was measured in the presence of trilostane (200 mg/kg, ip), an inhibitor of pregnenolone metabolism. In vivo, MDR activation by DBI, its putative endogenous peptide ligand, might mediate the action of ACTH in adrenal steroidogenesis. To investigate the mechanisms whereby DBI and MDR may accomplish this effect, hypophysectomized rats were treated with ACTH-R (ACTH in saline containing 16% gelatin; 15 U/kg, sc), which causes a long-term increase of adrenal DBI and MDR expression. Hours after ACTH-R administration the increase in DBI- and MDR-mRNA levels leads to an increase in their translation products. In addition to this, ACTH has a short-term effect on the rate of DBI-processing. Thirty minutes after the administration of ACTH (50 mU/kg, iv) in hypophysectomized rats, the immunoreactivity for DBI 17-50 (TTN) was increased more than three fold. Our results coupled to the demonstration that TTN is a DBI-processing product highly active in stimulating steroidogenesis, are consistent with the view that acute ACTH steroidogenesis may be mediated by TTN or other DBI-processing products with an high intrinsic efficacy on MDR induced steroidogenesis.

TRAUMA

457.1

ENDONUCLEASE ACTIVATION IN BRAIN INJURY OF RAT. T. Tominaga*, S. Kure and T. Yoshimoto. Div. of Neurosurgery, Inst. of Brain Diseases and Dept. of Biochem. Genetics, Tohoku Univ. Sch. of Med., Sendai, Japan 980

The main purpose of this study was to clarify the temporal profile of DNA degradation, especially DNA fragmentation, during the process of brain injury. Additionally, endonuclease activity responsible for DNA breakdown was investigated using nuclear protein fraction.

Adult male Wistar rats were used. Focal cerebral ischemia and freeze-injury were produced by the tandem occlusion of common carotid and proximal middle cerebral arteries, and the application of precooled metal probe to dorsal cortex, respectively, under halothane anesthesia. The DNA was extracted from the lesioned cortex and/or caudoputamen and electrophoresed on a agarose gel. Typical DNA fragmentation into oligonucleosomal sizes was found in the ischemic caudoputamen and the freeze-injured cortex, which amount reached maximum around 24 hours after the insults. Coexisting random degradation and specific fragmentation of DNA was observed in the cortex suffered from focal ischemia. To determine whether an endonuclease responsible for DNA fragmentation was present in the brain nuclei, nuclear proteins were extracted from normal brain nuclei and incubated with plasmid DNA or normal brain nuclei under various conditions. This demonstrated that brain nuclear proteins have Ca-dependent endonuclease activity which is related to DNA fragmentation. Moreover, the "gel assay" of endonucleases indicated the NUC1.8, which causes DNA fragmentation in lymphocyte, are also present in brain nuclei.

DNA fragmentation is not unique to programmed cell death and can occur as a result of brain injury, probably through the activation of Ca-dependent endonuclease.

457.2

NBQX PROTECTS RATS AGAINST TRAUMATIC BRAIN INJURY.
H. Bernert, P.-A. Löschmann and L. Turski*. Research Labs of Schering AG, Berlin, Germany.

Effect of the competitive AMPA (o-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) antagonist NBQX (6-nitro-7-sulphamoyl-benzo[f]quinoxaline-2,3-dione) was studied in a model of traumatic brain injury in rats. A stainless-steel circular footplate, diameter 4.5 mm, was positioned stereotaxically over the hindpaw area of the motor and somatosensory cortex of male Fisher 344 rats weighing 220-250 g. The footplate rested on the surface of the dura. The craniotomy was slightly larger than the diameter of the footplate to prevent brain contusion from herniating. The stainless-steel tube, 40 cm in length, guided a falling weight onto the footplate. A force of 0.04 N produced by a 20 g weight was selected for induction of injury in rats anesthetized with 260 mg/kg of tribromethanol (i.p.). The maximal depression of the brain surface was 2.5 mm. NBQX, 30 mg/kg i.p., was administered 1, 2 and 3 h after traumatic injury. Three days after traumatic injury, the rats were sacrificed by transaortic perfusion-fixation and the brains were serially sectioned and prepared for light microscopy. Morphological analysis of the brains from 20 vehicle-treated rats (cresyl violet stained sections) showed consistent damage in the neocortex, hippocampal subfields CA3 and CA4 and less frequently in the thalamus on the side of traumatic injury. Treatment of animals with NBQX (n = 20) led to the reduction of the volume of cortical lesion and a protection against hippocampal damage. These observations suggest that AMPA antagonists may be of benefit for the treatment of acute head injury.

457.3

RECOVERY OF METABOLISM IN THE LATERAL GENICULATE NUCLEUS AND SUPERIOR COLLICULUS FOLLOWING EXPERIMENTAL OPTIC NERVE INJURY. K.D. Steinsapir, D.L. Wirta, D.A. Hovda*, and R.A. Goldberg. Jules Stein Eye Inst., Div. Neurosurg., UCLA Sch. Med., Los Angeles, CA 90024

The effect of traumatic injury to the optic nerve on glucose metabolism within the lateral geniculate nucleus (LGN) and the superior colliculus (SC) was studied in male Sprague-Dawley rats (N=52, wt=250-300g). Injury to the optic nerve was performed under general anesthesia [33% O_2, 66% N_2O, enflurane (1.5-2.0 ml/,in)] by the surgical application of an aneurysm clip for 1, 5, 30 or 60 seconds. Local cerebral glucose utilization (lCMRglc, μmol/100g/min) was calculated using [^{14}C]2-deoxy-D-glucose (2DG) autoradiography at various times following injury. During the 2DG experiment animals were subjected to either ambient light or xenon arc strobe stimulation. Histological examination of the injured nerve indicated that as the duration of crush increase so to did the extent of morphological damage. In stimulated animals, lCMRglc in the LGN and the SC serving the injured optic nerve exhibited a pronounced and permanent (21 days) depression compared to the contralateral homologous structures; 51.0±17.4 vs 81.9±23.43 ($p<0.01$) and 58.0±12.19 vs 83.2±18.73 ($p<0.01$) in the LGN and SC respectively. However, in nonstimulated animals, particularly those sustaining the 1 sec injury, the lCMRglc depression was restricted to the SC. This metabolic depression spontaneously alleviated within 3 days with animals who sustained the most severe (60 sec) injury showed recovery by 10 days. Finally in 3 animals who had received methylprednisolone (30 mg/kg, i.p.) given every 6 hrs beginning 45 min prior to the insult, the recovery rate from metabolic depression was accelerated. These results suggest; (1) a difference in physiological response between the LGN and SC to optic nerve injury, (2) a role for methylprednisolone in the treatment of optic nerve trauma. (NS30308)

457.4

Perineuriotomy - A New Approach in Injection Neuropathies. J. Terzis*, T. Rath, H. Millesi. Microsurgical Res. Ctr, Eastern VA Med. School, Norfolk, VA 23501.

Intrafascicular fibrosis may be produced by intraneural injection of an agent. It is a rational assumption that release of the entrapping perineurium might improve nerve function. Purpose: 1) Establish an experimental model of injection neuropathies; 2) test the effect of toxicity of three agents, 0.9% saline, 0.125% marcaine + 1/400 000 epinephrine and 5% phenol; 3) evaluate the effect of epineuriotomy. Methods: Right tibial nerve of the guinea pig (200-300g) is used as the experimental model (N=60). Intraneural injection of three agents is performed by a micropipet and a micromanipulator to guarantee reproducibility. 60 guinea pigs are divided into 4 groups of 15 animals/5 per agent). Grp I: injection only, without treatment; Grp II: injection plus immediate perineuriotomy; Grp III: injection plus perineuriotomy after 1 week; Grp IV: injection plus perineuriotomy after 3 weeks. 32 days after injection, assessment is performed. Assessment parameters: 1) Gait analysis - A sciatic functional index, adapted to the gait patterns and foot prints of the guinea pig is used. 2) Electrophysiology - Compound action potentials are measured. 3) Histology - cross sectional biopsies are evaluated by light microscopy and electronmicroscopy to assess axonal re- and degeneration, myelin changes, response of the perineurium to the injection injury, intrafascicular scar formation. 4) Quantitative morphometry. Results: Preliminary data show that injection of 0.9% saline alone causes proliferation of the perineurium and endoneurial scar formation. These changes are far more pronounced after injection of a toxic agent. Phenol produces extensive pathologic changes which include severe epi- and perineurial proliferation, axonal degeneration and endoneurial scar formation. Injection of marcaine and epinephrine produces pathologic changes that range in between saline and phenol injected nerves. Conclusions: Preliminary data show possible beneficial effect of delayed perineuriotomy in nerves injected by a toxic agent.

457.5

HYPEREMIC RESPONSE TO PERCUSSIVE TRAUMA IN IMMATURE, MATURE, AND AGED RATS. K. V. Biagas, P. D. Grundl, J. K. Schiding, and P. M. Kochanek*. Depts. of Anes./Critical Care, Pediatrics, and the Head Injury Research Center, Univ. of Pittsburgh, Pittsburgh, PA 15213.

Clinical studies suggest that the cerebrovascular response to head trauma differs with age. Specifically, increased cerebral blood flow, or hyperemia, commonly found in children is less often seen in older adults after head injury. We hypothesize that reproducible posttraumatic hyperemia can be demonstrated in an animal model and that the extent of hyperemic response differs with age.

Wistar rats, sexually immature (3.5-4.5 wks, N=18), mature (9-15 wks, N=18), and aged (12.5-14 mo, N=8) were anesthetized and ventilated. Injury to the exposed right parietal cortex was produced by weight drop indexed to brain weight. Regional cerebral blood flow (rCBF) was determined by ^{14}C iodoantipyrine autoradiography in age-matched normal controls and in rats injured 24 h before. Percent of normal control rCBF was determined for 15 structures. Within group comparisons to controls were made by t-test.

At 24 h posttrauma, low rCBF was found in all groups in the zone of impact (20% of normal control in immatures, 5% in matures, and 11% in aged rats, all $p<0.05$ vs. respective normal controls). Hyperemia in the peri-trauma parietal cortex was greatest in immatures (270% of normal control, $p<0.05$), less in matures (165%, $p<0.05$), and not found in aged rats (106%, NS). Increased rCBF was found in structures distant to the trauma in all age groups (146 to 188% of normal control in immatures, 66 to 160% in matures, and 157 to 228% in aged).

These results demonstrate enhanced peri-trauma hyperemic response 24 h after brain injury in the immature. However, increased rCBF was found in distant structures in all ages suggesting that the diffuse posttraumatic hyperemic response is not age-dependent in this model.

457.6

FUNCTIONAL ANALYSIS OF EMBRYONIC NEOCORTEX GRAFTING AFTER GRADED SEVERE HEAD INJURY. O. Kopyov, D.B. Jacques,* R.W. Rand. The Neurosciences Institute, The Hospital of the Good Samaritan, Los Angeles, CA. 90017.

Adult male Wistar rats were subjected to a blow of measured intensity to the parietal area of the skull resulting in severe trauma. The majority of these animals (86%) died within 4-5 days. Some rats received embryonic neocortical grafts and debridement into the lesioned areas immediately after the trauma. This resulted in decreasing the post traumatic mortality to 4%. These animals showed a rapid restoration of their behavioral activities as demonstrated by the open field method. Food and water intake and motor activity returned to normal on the second post-trauma day. Debridement with no grafts reduced the post-traumatic death rate to 30%. Animals of this group showed reduced activity and food ingestion during the first post-trauma week. Histologic investigation three months after trauma and grafting revealed the presence of viable grafts in all hosts. The grafts were relatively well integrated with the host brain. No obvious signs of pathology were observed at the traumatized site of the brain in animals with grafts. The most important results of embryonic neocortex transplantation after severe head injury were mortality reduction, potent stimulation of post-traumatic brain tissue defect repair and the prevention of adhesive processes.

457.7

MIDDLE LATENCY AUDITORY EVOKED POTENTIALS FOR EVALUATION OF WAKEFULNESS. S. Yamada*, W. Hayward, L. Dayes, V. Morgese, B. Curtis, and R. Iacono. Division of Neurosurgery, Loma Linda University School of Medicine, Loma Linda, CA 92350.

The medial geniculate body is known to transform tonotopic impulses from brain stem to the hemispheric cognitive function. The authors studied the middle latency (MLAP) and brain stem auditory evoked potentials (BAR) at 3-day intervals in 100 patients who sustained head injuries and remained unconscious for certain periods. The results are: 1) Loss and no recovery of BAR wave 5 and MLAP indicates no return of consciousness (always Glascow Coma Scale 3 or 4); 2) Bilateral recovery of BAR and MLAP correlates with regaining consciousness including communicative capacity; 3) Unilateral recovery of the MLAP correlates with return of wakefulness but inadequate communicative capacity; 4) Normal BAR with loss of MLAP followed by its complete recovery correlates with rapid return of normal consciousness from continuous sleep.

The authors emphasize the importance of MLAP for evaluation and prognostication of wakefulness after head injury.

457.8

TIME COURSE OF TRAUMATICALLY INDUCED BLOOD-BRAIN BARRIER ALTERATION: A QUANTITATIVE AND ANATOMICALLY SPECIFIC ANALYSES. M. Kasapi, M.L. Giebel and J.T. Povlishock*. Dept. of Anatomy, Med. Col. of VA., VA. Com. Univ., Richmond, VA 23298.

To date, considerable interest has focused on the occurrence of traumatically induced blood-brain barrier (BBB) disruption, its temporal course and its possible impact upon outcome. Progress, however, has been hampered by inter-animal variability and by the difficulties associated with conducting, in the same animal, detailed quantitative and temporarily/anatomically specific assessments of BBB change. To address these issues, we developed new strategies using I^{125} labeled albumin, followed by the LM and TEM immunocytochemical visualization of this labeled substance. Anesthesized rats were subjected to moderate fluid-percussion brain injury. One-24 hr postinjury, I^{125} labeled human serum albumin was injected, followed 2 hr later by aldehyde perfusion. The brain was blocked into select foci, which were counted using a gamma counter. These regions were sectioned, and reacted for the immunocytochemical visualization of endogenous albumin as well as the exogenously administered albumin. In this fashion information was provided on both the initial as well as delayed altered BBB permeability to albumin. Moderate brain injury was observed to open the BBB within the dorsal neocortex and related hippocampi. Unexpectedly, the traumatically induced BBB change showed progression becoming maximal by 6 hr postinjury, with a diminution of albumin permeability and BBB closure by 24 hr. The permeability change seen immediately following injury was related to discrete petechial hemorrhage and foci of altered endothelial integrity. However, with increasing survival [3-6 hr], it appeared that WBC recruitment participated in the cleaving of interendothelial tight junctions resulting in increased permeability. These results suggest that BBB change postinjury is an evolving event, with multiple factors involved in its evolution. Supported by R01 NS29469.

457.9

CORRELATION OF NEUROFILAMENT IMMUNOHISTOCHEMISTRY WITH SILVER STAINING OF DAMAGED AXONS FOLLOWING HEAD INJURY IN HUMANS, SUB-HUMAN PRIMATES, AND RODENTS D.T. Ross*, D.I. Graham, J.H. Adams, and T.A. Gennarelli Head Injury Center, Div. of Neurosurgery, Univ. of Pennsylvania, Philadelphia, PA 19104, and Dept. of Neuropathology, Southern General Hospital, Glasgow, Scotland

Diffuse axonal injury in cases of severe head injury is defined by the presence of swollen axonal segments and axonal bulbs, classically visualized in reduced silver preparations. The present study sought to establish to what extent neurofilament immunohistochemistry correlated with reduced silver staining of damaged axons in the brains of fatal human head injury cases, sub-human primates subjected to experimental inertial head injury, and rats which received directed forebrain tensile strain injury.

SMI-31, a mouse monoclonal antibody (mAB) to *phosphorylated* epitopes on heavy and medium neurofilament subunits, labels axons in the corpus callosum, subcortical white matter and fascicles of axons radiating into the cortical gray matter in normal brains. Following head injury in man, baboons, and rats SMI-31 labeled large axonal bulbs in the corpus callosum, subcortical white matter, and deep cortical layers. The density of labeling of these axonal bulbs was virtually identical to that seen in adjacent sections stained with the Palmgren's reduced silver technique. Perikarya of damaged neocortical pyramidal cells in layers III and V were also positively labeled with SMI 31. SMI 32, a mAB to *non-phosphorylated* epitopes on heavy and medium neurofilament subunits, selectively labels neurons in layers III and V of normal human, baboon, and rat neocortex. Following head injury SMI-32 labeled small and medium sized retraction balls but most striking were the numerous axons characterized by labeled normal segments continuous with hypertrophied axonal segments disrupted by patches of granular labeling, suggestive of focal neurofilament proteolysis. SMI-31 and 32 are excellent markers of axonal injury which not only selectively label features of axonal pathology but also identify the perikarya of damaged axons and may also shed light on the cellular mechanisms of axonal pathology following head injury. (supported by NS-08803-21, NS-28852-02, and the Univ. of Pennsylvania Reserach Foundation)

457.10

ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL STUDY OF DIFFUSE AXONAL INJURY IN MAN. C.W. Christman, *[1] M.S. Grady,[2] J.T. Povlishock,[1] Dept. of Anatomy, Med. Col. of Virginia, Virginia Commonwealth Univ.[1] Richmond, VA 23298, and Dept. of Neurosurgery, Univ. of Washington[2]

Axonal injury is commonly observed in human traumatic brain injury, but its pathogenesis has remained controversial due in part to limitations associated with the use of traditional histological methods. Recently, in animals we have shown the utility of monoclonal antibodies to neurofilament subunits, particularly the 68 kD subunit, in detecting damaged axons (Yaghmai and Povlishock, 1992). In the present study, postmortem analysis was performed on ten human cases at various intervals of postinjury. The monoclonal antibody to the 68 kD subunit was used to identify sites of traumatically induced axonal change, which were analyzed at both the light microscopic and electron microscopic levels. This approach revealed at 6 h postinjury axons exhibiting focal swellings. Immunoreactivity was associated with neurofilaments, some of which exhibited clumping and misalignment. Localized infolding of the axolemma was also evident. By 12 h postinjury the continued expansion of sites of focal swelling had progressed in some cases to axonal disconnection. Neurofilaments associated with marked immunoreactivity were now more disordered and vacuolization was observed. At 30 h immunoreactive disconnected reactive swellings displayed convoluted arrays of neurofilaments, partially surrounded by a cap-like zone of electron-lucent organelles. In advanced stages of survival heterogeneity was evident. These findings reveal that, in head-injured man, axonal disconnection is the result of subtle cytoskeletal change that occurs over a period of time. Actual structural/functional factors underlying this reactive cytoskeletal change are unknown, but the observed increase in the 68 kD subunit reactivity appears compatible with some local change in phosphorylation state.

Supported by NIH Grant 20193.

458.1

SOURCES AND MECHANISMS OF $[Ca^{2+}]_i$ TRANSIENTS IN MYELINATING SCHWANN CELLS IN RESPONSE TO AXONAL ACTIVATION. V. Lev-Ram*[1], R. Y. Tsien[1&2], J. A. Airey[4], J. L. Sutko[4], and M. H. Ellisman[3] [1]Dept. of Pharmacology and [2]HHMI, [3]San Diego Microscopy and Imaging Resource, Dept. of Neurosciences, University of California San Diego, La Jolla, CA 92093; [4]Dept. of Pharmacology, Univ. of NV, Reno, NV 89557.

In order to better understand the roles of paranodal glia in the regulation of the ionic milieu, we have developed a system for *in vitro* studies, combining optical and electrophysiological recording methods. Such a methodology provides a unique facility to test hypotheses regarding the function of cell compartments at the node of Ranvier. We recorded single fiber physiological properties, conventionally and with fluorescent ion-sensitive dyes (fluo-3 or indo-1, both loaded as their AM-esters), and simultaneously observed single nodes at high optical resolution using Nomarski differential interference contrast optics (DIC) with time-lapse recording. Ratio imaging of frog sciatic nerve fibers loaded with indo-1 showed low $[Ca^{2+}]_i$ in myelinating Schwann cells which increased in response to axonal activation. To elucidate sources of $[Ca^{2+}]_i$, we tested the effects of drugs known to alter $[Ca^{2+}]_i$. Using fluo-3, $[Ca^{2+}]_i$-transients in Schwann cells were observed in response to axonal activation and were subsequently blocked by ryanodine if ryanodine was present during the first $[Ca^{2+}]_i$ transient. Short repetitive bath applications of caffeine induced $[Ca^{2+}]_i$ transients which could be blocked by ryanodine. Immunolocalization studies demonstrated the presence of ryanodine receptor-like immunoreactivity in Schwann cell paranodal loops supporting the physiological data regarding the presence of Ca^{2+}-activated Ca^{2+}-release channels. In contrast to caffeine, only small $[Ca^{2+}]_i$ transients were observed in response to application of thapsigargin. These findings indicate Ca^{2+}-activated Ca^{2+}-release in Schwann cells in response to impulse activity suggesting an active role for myelinating glial cells during axonal firing.

458.2

AXONAL REGULATION OF C-JUN EXPRESSION IN SCHWANN CELLS IN VIVO. M.E.Shy[1], L.Wrabetz[2], M.L. Feltri[1], J. Kamholz[2]*, S.S. Scherer[2]. Depts. of Neurology, Thomas Jefferson Univ.[1] and Univ. Penn[2].

The nuclear proto-oncogene c-*jun* is an "immediate early" gene in that its expression is rapidly altered in a variety of conditions both *in vivo* and *in vitro*. Monuki et al. (Neuron 3: 783-790, 1989) have shown that Schwann cells (SC) *in vitro* express high levels of c-*jun* mRNA, which is rapidly down-regulated by the addition of forskolin, which increases intracellular cAMP and is widely used as an *in vitro* mimic of axonal contact. The down-regulation of c-*jun* preceeds the up-regulation of SCIP mRNA, which in turn preceeds the up-regulation of the mRNAs of the myelin genes. We have examined the expression of c-*jun* in adult rat sciatic nerves that have been permanently transected (to cause Wallerian degeneration and prevent axonal regeneration) or crushed (to cause Wallerian degeneration but allow axonal regeneration) to examine whether axons regulate c-*jun* expression in SC *in vivo*. In unlesioned adult nerves, there was a low level of c-*jun* mRNA, and few if any SC nuclei were stained with antibodies against *jun*. After nerve-transection, the steady state level of c-*jun* mRNA increased dramatically between 1 and 24 days, and many SC nuclei were stained with the *jun* antibody. After nerve-crush, the steady state level of c-*jun* increased dramatically between 1 and 12 days, then decreased between 12 and 24 days post-crush as axons regenerated into the distal nerve-stump. Thus, the pattern of c-*jun* mRNA expression was remarkably similar to that of nerve growth factor receptor (NGFR) mRNA in both transected and crushed nerves, and indicates that axonal-SC interactions influence the expression of c-*jun*.

458.3

BETA-GALACTOSIDASE EXPRESSION IN OLIGODENDROCYTES IN TRANSGENIC MICE. P.A. Wight, C.S. Duchala, C. Readhead[1], W.B.Macklin*. M.R.R.C., UCLA Med. Ctr., Los Angeles CA 90024;[1] Calif. Inst. of Tech., Pasadena, CA 91125

Three transgenic lines were generated using the myelin proteolipid protein (PLP) gene. The transgene [PLP(+)Z] contained 2.4 kb of 5'-flanking DNA, exon 1, intron 1 (approx. 8 kb) and 37 bases of exon 2, fused to the lacZ gene. All three lines expressed beta-galactosidase in white matter areas, and when analyzed at the electron microscopic level, the enzyme was seen in the myelin sheath itself. Transgene expression was studied in pups at 2,5,7,9,12,15,18,21 and 25 days of age, and it was developmentally coordinated with myelination. Very young oligodendrocytes were stained, confirming our earlier studies indicating that the PLP gene is expressed quite early in oligodendrocyte differentiation. The transgene was also expressed in young oligodendrocytes in culture. Bipolar cells, presumably immature oligodendrocytes, expressed the transgene within five days of plating, and the transgene continued to be expressed in highly differentiated cells in culture. These studies demonstrate that the PLP(+)Z construct can be used to target DNA for expression in developing and mature oligodendrocytes. Because PLP(+)Z oligodendrocytes carry an unique, endogenous stainable marker, they should be useful in oligodendrocyte transplantation studies. Support: Natl. Mult. Scler. Soc. and NS08774.

458.4

CONTACT WITH MYELIN CAUSES A RELEASE OF CALCIUM FROM INTERNAL STORES IN OLIGODENDROCYTES. S.J. Moorman* and R.I. Hume. Dept. of Biology, University of Michigan, Ann Arbor, MI 48109.

We are interested in whether interactions between adjacent oligodendrocytes play a role in the process of myelination. As an initial approach, we determined the reaction of neonatal-rat oligodendrocytes in culture to an application of crude myelin extract. Myelin extracts were made from either adult rat spinal cord (CNS myelin) or adult rat ischiatic nerve (PNS myelin). Oligodendrocytes were identified based on their characteristic morphology; some cells with this morphology were confirmed as oligodendrocytes by staining with an antibody to galactocerebroside. We monitored morphology of the leading edge of oligodendrocyte processes using time-lapse video-microscopy, and monitored internal free calcium concentration ([Ca]i) using FURA-2. Time-lapse experiments were done at 37° in the normal medium. FURA-2 experiments were done at room temperature in the normal medium. Contact with either CNS myelin or PNS myelin resulted in collapse and retraction of the leading edge of oligodendrocytes. Because collapse of the neuronal growth cone has been associated with increases in [Ca]i, we tested whether collapse of the oligodendrocyte leading edge was associated with changes in [Ca]i. Oligodendrocytes had an average resting [Ca]i of 71(+-2)nM. Contact with CNS myelin caused a relatively rapid [Ca]i increase. The average maximum [Ca]i level reached after contact with CNS myelin was 431(+-27)nM. The [Ca]i increase was reduced but not blocked by 5mM EGTA. This result supports the idea that at least part of the [Ca]i increase was due to a release of calcium from internal stores. A similar [Ca]i increase was induced by contact with PNS myelin. These results suggest that oligodendrocytes might be able to recognize and react to specific molecules on the surface of other oligodendrocytes. This type of cell-cell interaction might play a role in preventing overlap of processes of adjacent oligodendrocytes at the developing Node of Ranvier. (Supported by the American Paralysis Assoc., the Marine Biological Laboratory at Woods Hole, and N.I.H.)

458.5

ELEVATED EXTRACELLULAR K^+ AND TUMOR NECROSIS FACTOR DECREASE THE PHOSPHORYLATION OF OLIGODENDROGLIAL PROTEINS. B. Soliven*, M. Takeda, D.J. Nelson and S. Szuchet. Dept. of Neurology, The University of Chicago, Chicago, IL 60637.

Extracellular K^+ regulates glial metabolism. We examined the effect of high K_o on phosphorylation of myelin basic protein (MBP) and 2', 3'-cyclic nucleotide phosphodiesterase (CNPase) in pure cultures of adult oligodendrocytes (OLGs). These proteins were chosen because we have previously shown that their phosphorylation is linked to myelinogenesis. OLGs (7-14 days old) were labelled with ^{32}P for 1 hr in the presence of 5.4 mM K^+ or 24 mM K^+. We found that exposure of OLGs to 24 mM K^+ (n=5) for 1 hr decreased the phosphorylation of MBP by 57 ± 3.9% and CNPase by 64.2 ± 3.7% compared to control OLGs (N=6, $p < 0.01$). Inhibition of the inward rectifier K^+ channels with 1 mM Ba^{2+} gave no consistent results (n=3). One hr exposure of OLGs to 1 nM recombinant human tumor necrosis factor (rhTNFα) (n=4) mimicked the effect of high K_o on MBP and CNPase phosphorylation. Whether or not the effect of rhTNFα is related to its ability to cause membrane depolarization and to decrease OLG K^+ current amplitudes (Soliven *et al.*, J. Memb. Biol. 124: 127-137, 1991) or is due to a separate mechanism is under investigation. The effects of high K^+ and rhTNFα can be counteracted by activators of protein kinase C (PKC) such as phorbol 12-myristate 13-acetate. Neither high K^+ nor rhTNF-α had any significant effect on MBP or CNPase synthesis. We postulate that PKC may be implicated in the inhibition of MBP and CNPase phosphorylation by high K_o and TNF. These observations differ from those reported in optic nerves (Murray & Steck, J. Neurochem. 41: 543-548, 1983), suggesting that additional factors activated by high K^+ in intact nerves modulate MBP phosphorylation. Supported by grants NIH PO1 NS24575 & MS Soc. RG-2195-A-2.

458.6

PHAGOCYTOSIS AND PROCESSING OF MYELIN BASIC PROTEIN. J. McLaurin, K. Williams, H.D. Durham*, and J.P. Antel, Dept. of Neurology and Neurosurgery, Montreal, P.Q. H3G 2B4.

The role of phagocytic cells may be key in understanding the mechanisms involved in immune-mediated demyelination in the central nervous system. It has been hypothesized that myelin basic protein (MBP) may be the antigen most likely to be processed and presented by MHC class II molecules to elicit an immune mediated response. Although MBP has been used extensively in vitro and in vivo, the mechanism of phagocytosis and processing has not been examined. We have used a human EBV transformed B cell line, a human histiocytic lymphoma cell line, U937, and human microglia to investigate the phagocytosis of MBP. To be able to follow MBP ingestion, MBP was either radiolabelled by reductive methylation or conjugated with FITC then monitored by fluorescence and confocal microscopy. Activated EBV-B cells were shown to be the most efficient at phagocytosis of MBP as demonstrated by both ^{14}C-MBP and FITC-MBP uptake. U937 and microglia were shown to ingest MBP albeit at slower rates. All three cell types failed to ingest MBP when incubated at 4°C, a characteristic of phagocytosis rather than pinocytosis. The rate and extent of MBP phagocytosis was markedly lower than that of celite beads and bovine serum albumin. The processing of MBP is associated with the endosomal fraction of U937 after 2 hours and EBV-B cells after 8 hours. This is consistent with processing for binding to MHC class II molecules.

458.7

EVIDENCE FOR SUBTYPES OF THE PERIPHERAL-TYPE BENZODIAZEPINE RECEPTOR IN CULTURED ASTROCYTES. M.D.Norenberg* and Y.Itzhak, Vet Admin Med Ctr, & Dept of Biochemistry & Molecular Biology, Univ of Miami, Miami, Florida 33135.

Two types of benzodiazepine (BZD) receptors have been identified in brain: central (C) and peripheral (P). The present study was undertaken to characterize the PBZD receptors in cultured astrocytes from neonatal rat cerebral cortex. In homogenate cell preparations, the selective PBZD ligands, [^{3}H]PK 11195 and [^{3}H]Ro5-4864, labeled high affinity sites (Kd-0.82 and 2.4 nM, respectively) that appear to display only a partial overlap. The maximal number of sites (Bmax) labeled with [^{3}H]Ro5-4864 was only 42% of the number of sites labeled with [^{3}H]PK 11195 (1.8 pmoles/mg protein). Unlabeled Ro5-4864 competed for [^{3}H]PK 11195 binding sites in a biphasic manner, suggesting the existence of PBZD sites that are not readily accessible to Ro5-4864. BZD ligands that bind to both CBZD and PBZD receptors competed for [^{3}H]PK 11195 and [^{3}H]RO5-4864 binding in a monophasic manner, and with similar affinities. These findings suggest that Ro5-4864, but not other BZD ligands tested, distinguishes between putative subtypes of PBZD receptors. Subcellular distribution studies indicated no apparent difference between the localization of [^{3}H]Ro5-4864 and [^{3}H]PK 11195 binding sites. The mitochondrial fraction of astrocytes revealed a major enrichment in the binding of the two PBZD ligands tested. This study suggests the existence of putative subtypes of PBZD receptors in astrocytes, and indicates that their primary localization in these cells are in mitochondria.

458.8

REGULATION BY NOREPINEPHRINE OF NITRIC OXIDE SYNTHASE INDUCTION IN GLIA. D.L. Feinstein*, E. Galea, and D.J. Reis Div. of Neurobiol., Dept of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

Norepinephrine (NE) inhibits the induction by IFN-γ of class II antigen expression in cultured astrocytes (Frohman et al. *PNAS* 85:1292, 1991). We examined whether NE would also inhibit the LPS-induction of NOS in astrocytes as determined by nitrite accumulation. Primary astrocyte cultures exposed to LPS (500 ng/ml, 24 hr) but not NE (100 μM, 24 hr) increased NOS activity 15-fold over basal levels. However, NE elicited a dose-dependent inhibition of induction by LPS, with a threshold of 1 nM and maximal inhibition of 50-80% at 100 μM. Exposure to NE (100 μM) for 10 min was as effective as 24 hr continuous exposure in decreasing LPS-induction. In contrast, NE did not alter NOS activity pre-induced in astrocytes suggesting that NE inhibition occurs at the levels of transcription not by protein modification. The induction of NOS by LPS was also blocked by the β-adrenergic agonist isoproterenol (10 μM) but not by the α-agonist phenylephrine. The response to NE was partially reversed by the β-antagonist propranolol, but not by the mixed α-antagonist phentolamine. VIP (1 μM), but not NPY (1 μM), could also inhibit the induction due to LPS. These results suggest that induction of glial NOS by LPS can be regulated by catecholamines acting upon astrocyte β-receptors via elevation of cAMP levels. Conceivably the release of neurotransmitter in vivo may modulate the expression of inducible NOS in astrocyte possibly in response to injury or disease.

458.9

GROWTH FACTOR REQUIREMENTS FOR CELL CYCLING OF SYNCHRONIZED ASTROGLIA. T.J.Langan* and M.C.Slater, Dept. of Neurology, SUNY Sch. Med., Buffalo NY 14222

Polypeptide growth factors (PGFs) generally act in sequential pairs- competence factors in early G_1, and progression factors, particularly insulin (INS), in mid- to late G_1. Newborn rat astrocytes were synchronized by the addition (at time 0) of 10% serum to serum-depleted cultures. Between 12 h (G_0 /G_1) and 20 h, S phase nuclei (bromodeoxyuridine immunofluorescence) increased from 19 ± 4% to 72 ± 10%. DNA synthesis (thymidine incorporation) increased from 48 ± 6 to 205 ± 18 CPM/ ug protein. Even at 12 h, reduction of serum content from 10% to 0.1% arrested cell cycling (S phase nuclei 28 ± 6 % at 20 h). Cycle kinetics were identical in G_0 cultures transferred to a defined medium (DM) having fibroblast growth factor (FGF), INS and epidermal growth factor (EGF) as the sole PGFs. Cultures in DM were exposed selectively to combinations of PGFs from 9 to 12 h, i.e, in late G_1. No single PGF was sufficient to permit cycle progression (≤ 20% S phase nuclei at 20 h). The presence of any 2 PGFs (INS/FGF, INS/EGF, FGF/EGF) resulted by 20 h in ≥ 70% S phase nuclei. Synergism was noted for each of these pairs. Consequently, the competence/progression model is not strictly applicable to these synchronized primary astrocytes. Rather, simultaneous activation of at least 2 PGF-stimulated pathways is necessary in late G_1, although each pathway may recognize multiple ligands.

GENE STRUCTURE AND FUNCTION VI

459.1

ORGANISATION AND EXPRESSION OF THE FMRFamide GENE IN THE MOLLUSC *LYMNAEA*. E. Kellett, S. Saunders, N. Santama, P. Benjamin, J.F. Burke* Sussex Centre for Neuroscience, Biological Sciences, University of Sussex, Brighton, E Sussex BN1 9QG (UK)

The FMRFamide gene in *Lymnaea* contains two major peptide-encoding exons. One encodes the peptides FMRFamide and related peptides. The other encodes GDPFLRFamide and related peptides. Expression of these two exons is mutually exclusive in individual cells, however both exons are spliced onto a common hydrophobic leader sequence. Cytoplasmic expression of these two peptide-encoding exons is regulated by differential RNA splicing. We have recently found that the FMRFamide gene contains at least two other peptide-encoding exons which are spliced onto the GDPFLRFamide exon in the messenger RNA. One of these exons contains the tetrabasic cleavage sequence RRKR and also encodes a novel peptide SKPYMRFamide. Genomic sequence of 5783 base pairs has been determined and, on comparison with cDNA sequences, shows that the FMRFamide and GDPFLRFamide exons are separated by an intron of 2914bp. The mRNAs encoding FMRFamide and GDPFLRFamide are 1647bp and 1623bp respectively. By the use of immunocytochemistry, protein purification and sequencing we have shown that peptides encoded by each exon are translated and processed. This work is supported by the SERC.

459.2

Isolation and characterization of FMRFamide-like peptides from *C. elegans* and mapping of promoter elements for the corresponding gene. M. Rosoff* and C. Li, Dept. of Biology, Boston University, Boston MA 02215.

In the free living soil nematode *Caenorhabditis elegans*, FMRFamide-like immunoreactivity has been localized to approximately thirty neurons in the adult hermaphrodite [Schinkmann and Li (1992) J. Comp. Neurol. 316:251-260]. A gene, *flp-1*, that encodes multiple FLRFamide-containing peptides has been isolated and characterized from *C. elegans* [Rosoff et al., J. Neurosci., in press]. Two different transcripts are produced as a result of alternative splicing; analysis of the corresponding cDNA clones revealed that one transcript encodes six distinct FLRFamide-containing peptides while the other encodes seven.

In collaboration with David Price, five of the seven FLRFamide-containing peptides predicted from the cDNA sequence have now been isolated from whole animal extracts by HPLC purification. Four additional minor peaks show FMRFamide-like immunoreactivity by RIA analysis. Efforts are underway to isolate and characterize these additional peaks.

Using *lacZ* as a reporter gene under the transcriptional control of the *flp-1* promoter region, transgenic animals have been constructed and stained for expression of the transgene. A promoter element that seems to be sufficient to elicit expression in specific cells in the head of the animal has been mapped to within 300 bp of the start site of transcription. Deletion analysis on this region is being performed to map further this element. Finally, to determine whether the occurrence of the two transcripts is developmentally regulated, reverse transcription/PCR experiments of staged RNA are currently being performed.

459.3

TOWARD DEVELOPMENT OF A MASTER SET OF THE cDNAS EXPRESSED IN HUMAN BRAIN. J.M. Sikela*, T.J. Stevens, A.S. Khan, A.S. Wilcox, M. Polymeropoulos, A. Orpana, J.A. Hopkins and M. Robinson. Univ. of Colorado Health Sci. Ctr. and VA Research Ctr., Denver, CO 80262

The generation of a master set of the cDNAs expressed in the human brain, knowledge of the chromosomal location of each cDNA and the complete DNA sequence of the protein coding region of each expressed brain gene would constitute a tremendous resource to human biology and neuroscience. Our goal is to significantly contribute to the development of such a resource through the large scale collection, sequencing and mapping of human brain cDNAs. We have developed a method for enriching for cDNAs that are unique from one another, and have refined strategies for automated single-pass and full-length sequencing of each cDNA and for physical mapping of each cDNA to a location in the genome. We have also identified a subset of cDNAs that contain polymorphic microsatellite sequences and demonstrate how they can be converted to highly informative (PIC value > 0.7) gene-associated genetic markers. Single-pass sequencing data from the cDNAs is being used to search DNA and protein databases using the BLAST programs. At present, most of the sequenced cDNAs correspond to potentially new human brain genes, while a significant number of cDNAs appear to represent human homologs of interesting genes found in other species. Among these are the Drosophila *brahma* gene, a regulator of a number of homeotic genes, several cDNAs related to known neurotransmitter receptor genes, ERK3, an extracellular signal regulated kinase and a potentially novel sodium channel gene. Currently in our laboratory automated single-pass sequencing is being carried out at a rate of several thousand cDNAs per year. Therefore, coordination of this effort with other laboratories doing similar work should permit the sequence identification of most of the genes expressed in the human brain within the next few years.

459.4

TETANIC POTENTIATION ALTERS THE ABUNDANCE OF SEVERAL mRNAS IN SINGLE LIVE CA1 HIPPOCAMPAL NEURONS. S.A Mackler*, B.P. Brooks & J.H. Eberwine Univ of PA Phila PA 19104

We examined the effects of use-dependent changes in synaptic transmission on the relative amounts of different mRNAs in individual neurons. The approach combined whole-cell recordings in rat hippocampal slices with amplification of the polyA+ RNA population from single CA1 cells. The microelectrodes contained all of the reagents necessary for cDNA synthesis and the polyA+ RNA was amplified using the T7 RNA polymerase promoter sequence and 32P-αCTP.

Screening of multiple candidate cDNA neuronal clones with the amplified probe revealed several differences in relative mRNA abundances between control cells and those cells 30 mins or more after potentiation of evoked e.p.s.cs. A 300% increase in CamKII mRNA occurred along with a 50% decrease in protein kinase C (β1) mRNA. This 6-fold change in mRNA levels may accentuate the activity of the CamKII second messenger system compared to PKC. Phospholipase A2 mRNA was unaffected. A 2-fold increase for *zif-*268 mRNA, without consistent changes in *c-fos,c-jun* and HSP70, suggests that transcriptional activators are selectively activated by synaptic use. The above changes were prevented by the prior addition of APV, a NMDA receptor antagonist.

The total of the described changes of mRNA levels from individual cells provide a detailed picture of a neuron after synaptic potentiation and in the context of synaptic connectivity and glial association.

459.5

CALCIUM CALMODULIN DEPENDENT PROTEIN KINASE AND MAP KINASE ARE DIFFERENTIALLY REGULATED BY SYNAPTIC ACTIVITY. T.H. Murphy*, R.V. Bhat, R. Fiore, and J.M. Baraban. Johns Hopkins Univ. Sch. of Med., Dept. of Neuroscience, Baltimore, MD 21205.

Protein kinases are key intermediates in stimulus-induced neuron plasticity. However, the synaptic stimuli needed to activate specific kinases is unclear. Using primary cultures of cortical neurons, we have investigated synaptic activation of Ca^{++}-calmodulin dependent protein kinase (CaMK) and mitogen activated protein kinase (MAPK). Bursts of spontaneous synaptic activity were induced and monitored with Ca^{++} indicators. Phosphorylation of these kinases during their activation allows activity to be preserved and assayed in cell extracts. We report that although both these kinases are activated by synaptic activity, they have different kinetics. While 90% of maximal CaMK activation was observed after only 10 s of burst activity, MAPK is not affected at this early time and is only activated to 30% of maximal after 2 min of stimulation. In parallel experiments, we examined the rate of decay of activity following synaptic stimulation. The half life of stimulated CaMK was 10-30s, while MAPK decayed by 50% within 6-10 min. These data provide evidence for differential regulation of these kinases by synaptic activity.

We thank H. Schulman and M. MacNicol for advice and reagents.

459.6

THE DIFFERENTIAL EXPRESSION OF FOUR CLASS III POU-DOMAIN GENES IN THE CENTRAL NERVOUS SYSTEM OF THE MOUSE A.L. Hartman#†, D.J. Bradley#, Y. Hara§, M. Nirenberg§, and W.S. Young, III#*. #Lab. Cell Biology, NIMH; §Lab. Biochemical Genetics, NHLBI, Bethesda, MD 20892. †Howard Hughes Medical Institute-NIH Research Scholars Program.

One of the most fascinating issues in the study of the developing and adult CNS is transcriptional regulation of genes. POU proteins are trans-acting factors which contain two highly conserved DNA binding domains, the POU-homeodomain and the POU-specific domain. We used *in situ* hybridization histochemistry to examine the distribution of mRNA encoding four Class III POU proteins recently cloned in our labs (PNAS 89:3280; PNAS 89:3285). Serial adjacent sections from adult male Swiss-Webster mice were studied. For Brain-1, Brain-2, and SCIP, we used ^{35}S-labelled riboprobes (cRNA) targeted to the 3' end of the coding region and the adjacent untranslated region of each transcript. For Brain-4, the probe was targeted to the mRNA encoding the translated region 5' up to the POU-specific domain. The sense strand of the Brain-4 probe was used as a negative control.

Brain-1 was the most abundant transcript of the four transcripts, being especially prominent in the piriform cortex, olfactory tubercle, and lateral ventricular ependyma. Brain-2 was prominent in the piriform cortex, vertical limb of the diagonal band of Broca, lateral ventricular ependyma, paraventricular and supraoptic hypothalamic nuclei, and substantia nigra, pars compacta. SCIP was abundant in the piriform cortex, striatum, accumbens nucleus, Islands of Calleja, vertical and horizontal limbs of the diagonal band of Broca, nucleus of the lateral olfactory tract, CA1 and CA2 pyramidal cells of the hippocampus, medial habenula, and the inferior colliculus. Brain-4 was expressed in the olfactory bulb, striatum, nucleus accumbens, olfactory tubercle, lateral ventricular ependyma, medial habenula, and the paraventricular and supraoptic nuclei. All four Class III genes were expressed in laminar patterns in the neocortex. More detailed analysis is being pursued in the adult and developing mouse.

459.7

C_2H_2 ZINC FINGER GENES EXPRESSED IN THE MOUSE NERVOUS SYSTEM. N. Mazarakis, N. Galjart, J. Brockes*† and F. Grosveld, Lab of Gene Structure and Expression, National Institute for Medical Research, The Ridgeway, London NW7 1AA, UK; †Ludwick Inst.Cancer Res, London W1P 8BT, UK.

The C_2H_2 zinc finger motif is a structure that contains highly conserved pairs of cysteine and histidine residues first identified in the Xenopus transcription factor TFIIIA. It constitutes a sequence specific, nucleic acid-binding domain that is found in a superfamily of invertebrate and vertebrate genes. Some of these genes encode *trans*-activating factors with regulatory roles in cellular growth and differentiation. In an attempt to study the regulation of neuronal differentiation a mouse brain cDNA library was constructed and screened under low stringency conditions with a zinc finger probe from a mouse gene that was shown by Northern blot analysis to generate a 3.7 kb brain specific transcript present during neurogenesis. *In situ* hybridization studies indicated that this message had a pan-neuronal pattern of expression. A large pool of cDNA clones was isolated that contained this and other highly homologous C_2H_2 zinc fingers. At least two novel C_2H_2 zinc finger genes have thus been identified. These data indicate that several members of the zinc finger family are being expressed in the developing mouse nervous system.

459.8

DISPROPORTIONATE REGULATION OF MITOCHONDRIAL AND NUCLEAR GENE EXPRESSION FOR CYTOCHROME OXIDASE SUBUNITS IN NEURONS. R. Hevner* and M. Wong-Riley. Dept. of Cellular Biol. & Anatomy, Med. Coll. of Wis., Milwaukee, WI 53226.

The mitochondrial enzyme cytochrome oxidase (CO) is a model for studying coordination of nuclear and mitochondrial gene expression. The enzyme contains 3 mitochondrial-encoded, and 10 nuclear-encoded subunits. We used *in situ* hybridization to study mitochondrial DNA (mtDNA), CO1 mRNA (mitochondrial-encoded), and CO4 and CO8 mRNAs (both nuclear-encoded), in the visual system of normal and 3-7 day monocular TTX-treated macaques. In all animals, mtDNA and CO1 mRNA were detected in neuropil as well as neurons, while CO4 mRNA and CO8 mRNA were mainly localized in perikarya, confirming our previous findings (J. Neurosci. 11:1942). Compared with normals, TTX-treated animals had not only decreased CO activity (shown histochemically) and CO protein (shown immunohistochemically), but also decreased mtDNA and subunit mRNA levels, in functionally deprived laminae of the lateral geniculate nucleus (LGN) and ocular dominance columns of area 17. After 7 days of TTX, mtDNA fell by 26%; CO1 mRNA by 49%; CO4 mRNA by 18%; and CO8 mRNA by 29%, as determined by computer-assisted grain counting in LGN neurons. CO activity, measured densitometrically, decreased 23%. These results indicate that CO subunit mRNAs are disproportionately regulated in brain, subsequent to functional deprivation. The major acute control over CO activity is probably exerted by regulation of mRNAs for mitochondrial-encoded subunits, which form the catalytic core of the enzyme. (Supported by NIH grants NS18122 and EY05439 to MWR, and by an MCW MSTP fellowship to RFH.)

459.9

GENES WITH TRINUCLEOTIDE REPEATS: POTENTIAL CANDIDATES FOR NEUROPSYCHIATRIC DISORDERS. Ross, C. A.,* Li, S., McInnis, M.G. and Antonarakis, S.E. Departments of Psychiatry and Neuroscience, and Center for Medical Genetics. Johns Hopkins Univ. Sch. of Med., Baltimore, MD. 21205

Expansions of trinucleotide repeats (CGG or CTG) in mRNA underlie three neuropsychiatric disorders: Fragile-X syndrome, X-linked spinal and bulbar muscle atrophy, and Myotonic dystrophy. In addition, in unaffected individuals, the number of repeats is highly polymorphic. In an attempt to find additional genes with trinucleotide repeats, we have screened a human cerebral cortex cDNA library with CGG and CTG repeat oligonucleotides. There were dozens of positives per large plate (~50,000 plaques per plate). Out of an initial ten clones, one was myotonin-protein kinase of myotonic dystrophy (Fu, et al. Science 255 1256) with 13 CTG repeats, two were 28S ribosomal RNA and two were a human homolog of dog SRP (both of which are known to have repeats). In addition, two appear to represent repeat-containing cDNAs with novel sequences. In one (A3) and possibly both, the repeat is within an open reading frame. Genotyping using PCR across the repeat identified one fragment length polymorphism out of six individuals screened. We are now studying an additional series of clones, generated by screening the library at higher stringency, several of which appear to be novel sequences. Genes with trinucleotide repeats may provide markers with high polymorphic index for linkage studies and are potential candidate genes for neuropsychiatric disorders.

459.10

EXPRESSION CLONING OF A RESERPINE-SENSITIVE MONOAMINE TRANSPORTER. B.J. Hoffman*, L.E. Eiden and J.D. Erickson, Lab of Cell Biology, NIMH, Bethesda, MD 20892.

Monoamine transporters are responsible for the accumulation of biogenic amines into secretory organelles of neurons, platelets, basophils and chromaffin cells. Using an expression cloning strategy for transport proteins, we have isolated a novel rat cDNA encoding a monoamine transporter (MAT). Transient expression of the 3.0 kb MAT cDNA resulted in ^{3}H-5HT uptake which was saturable, temperature-sensitive and greatly affected by the pH of the incubation medium. ^{3}H-5HT uptake was inhibited by nigericin and NH_4^+ ions, agents known to dissipate transmembrane pH gradients. ^{3}H-5HT uptake was inhibited by various compounds with the following rank order of potencies: reserpine > tetrabenazine (TBZ) = ketanserin > 5HT > DA > NE > histamine. Photo-labelling of membranes expressing the MAT cDNA with ^{125}I-azido-iodoketanserin followed by SDS-PAGE and autoradiography revealed irreversible labeling of two membrane components with apparent molecular weights of 75,000 and 50,000. The labelling of both bands was blocked by TBZ and was not present in membranes from untransfected control cells. The MAT cDNA has properties consistent with those of the vesicular monoamine transporter.

459.11

NAP, A HUMAN CEREBELLAR DEGENERATION ANTIGEN, IS A NOVEL, NEURON SPECIFIC, ADAPTIN FAMILY MEMBER. M.O. MCKEEVER AND R.B. Darnell. * Lab of Neuro-Oncology, Memorial Sloan Kettering Cancer Center, NY, NY 10021.

We have previously shown that antiserum from a patient with atypical cerebellar degeneration recognizes antigens expressed in cerebellar Purkinje cells and some neocortical neurons (Darnell et al., J. Neurosci 1991;11:1224-1230). Using this antiserum we have isolated a clone from a human cerebellar expression library, which, together with overlapping cDNA's, predict a protein sequence of 851 amino acids, of Mr 96kDa. Antibody affinity purified with the cloned fusion protein recognized the same Purkinje antigens as native patient's antiserum. Sequence analysis revealed homology (31% identity over 296 amino acids) with the clathrin binding domain of the adaptin family of proteins. Northern blot analysis using human polyA+ RNA demonstrated that this gene is expressed only in brain. These results identify a novel neuron specific member of the adaptin family, termed NAP, which is recognized by serum from a patient with human cerebellar degeneration. NAP may be involved in regulating neuronal specific aspects of vesicular trafficing, particularly receptor mediated endocytosis.

POTASSIUM CHANNEL EXPRESSION AND LOCALIZATION

460.1

EXPRESSION OF POTASSIUM CHANNEL TRANSCRIPTS IN THE EMBRYONIC AMPHIBIAN NERVOUS SYSTEM. A. B. Ribera*. Department of Physiology C-240, University of Colorado Health Sciences Center, Denver, CO 80262.

Differentiation of the voltage dependent potassium current in amphibian spinal neurons is delayed with respect to the maturation of calcium current, regulating action potential duration. The molecular basis for this program of electrical excitability is being analysed using probes for voltage dependent ion channel genes. Maturation of potassium current is pivotal for action potential differentiation, and thus efforts have focused on this ion channel.

A second *Xenopus* potassium channel gene expressed in the developing nervous system has been cloned. This *Xenopus* sequence is most related to the mammalian sequences RCK1 and MBK1 and is thus called XSha1. RNase protection assays indicate that XSha1 is expressed in the embryonic nervous system at levels comparable to that of the previously reported potassium channel gene, XSha2. The predicted peptide has 490 amino acids. At the amino acid level, XSha1 is 88% and 75% identical to MBK1 and XSha2, respectively. In the 3' untranslated region of XSha1, there is a region of 60 nucleotides that shows 70% similarity to sequences found in homologous locations in the 3' untranslated regions of MBK1 and RCK1. Functional expression of XSha1 in oocytes induces a delayed rectifier potassium current. The induced current shows high sensitivity to the potassium channel blocker TEA, being reduced by >90% by 15 mM TEA.

The functional properties of XSha1 in addition to its tissue and temporal patterns of expression of XSha1 are consistent with its putative role in regulating excitability of developing spinal neurons.

Supported by NIH NS25217 and NS01531.

460.2

ALTERED POTASSIUM CHANNEL GENE EXPRESSION AND NEURAL DIFFERENTIATION. S. M. Jones* & A. B. Ribera. Department of Physiology C-240, University of Colorado Health Sciences Center, Denver, CO 80262.

Several voltage dependent potassium currents are expressed in embryonic vertebrate neurons and their differentiation regulates the development of action potentials in amphibian spinal neurons. XSha1 and XSha2 are two *Xenopus Shaker*-like potassium channel genes that are normally expressed in the embryonic nervous system. In order to determine how the program for electrical excitability is established, XSha1 and XSha2 were overexpressed in developing embryos.

Overexpression was achieved by injecting capped XSha1 or XSha2 transcripts prepared *in vitro* into 1 cell of a 2 cell stage embryo. Rhodamine-conjugated dextran was included in the injection solution to follow the progeny derived from the original injected cell. Inclusion of potassium channel RNA, but not β-galactosidase RNA, in the injection solution leads to a dramatic decrease in the number of morphologically differentiated neurons *in vitro* that are rhodamine fluorescent. This effect was dose dependent; at concentrations less than 0.2 mg/ml, the potassium channel RNA lost its effectiveness. Neurite extension in whole mount embryos was examined immunocytochemically with acetylated α-tubulin antibodies; these studies indicated that at concentrations greater than 0.2 mg/ml, overexpression of potassium channel RNA led to abnormal motor nerve development.

Electrophysiological analysis of potassium currents in fluorescent and nonfluorescent neurons prepared from embryos injected with this low dose of potassium channel RNA did not reveal any difference in current densities. We are now studying the effects on current density of injection of higher concentrations of XSha1 or XSha2 RNA. Supported by NIH NS25217 and NS01531.

460.3

REGULATION OF mRNA FOR THE Kv3.1 POTASSIUM CHANNEL *IN VITRO* AND *IN VIVO*. T.M. Perney*, X. Li, S. Kang, L.K. Kaczmarek and N.C. Birnberg. Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Transfection of AtT20 cells with the H-*ras* oncogene causes a decrease in the width of action potentials and is associated with the induction of the potassium channel gene, Kv3.1 (Hemmick et al., J. Neurosci., 1992). In this study we have examined the e 's of transmembrane signalling pathways on Kv3.1 gene expressi in wild-type (WT) and *ras* transformed (R1) AtT20 cells. Long-term treatment (24-48 hrs) of WT cells with 10 ng/ml basic fibroblast growth factor (bFGF) resulted in the induction of Kv3.1 mRNA. bFGF treatment also caused a substantial decrease in action potential width consistent with a role for Kv3.1 encoded channels for rapid repolarization. bFGF treatment had little effect on Kv3.1 mRNA levels in R1 cells. In contrast, long-term treatment with 5 mM dibutryl cAMP or 50 mM K^+ increased Kv3.1 mRNA levels in both WT and R1 cells. The effect of depolarization was inhibited by the Ca^{2+} channel blocker, nimodipine, suggesting that the influx of Ca^{2+} is important in regulating Kv3.1 expression. Interestingly, R1 cells treated with high K^+ or dibutryl cAMP display a transient decrease in Kv3.1 mRNA levels at 2-8 hr. Both of these treatments cause a rapid induction of the c-Fos gene and an increase in AP-1 DNA binding activity. It is possible, therefore, that the Kv3.1 gene is regulated in a biphasic manner, acutely through the actions of second messengers and delayed through the action of immediate early genes. Because these same signaling pathways may also be activated by neuronal activity, we examined the effects of seizures on Kv3.1 gene expression. We found that 6 hr after a metrazole-induced seizure levels of Kv3.1 mRNA were decreased approximately 50% in rat hippocampus. We are currently investigating the long-term effects of seizure activity on the expression of the Kv3.1 gene.

460.4

STABLE EXPRESSION OF A RAT BRAIN K+ CHANNEL (K_v3.1) IN HUMAN EMBRYONIC KIDNEY CELLS. S.D. Critz*, B.A. Wible, D.J. Arnold, H.S. Lopez, and A.M. Brown. Dept. of Molecular Physiology & Biophysics, Baylor College of Medicine, Houston, TX 77030.

Biophysical analysis of K+ channels is often made difficult by the diversity of K+ channels found in cells. To address this issue, we have begun to express individual K+ channels using stable transfection of human embryonic kidney (HEK 293) cells. In this study, the biophysical properties of one such stably transfected channel, K_v3.1, were compared to those produced by transient expression following the microinjection of K_v3.1 cRNA into *Xenopus* oocytes.

The region of DNA coding for the K+ channel K_v3.1 was ligated to a mammalian vector, pRC/CMV (Invitrogen ®), which contained a polylinker region, a cytomegalovirus promoter and an antibiotic resistance gene. K_v3.1 DNA was incorporated by lipofection (Lipofectin ®, BRL) at 1 ug DNA /10^6 HEK 293 cells. Twelve out of 15 antibiotic-resistant clones randomly selected for biophysical analysis contained detectable levels of voltage-activated outward current (10.9 ± 2.2 nA at +90 mV, mean ± SEM). In contrast, endogenous currents were less than 200 pA (120 ± 20 pA at +90 mV, n=6) in these cells. The reversal potential of HEK K_v3.1 shifted in Nernstian manner with external [K+] ion concentration. Moreover, the channels were sensitive to concentrations of TEA (IC_{50} 165 μM) that block K_v3.1 channels in *Xenopus* ooctyes. The voltage-dependence of activation of K_v3.1 in HEK cells was similar to that observed in the oocyte ($V_{0.5}$ ≈+5 mV). The voltage-dependence of inactivation appeared to be different, however (+11 mV vs -20 mV, respectively). Single channel data indicate a unitary conductance of approximately 25 pS, comparable to the 22 pS K_v3.1 channels expressed in *Xenopus* oocytes. HEK 293 cells are a suitable mammalian system for the expression of K+ channels and may provide insights into the posttranslational modification of these channels. (Support: F32-NS08579 SDC, NS23877 AMB).

460.5

DIFFERENTIAL EXPRESSION OF K^+ CHANNEL mRNAS IN THE RAT BRAIN AND DOWN-REGULATION IN THE HIPPOCAMPUS FOLLOWING SEIZURES. M.L. Tsaur, M. Sheng, D.H. Lowenstein, Y.N. Jan & L.Y. Jan*. Howard Hughes Medical Institute and Departments of Physiology and Biochemistry, Univ. of California, San Francisco, San Francisco, CA 94143

K^+ channels are major determinants of membrane excitability. Differences in neuronal excitability within the nervous system may arise from differential expression of K^+ channel genes, regulated spatially in a cell-type specific manner, or temporally in response to neuronal activity. We have compared the distribution of mRNA of three K^+ channel genes, Kv1.1, Kv1.2 and Kv4.2 in rat brain, and looked for activity-dependent changes following treatment with the convulsant drug pentylenetetrazole. Both regional and cell-type specific differences of K^+ channel gene expression were found. In addition, neuronal activity caused a reduction of Kv1.2 and Kv4.2 mRNAs in the dentate granule cells of the hippocampus, raising the possibility that K^+ channel gene regulation may play a role in long-term neuronal plasticity.

460.6

ALTERNATIVE SPLICING OF THE 5'-UNTRANSLATED REGION OF A GENE ENCODING K^+ CHANNEL COMPONENTS. C. Kentros*, M. Weiser, E. Vega-Saenz de Miera, K. Morel, H. Baker, and B. Rudy. Dept. Physiology, NYU Med. Ctr., N.Y. 10016 and W.M. Burke Medical Res. Inst. White Plains, N.Y. 10605

Transcripts of the rat KShIIIA gene, one member of the Shaker III gene subfamily, express delayed rectifier voltage-dependent K^+ currents in *Xenopus* oocytes. We and others have previously reported the isolation of cDNAs encoding several alternatively-spliced versions of this and other genes of this subfamily predicting proteins differing in their carboxyl ends. The *Drosophila* Shaker gene encodes alternatively-spliced transcripts with different 3' and 5' coding regions. We now report the isolation of two types of transcripts of the KShIIIA gene with divergent 5'-untranslated regions (5' UTR). Their 5' sequences differ up to a point 5 bases upstream of the putative start codon (position -5). The presence of an AG, the dinucleotide characteristic of the exon boundary of 5' splice junctions, at position -4 supports the premise that these different 5' UTRs result from alternative splicing. Unlike the fly Shaker gene, these 5' alternative exons encode only untranslated sequence. Northern blot analysis of rat brain mRNA with probes specific to KShIIIA show a complex banding pattern that is not resolved by the utilization of probes specific to the 3' alternatively-spliced exons of this gene. However, Northern blots hybridized with probes specific to the two alternatively-spliced 5' UTR exons, designated alpha and beta, produce distinct banding patterns. On the basis of both Northern blot analysis and corroborating in situ hybridization studies, alpha is more highly expressed than beta in the rat CNS. As it is thought that 5' UTRs are involved in regulation of the processing and translation of mRNA, these results raise the intriguing possibility of differential post-transcriptional regulation of K^+ channel expression at the RNA level.

460.7

COMBINATORIAL ASSEMBLY OF THE EAG POLYPEPTIDE WITH K^+ CHANNEL SUBUNITS FOR MEDIATING cGMP-DEPENDENT MODULATION OF K^+ CURRENTS IN DROSOPHILA. Y. Zhong* and C.-F. Wu. Dept of Biology, Univ. of Iowa, Iowa City, IA 52242.

Co-expression of different (*Shaker*) *Sh* subunits in the *Xenopus* oocyte has confirmed the oligomeric assembly of the voltage-activated I_A channels. However, the transient K^+ current I_A in *Drosophila* muscles is affected not only by *Sh* mutations but also by those of the *ether à go-go* (*eag*) locus, which encodes a polypeptide homologous to, but distinct from, *Sh* subunits. This suggests participation of products from different genes in the assembly of native K^+ channels. Existence of multiple *eag* and *Sh* alleles enabled the testing of this idea in various double-mutant combinations and allowed an investigation of the functional role of different types of subunits in the channel.

Voltage-clamp analysis of amplitude, time course, and steady-state inactivation revealed that the effect of eag^1, eag^{4PM}, eag^{24} on I_A varied with *Sh* alleles in the background. For instance, the amplitude of I_A is increased in eag^1 Sh^+, decreased in eag^1 Sh^5, but unchanged in eag^1 Sh^{120}, as compared to each of the corresponding *Sh* background. The allele-specific interactions in *eag Sh* double mutants reflect a close association between the *Sh* and *eag* subunits within the I_A channel. Since *eag* mutations affect four identified K^+ currents in *Drosophila* muscles, including the voltage-activated delayed I_K, the *eag* subunit may be present in multiple K^+ channels.

The functional significance of such heteromultimeric assembly of K^+ channels is suggested by our finding that modulation of I_A and I_K in response to application of cGMP (2 mM) and its analogs, 8-Br cGMP (500 μM) and dibutyryl cGMP (500 μM), is either altered or eliminated by different *eag* mutations. The role of the *eag* subunit in channel modulation, as opposed to that of *Sh* subunits in determining the channel gating and conductance, supports the notion that at least two classes of subunits, each dedicated to different functions, co-assemble into a variety of K^+ channels.

460.8

MEMBERS OF A MOUSE SUBFAMILY OF GENES ENCODING VOLTAGE-GATED POTASSIUM CHANNEL SUBUNITS FORM HETEROMULTIMERS WHEN COEXPRESSED IN *XENOPUS* OOCYTES. W.F. Hopkins* and B.L. Tempel, Depts. of Pharmacology and Medicine, Univ. of Washington and VA Medical Center, Seattle, WA 98108.

Potassium channels with diverse functional properties could arise, in principle, by the aggregation of nonidentical subunits. We wished to determine if heteromultimer formation was possible for the products of MK1 and MK3 mouse genes, members of the mammalian Shaker-like subfamily, that encode voltage-gated potassium channels. We also sought to test the hypothesis that potassium channels are comprised of 4 subunits. When MK1 and MK3 were expressed in *Xenopus* oocytes using the two-electrode voltage-clamp technique, we observed potassium currents that differed in their sensitivity to fraction I of dendrotoxin (DTX-I). The Ki's for DTX-I in oocytes expressing MK1 (N=7) and MK3 (N=9) were 3 and 4533 nM, respectively. We exploited this large difference in DTX-I sensitivity to test if heteromultimers comprised of MK1 and MK3 subunits form when coexpressed in oocytes. When MK1 and MK3 were coexpressed in several different ratios, the DTX-I sensitivities of the resulting currents were always greater than would be predicted by an additive model based on the hypothesis that MK1 and MK3 form only homomultimers. With a MK1:MK3 = 1:3 ratio, the Ki was 14 nM (N=4), which is much less than the Ki of 3100 nM predicted by the additive model. This suggests that heteromultimers composed of MK1 and MK3 subunits with intermediate DTX-I sensitivities can form in oocytes and that they have DTX-I sensitivities much closer to MK1 than to MK3. This implies that one MK1 subunit confers nearly full DTX-I sensitivity on a channel complex. The approach of MacKinnon (Nature 350: 232, 1991) was used and the results were consistent with a channel complex composed of 4 subunits. Supported by NIH NS27206 and the VA.

460.9

FUNCTIONAL INTERACTIONS BETWEEN SUBUNITS OF A CHIMERIC K^+ CHANNEL. G.E. Kirsch*, J.A. Drewe, M. DeBiasi, M. Taglialatela, H.A. Hartmann, and A.M. Brown. Departments of Anesthesiology, and Molecular Physiology and Biophysics. Baylor College of Medicine, Houston TX 77030.

We have shown previously that in a chimeric K^+ channel (22 pS K^+ conductance, expressed in *Xenopus* oocytes) the point mutation V369I shortens channel open time and causes fast inactivation, without affecting conductance (De Biasi, this meeting); whereas, L374V shortens channel open time and markedly reduces conductance (5 pS) with little effect on inactivation. Furthermore, the double mutation V369I+L374V increases channel open time and produces an intermediate level of conductance (10 pS). Here we show that the fast inactivation produced by V369I is abolished in the double mutant by increased burst duration. We tested for functional interaction of non-consecutive pore residues across subunit boundaries by co-injecting a mixture of cRNAs encoding the point mutants V369I and L374V. Two major classes of channels with intermediate conductances (8 and 14 pS) were observed in addition to the expected homotetrameric channels. Unlike the short-opening homotetramers, both classes of putative heterotetrameric channels had burst durations and open times approaching those of the double mutant. These results suggest that pore residues residing in adjacent subunits form a closely packed structure which determines both ion conductance and stability of the open state of the channel. Supported by NIH grants NS29473 (GEK), and NS23877 and HL37044 (AMB).

460.10

TARGETING OF WILD TYPE AND MUTANT DRK1 K^+ CHANNEL POLYPEPTIDES IN POLARIZED AND NONPOLARIZED MAMMALIAN CELLS. J. S. Trimmer, M. G. Arreaza, D. A. Brown, S. Halegoua, N. V. Marrion, P. Brehm*, and A. K. Kleinklaus. Depts. of Biochemistry & Cell Biology, and Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

Spatial segregation of voltage-sensitive ion channels in surface membranes of neurons and muscle fibers is critical to the conduction of electrical impulses. We have begun to investigate the molecular mechanisms that govern this restricted expression using the drk1 (K_v2.1) K^+ channel polypeptide as a model. We have expressed wild type and mutant drk1 polypeptides in polarized and nonpolarized cells by transfecting drk1 cDNAs into fibroblast (COS), epithelial (MDCK) and neuronal (PC12) cell lines, and have begun to characterize the targeting and functional characteristics of these drk1 polypeptides in these different cellular backgrounds. Expression of wild type and mutant drk1 polypeptides in COS cells is highly efficient, with the majority of expressed polypeptide found on the cell surface as judged by: 1) vectorial labelling with membrane impermeant biotinylation reagents; 2) plasma membrane localization using immunostaining; 3) presence of large (20 nA = 500 pA/pF) whole cell K^+ currents. Studies with membrane impermeant, reducible crosslinking reagents show that both wild type and mutant drk1 polypeptides form multisubunit channel complexes on the cell surface. MDCK and PC12 cell lines stably expressing drk1 polypeptides have been generated. Studies to determine the cell surface localization of drk1 in these cells are in progress.

460.11

SUBCELLULAR SEGREGATION OF A-TYPE POTASSIUM CHANNELS IN RAT CENTRAL NEURONS. Morgan Sheng, Meei-Ling Tsaur, Yuh Nung Jan,* and Lily Y.Jan Howard Hughes Medical Institute, Depts. of Physiology and Biochemistry, Univ. California, San Francisco, CA 94143-0724.

In the mammalian nervous system, K channels regulate diverse aspects of neuronal function and are encoded by a large set of genes. The roles of the many different K channels that are expressed in individual neurons could be dictated by their localization to specific subcellular domains. Using gene-specific antibodies, we show that two K channel polypeptides, Kv1.4 and Kv4.2, that give rise to A-type currents when expressed in Xenopus oocytes, are segregated in rat central neurons. Kv1.4 is targeted to axons and possibly terminals, while Kv4.2 is concentrated in dendrites and somata. Translation of both proteins, however, occurs in the cell body. The differential distribution implies distinct roles for these channel proteins in vivo; their localizations suggest that Kv1.4 and Kv4.2 may regulate synaptic transmission via presynaptic, or postsynaptic mechanisms, respectively.

460.12

CONTRASTING IMMUNOHISTOCHEMICAL LOCALIZATIONS IN THE RAT BRAIN OF TWO NOVEL K^+ CHANNELS OF THE *SHAB* SUBFAMILY. P.M. Hwang*, M. Fotuhi, D.S. Bredt and S.H. Snyder. Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

A number of mammalian voltage-dependent K^+ channels with closely similar amino acid sequences and electrophysiologic properties have been cloned. However, little is known about why multiple K^+ channels genes are needed to subserve similar functions. We have recently identified, cloned and characterized a novel K^+ channel, designated CDRK (Hwang *et al.*, Neuron, 8:473-481, 1992). CDRK appears to be a member of the *Drosophila Shab* K^+ channel subfamily, most closely resembling DRK1 (Frech *et al.*, Nature, 340:642-645, 1989), the only other rat homolog of *Shab*. Electrophysiologic analysis of expressed CDRK reveals delayed rectifier properties similar to those of DRK1. We now have made synthetic peptide antibodies specific for CDRK and DRK1 proteins. Remarkable contrasting immunohistochemical localizations in the brain as well as in peripheral tissues have been observed for both channels, suggesting that these highly homologous K^+ channels with distinct localizations may play unique roles in the control of membrane potential in various excitable tissues.

PROCESS OUTGROWTH, GROWTH CONES AND SPROUTING V

461.1

CENTRAL vs PERIPHERAL MECHANISMS FOR SPROUTING OF THINLY AND UNMYELINATED PRIMARY AFFERENT FIBERS IN THE PARTIALLY DENERVATED SPINAL CORD. **J.X. BAO,* P.J. Reier and J.B. Munson Dept. of Neuroscience, Univ. of Florida, Gainesville, FL 32610.**

The goal of this study is to study the possible central and peripheral mechanisms for the sprouting of primary afferent fibers in the partially denervated spinal cord.

The first group of adult Sprague-Dawley rats (n=5) received unilateral dorsal root ganglionectomies of L1-L4 on one side and rhizotomies of L1-L4 dorsal roots on the other side. The second group of rats (n=3) received unilateral dorsal root rhizotomies of L1-L4 dorsal roots on the right or left side followed seven weeks later by contralateral rhizotomies. Two months after the first surgery, all rats were anesthetized, and stimulated by immersing both of their hindlimbs in 52 ^{0}C water for 20 seconds. The rats were perfused two hours later. CGRP and c-fos immunocytochemical staining was then performed.

Preliminary results indicate that CGRP immunoreactive fibers had sprouted into a new area (lamina III) on the chronic ganglionectomized sides. Also, the area of CGRP immunoreactive fibers and the area occupied by c-fos immunoreactive cells were much greater on the ganglionectomized sides in the first group of rats, and much greater on the chronic side in the second group of rats. These results suggest both central and peripheral denervation contribute to the sprouting of thinly myelinated and unmyelinated primary afferent fibers in the partially denervated spinal cord. Furthermore, combined central and peripheral denervation (via ganglionectomy) induces sprouting of small primary afferent fibers into novel regions. **Supported in part by NS15913 and NS27511.**

461.2

THE SPATIOTEMPORAL EXPRESSION PATTERN OF THE CANDIDATE CELL ADHESION MOLECULE NEUROLIN IN GOLDFISH. [1]C.A.O. Stuermer*, [2]F. Lottspeich and [1]K.A. Paschke. [1]Faculty Biology, Univ. Konstanz, 7750 Konstanz, [2]Max-Planck-Inst., 8033 Martinsried,Germany

Using the monoclonal antibody E21 (Soc. Neurosci. Abstr. 16, 1990) we identified an 86kd cell surface glycoprotein (Neurolin) in the goldfish brain. Neurolin exhibits a striking spatiotemporal expression pattern.

The retinal ganglion cells (RGCs) of embryos and their axons, as well as those of the marginal retinal growth zone in adults, carry E21 staining over their entire surface. On mature RGCs E21 staining is restricted to contact sites between the cells and to the zone of RGC axon terminal arbors in the tectum. After optic nerve transection the regenerating axons re-express Neurolin throughout their path while the RGCs keep it restricted to their contact sites. The NH2-terminal aminoacid sequence of Neurolin has similarities to that of the recently discovered cell adhesion molecule DM Grasp (Burns et al., Neuron 7, 1991) and SC1 (Tanaka et al., Neuron 7, 1991) of the chick spinal cord.

In fact, E21 also stained motoneurons, floorplate cells and the DRG entry zone and tract in the developing goldfish spinal cord and only the latter in adults.

Neurolin may represent either the goldfish homologue of DM-Grasp/SC1 or another member of that family.

461.3

ASTROCYTES DEMONSTRATE REGIONAL DIFFERENCES IN PROMOTING DENDRITIC GROWTH. [1]P. Le Roux* and [2]T. Reh. Depts. of [1]Neurol. Surg., and [2]Biol. Struct., University of Washington, Seattle, WA 98195.

Glia from different regions of the CNS have been shown to be heterogenous in their ability to promote neurite outgrowth from neurons. However, it is not known whether glia from different CNS regions differ in their abilities to promote axons vs dendrites from neurons. Therefore we compared total dendritic and axonal growth from embryonic mouse cortical neurons (E 18) grown *in vitro* on early postnatal rat (P4) astrocytes purified from cortex, mesencephalon, striatum, olfactory bulb, spinal cord (GFAP> 90%+); Muller glia from retina; and fibroblasts. Double immunolabelling (MAP2, M6, NF-H) was used to identify mouse cortical dendrites and axons after 5 DIV. While axon length was similar on all glial monolayers, dendritic growth was nearly threefold greater on cortex, retina and olfactory bulb than from other areas of the CNS. These findings demonstrate regional differences in the ability of astrocytes to support dendrogenesis. Supported by NIH NS 30305.

461.4

PP60C-SRC REGULATES L1-MEDIATED AXON OUTGROWTH M.A. Ignelzi, D.R. Miller, P. Soriano, and P.F. Maness*. Dept. Biochemistry. Univ. North Carolina, Chapel Hill, NC 27599.

We have recently shown that triggering of the neural cell adhesion molecule L1 with specific ligands or antibodies inhibits tyrosine phosphorylation by pp60c-src of tubulin isoforms in a subcellular membrane fraction of nerve growth cones from fetal rat brain, whereas triggering of integrins does not (Atashi et al., Neuron 8: 1, 1992). To determine whether pp60c-src was a specific transducer of signals important for regulation of axonal extension on L1, cerebellar neurons from wild type and homozygous mice lacking a functional c-src gene (Soriano et al., Cell 64: 6903, 1991) were analyzed for neurite outgrowth on purified L1 or laminin adsorbed to nitrocellulose-coated culture dishes. Cerebellar neurons from src-deficient mice extended neurites on both substrates, indicating that pp60c-src was not required for process outgrowth. However, average neurite length measured 10 hr after plating on L1 was significantly reduced in cultures of src-deficient neurons (26.6 μm + 0.4) compared to wild type neurons (49.5 μm + 1.3). No differences in neurite lengths were measured for neurons plated on laminin in src-deficient (29.4 μm + 1.2) or wild type cultures (28.9 μm + 1.2). Adhesion of src-deficient neurons on L1, but not laminin was also reduced compared to wild type neurons. Differences in neurite outgrowth and cell adhesion persisted 20 hr after plating. Computer-assisted morphometric analysis of live growth cones revealed no differences in growth cone area or filopodial number between src-deficient and wild type neurons growing on L1. These results demonstrate that pp60c-src acts as a positive regulator of L1-mediated axon outgrowth.

461.5

ACTIVITY-SENSITIVE SIGNALLING BY INSULIN-LIKE GROWTH FACTORS IN THE DEVELOPING AND REGENERATING NEUROMUSCULAR SYSTEM. P. Caroni* and C. Schneider. Friedrich Miescher Institute, P.O. Box 2543, CH-4002 Basel.

Synapse elimination during development and nerve sprouting in the adult affect the arrangement of presynaptic terminal branches and are coupled to postsynaptic activity. Thus, the prolonged absence of postsynaptic activity leads to sprouting in the adult and prevents the retraction of collaterals during development. In the neuromuscular system, lack of muscle activation leads to a rapid elevation of muscle fiber-derived IGF1 in the adult and prevents the developmental downregulation of muscle IGFs during synapse elimination. We have searched for motoneuron mRNAs and proteins whose levels are controlled by muscle activity and have determined whether muscle-derived IGFs are sufficient and/or necessary to mediate activity-sensitive retrograde signalling from muscle to spinal motoneurons. We report that: 1) the mRNAs coding for the growth-associated proteins (GAPs) GAP-43 and tubulin-a1 are downregulated in rat and chick spinal motoneurons at the onset of the synapse elimination process; levels of terminal-associated GAP-43 decline during the elimination process; 2) motoneuron GAP downregulation is controlled by the periphery: it is prevented by local muscle paralysis or by elevated levels of IGFs in the muscle; 3) muscle-derived IGFs accumulate at the neuromuscular junction and are taken up and transported in motor nerves; 4) blockade of IGFs in the muscle by local applications of recombinant IGF-binding protein prevents the effect of paralysis on motoneuron GAP downregulation and on the pattern of intramuscular nerve growth.Therefore, IGFs mediate activity-sensitive retrograde signalling from skeletal muscle to spinal motoneurons.

461.6

CILIARY NEUROTROPHIC FACTOR (CNTF) INDUCES SPROUTING BY ADULT LYMNAEA NEURONS IN VITRO. A.G.M. Bulloch*, N.I. Syed and P.M. Richardson. Dept. Med. Physiol., Univ. Calgary, Fac. of Med., Calgary, AB and Neurosurg. Dept., Montreal Gen. Hosp., Montreal, Que.

This study is part of a series attempting to identify endogenous neurotrophic factors in molluscs. Neurons from the freshwater snails Lymnaea and Helisoma are usually cultured in vitro in brain conditioned medium. Recently we showed that Lymnaea conditioned medium contains NGF-like molecules and that murine NGF evokes sprouting from specific neurons. Here we extend these studies to another mammalian neurotrophic factor, ciliary neurotrophic factor (CNTF). Motoneurons, interneurons and neurosecretory cells from Lymnaea were isolated and cultured in vitro in defined medium with or without CNTF. One class of motoneurons (Pedal A cluster) and some interneurons exhibited sprouting in response to CNTF over a dose range of 250-1000 pM. Neurons of adult molluscs are thus now known to respond to two classes of mammalian neurotrophic factors (the neurotrophins and CNTF), neither of which is required for survival. Supported by MRC (Canada) and the National Centres of Excellence.

461.7

EFFECT OF CNTF ON NEURITE OUTGROWTH FROM CULTURED SPINAL NEURONS. N.M. Oyesiku* and D.J. Wigston. Program in Neuroscience and Dept. of Physiology, Emory University School of Medicine, Atlanta, GA 30322.

There is a serious need for agents that may promote axonal growth after spinal cord injury. Although ciliary neurotrophic factor (CNTF) can enhance the survival of a class of spinal neurons, motoneurons, its effects on the growth or regeneration of axons from motoneurons or other spinal neurons are not known.

To investigate the effect of CNTF on neurite outgrowth from spinal neurons, we dissociated neurons from the lumbar spinal cords of stage 30 (~E6) chick embryos and plated them on laminin-coated glass coverslips in medium with or without CNTF (recombinant human CNTF; Synergen). By 48 hr, we found that neurites were about 65-100% longer and the total neurite length per neuron was 60-150% greater in cultures exposed to CNTF ($p<0.025$). In addition, CNTF enhanced neuronal survival. The enhanced neurite outgrowth was not a consequence of the higher cell density in CNTF-treated cultures: untreated cells that were plated so that their final density matched that of CNTF-treated cultures did not show enhanced neurite outgrowth. To examine the effect of CNTF on subpopulations of spinal neurons, we first compared cultures derived from the ventral spinal cord, which contains motoneurons, with cultures derived from the dorsal spinal cord which lacks motoneurons. CNTF enhanced neurite outgrowth to about the same extent in ventral and dorsal cultures. We also identified motoneurons in some experiments by labelling them retrogradely with diI. The effect of CNTF on neurite outgrowth was similar for labelled and unlabelled neurons, and was therefore not specific to motoneurons.

We conclude that CNTF promotes neurite outgrowth from spinal cord neurons *in vitro*. CNTF may have a similar effect on neurons in the injured spinal cord. (Supported by the American Paralysis Association.)

461.8

SEROTONIN AND ACETYLCHOLINE ALTER BRANCHING DECISIONS AND SOMA SIZE OF DEVELOPING LEECH RETZIUS NEURONS. S.M. Elsas and G.S. Stent*. Dept. of Molecular and Cell Biology, Univ. of California, Berkeley, CA 94720.

Embryonic axons of Retzius neurons (Rz), one of 5 types of 5-HT neurons in leech segmental ganglia, show a stereotyped sequence of pathway and branching decisions. Since synthesis of 5-HT and receptors for 5-HT and ACh appear early in Rz axon outgrowth, we asked whether 5-HT or ACh help regulate these decisions.

In *Theromyzon rude*, whose normal sequence of Rz axonal branching differs from that previously described in *Hirudo*, the primary axon forms a T-branch; the posterior arm of the T grows into the DP peripheral nerve. Then a secondary branch from the posterior arm grows into the posterior interganglionic connective nerve, and the anterior arm grows into the anterior connective nerve. When Rz connective axons from adjacent segments meet, they fasciculate. Then a new axon grows from near the branch point of the T into the MA peripheral nerve. Later all Rz somata except those in segments M5 and M6 enlarge to become the largest somata in a ganglion.

Embryos of *T. rude* were incubated for 2-5 days, during the period of axon outgrowth, in media containing 10mM 5HT or 10mM ACh with 50mM eserine. Rz morphology was visualized by anti-5HT antibody staining or by lucifer yellow injection.

Following ACh incubation, initial axonal outgrowth was normal. But when the connective axons of Rz neurons in adjacent ganglia would normally meet and fasciculate, all connective axons disappeared. Then a new anterior connective axon arose from the posterior arm of the T (instead of from the anterior arm), and the MA axon arose from this new branch (instead of from the T). A new posterior connective axon regrew in the original position. ACh incubation also led to a substantial increase in anti-5HT staining intensity in Rz neurons, but not in other 5-HT neurons.

Prolonged 5-HT incubation reduced anti-5HT staining in Rz neurons to near invisibility. Staining of other 5-HT neurons was unaffected. This selective effect on Rz reflects a change in transmitter metabolism, since uptake of 5-HT during brief (1h) incubation increases anti-5HT staining intensity. Prolonged 5-HT exposure also caused all Rz somata to remain small, which is normal for segments M5 and M6.

The results show that exogenous 5-HT or ACh can induce changes in transmitter content, soma size and axon branching in Rz neurons. Therefore these transmitters are likely to play a role in the regulation of these decisions in Rz development.

461.9

ANALYSIS OF ASYMMETRY IN THE DEVELOPMENT OF IDENTIFIED NEURONS IN THE GRASSHOPPER. P.Z. Myers*, C.L. Byars, and M.J. Bastiani. Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

One possible role for interactions between growth cones and their substrates is in directly influencing pathfinding choices. Another potentially significant but more subtle function may be in regulating rates of growth cone migration; growth cones that are delayed by 3% or more may fail to find appropriate cues, leading to pathfinding aberrations. Using time-lapse video microscopy, we have quantified migration rates of an identified growth cone, that of the grasshopper neuron Q1. We have correlated one specific and reproducible change in growth rate, a pause at the midline, with a specific interaction with the growth cone of Q1's contralateral homolog. We have also developed a novel methodology, analysis of asymmetry between contralateral homologs of individually identifiable neurons, that allows us to identify interactions that affect growth cone velocity and also quantifies the amount of variability inherent in a simple developmental system.

The basic principle behind the analysis is that substrates that reduce growth rates act as bottlenecks that reduce any disparity in axon length between contralateral homologs; similarly, substrates that permit accelerated growth would increase the disparity. In agreement with our time-lapse observations, we see that the mean disparity in axon length between the two Q1 axons within a single segment decreases from an initial 16.7 μm to 3.5 μm upon contact at the midline. One function of this interaction between the two Q1s at the midline may be in synchronizing outgrowth.

We have also quantified the behavior of another identified neuron, MP1, and derived information about the general level of variability in the growth of both MP1 and Q1. The only events that reset the growth rates of these neurons are 1) the midline interaction by Q1, and 2) the arrival of the MP1 growth cone at the next caudal segment. Homologous axons vary in the time of initiation by 2.4±2 hours, or 0.5±0.4% of development. Most of the variability between homologs arises by this difference in initiation time; the growth rates of the two MP1 neurons in a segment vary by less than 0.4 μm/hr from one another, and the two Q1s vary by up to 1.2 μm/hr.

Supported by NS25378 and the McKnight Foundation.

461.10

SHAPE CHANGES IN EMBRYONIC LEECH GROWTH CONES REFLECT TRANSITIONS IN OUTGROWTH. D.M. Kopp* and J. Jellies. Neurobiology Research Center and Dept. of Physiol. and Biophysics, Univ. of Alabama at Birmingham, Birmingham, Al, 35294.

Correlations between growth cone shapes and molecular and cellular guidance signals suggest that studying changes in morphology may help characterize these underlying guidance cues *in situ*. We have analyzed the morphology of a set of growth cones on the Comb- or C-cell in the embryonic medicinal leech, *Hirudo medicinalis*, as they undergo a transition to rapid extension. The C-cell is a transient cell found as a bilateral pair in each midbody segment. Each C-cell adds and orients about 70 parallel growth cones which remain relatively non-motile until E12 when rapid and directed process outgrowth is initiated. C-cells from the same segment at E10, E11, E12, and E14 were injected with Lucifer Yellow and processed with antibodies to visualize their growth cones in whole mounts. A central subset of obliquely-oriented growth cones (n=583) were traced using DIC optics and camera lucida. Morphology was digitized from these tracings and a computer program (MacMeasure) was used for analysis and computation. Contrary to expectations for growth cones that become more rapidly advancing, lamellar regions and filopodial sampling increased with age. Young, relatively non-motile growth cones had numerous short filopodia in many orientations, while the more rapidly advancing growth cones showed a decrease in filopodial number, an increase in filopodial length, and a striking restriction of filopodial orientation to the direction of process outgrowth. However, the average filopodial angle was predictive of the direction of outgrowth at all stages, suggesting that while direction of growth could always be predicted by a vector sum of filopodial trajectories, younger quiescent growth cones respond to different cues (or similar cues differently) than older, more rapidly extending ones. These results support the view that C-cell growth cones are not fasciculating and further suggest that they progressively alter filopodial extension/retention in the manner predicted if their affinity for local cellular cues was superceded by a more distributed set of extrinsic guidance signals. Supported by NIH NS 28603 (JJ).

PATTERN FORMATION, COMPARTMENTS AND BOUNDARIES III

462.1

THE ROLE OF THE ZEBRAFISH SCLEROTOME IN PERIPHERAL NERVOUS SYSTEM SEGMENTATION. Elizabeth M. Morin-Kensicki* and Judith S. Eisen, Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Sclerotome has been implicated in patterning peripheral nervous system (PNS) segmentation in vertebrate embryos. We have identified sclerotome cells in embryos of the zebrafish, *Brachydanio rerio*, based on the following criteria: these cells 1) delaminate from the ventromedial aspect of each somite, 2) become mesenchymal, and 3) migrate to positions where vertebrae later form. In each embryonic zebrafish trunk segment, sclerotome cells migrate along a pathway that later is traversed by the growth cones of axial motoneurons and by some neural crest cells. We tested the role of sclerotome in patterning zebrafish dorsal root ganglia (DRGs) and axial motor nerves by ablating premigratory sclerotome by aspiration and observing the pattern of these PNS structures at later stages by staining with specific antibodies. Removal of sclerotome did not disrupt the pattern of the DRGs or the axial motor nerves. These findings suggest that zebrafish sclerotome is not required for proper formation and segmentation of DRGs or axial motor nerves. Our results are in marked contrast to findings in avian embryos where sclerotome does appear to be required for these processes and thus, we suggest that there are different mechanisms for establishing PNS segmentation in different vertebrate species.

NIH HD07348, HD22486, GM070257, NS23915

462.2

PATTERNING AND INDUCTION IN A ZEBRAFISH MUTANT THAT LACKS A NOTOCHORD. M. E. Halpern*, R. K. Ho, S. Schulte-Merker[1], C. Nüsslein-Volhard[1], C. Walker, and C. B. Kimmel. Institute of Neuroscience, University of Oregon, Eugene, Oregon, 97403 and Max-Planck-Institut für Entwicklungsbiologie[1], 7400 Tübingen, Germany.

In the zebrafish embryo, the notochord is derived from precursor cells that involute during gastrulation to form the dorsal mesoderm. As in other vertebrate embryos, dorsal mesoderm may play an important role in the induction of the central nervous system (CNS).

We have recovered two allelic, recessive lethal mutations that produce embryos with no notochords or tails (*ntl*). Cell lineage studies suggest that dorsal mesoderm is present in *ntl* embryos, although subsequent notochord differentiation is blocked. Phenotypically, *ntl* embryos resemble mouse *Brachyury* (or T gene) mutants. They also fail to express a protein homologous to the mouse T protein, which is an early marker of notochord development. Molecular analysis confirms that *ntl* is in fact the zebrafish homologue of the T gene.

Although the notochord is absent in *ntl* mutants, the induction and differentiation of the CNS appears normal. In contrast, patterning of somitic mesoderm is abnormal due to the absence of engrailed expressing muscle pioneer cells. However, the somite defects can be rescued through transplantation of wild-type (WT) cells into *ntl* hosts. These experiments demonstrate that mutant host mesoderm is capable of forming muscle pioneers in the presence of notochord derived from WT donor cells.

We propose that the dorsal mesoderm has multiple cell signalling roles in the development of the zebrafish embryo. In *ntl* mutants, the signals required for CNS induction are retained, while the signals required for muscle pioneer induction and somite patterning are disrupted.

462.3

EXPANDED ROLE OF MIDLINE SIGNALING IN THE DORSO-VENTRAL BRAIN POLARITY OF ZEBRAFISH. Kohei Hatta, Andreas Püschel, Monte Westerfield and Charles B. Kimmel*, Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

The zebrafish *cyclops* mutation deletes the entire ventral midline of the central nervous system (CNS), including the ventral forebrain and floor plate in the brain and spinal cord, without disturbing surrounding mesodermal structures. In the spinal cord and caudal hindbrain, positioning of most neurons is unaffected although pathfinding of some axons is severely disrupted. The phenotype is more severe in the anterior CNS; in the diencephalon, the ventral neuronal groups and longitudinal axonal tracts are deleted. To characterize the phenotype in more detail, we examined four homeobox genes (*eng*, *hox3.3*, *pax2*, *pax6*) that are expressed in specific positions in the CNS. Among them, *pax6* is expressed in the wild type as a bilateral pair of domains in the diencephalic primordium just after gastrulation. These domains are fused at the midline in *cyclops*. At later stages, the mutant expression pattern of each of the homeobox genes shifts ventrally in more anterior regions of the brain. These results suggest that *cyclops* alters the early fate map and dorso-ventral positional information of the CNS.

Transplantation of a single wild-type cell that comes to occupy the ventral midline in the mutant locally rescues the forebrain and eye phenotypes. We argue that proper establishment of positional information in the forebrain requires inductive signaling from ventral midline cells, whose specification depends on the *cyclops* gene product. (Supported by NIH grants NS17903, HD22486, and NS21132)

462.4

EXPRESSION OF Tes-1(Dlx-2): A HOMEODOMAIN-CONTAINING GENE THAT IS EXPRESSED IN DISCRETE DOMAINS OF THE EMBRYONIC MOUSE VENTRAL FOREBRAIN. J.L.R. Rubenstein*, A. Bulfone, M. Frohman#, M.H. Porteus, D. Xu. Neurogenetics Laboratory, LPPI, Box F, 401 Parnassus, UCSF, San Francisco, CA 94143-0984. #Department of Pharmacology, SUNY, Stony Brook, NY.

Using subtractive hybridization and screening with homeodomain-encoding probes, we isolated, from an E14.5 mouse telencephalon cDNA library, a 2.4 kb cDNA encoding a novel homeodomain-containing protein called Tes-1. The Tes-1 homeodomain is identical in 53 out of 61 amino acids with the homeodomain of the Drosophila *distal-less* gene, and is part of the newly discovered Dlx family of homeobox-containing genes. It is also named Dlx-2. Unlike other vertebrate homeodomain-containing genes, Tes-1 is predominantly expressed in the head. Within the brain of the midgestational embryo, Tes-1 is expressed in the ventral forebrain. *In situ* RNA hybridization shows that Tes-1 is expressed in discrete domains within the embryonic forebrain. In the diencephalon, these domains form sharp borders with the expression pattern of another homeobox gene, and a gene encoding a putative growth factor.

462.5

NEUROGENESIS AND NEURITE GROWTH DEFINE A DISTINCT DOMAIN IN THE VENTROLATERAL WALL OF THE DEVELOPING MOUSE FOREBRAIN. A.K. McAllister*, J. Whitesides, A.-S. LaMantia. Department of Neurobiology, Duke University, Durham,NC 27710.

Segmentation has been implicated in the development of the spinal cord and hindbrain. In the forebrain, however, evidence for distinct embryonic domains—a hallmark of segmental development—has remained elusive. We report here a distinct domain in the developing mouse forebrain that is defined by early neurogenesis and specific axon ingrowth. This unique but transient domain appears in the outer 2/3 of the ventrolateral wall of the forebrain soon after the formation of two distinct telencephalic vesicles from the single prosencephalon. The earliest neurogenesis in the forebrain, indicated by MAP-2 staining and Di-I labelling, occurs in this zone. In addition, the first neurite growth in the developing forebrain is limited to this region as shown by immunostaining for neurite specific molecules GAP-43 and L1 as well as by EM analysis. Di-I tracing indicates that many of these neurites originate in the olfactory epithelium. The ventrolateral domain disappears as rudiments of the major forebrain subdivisions—the neocortex, hippocampus, basal ganglia, basal forebrain, and olfactory bulb—emerge.

This temporally and spatially restricted pattern of neurogenesis and neurite growth delineates a distinct domain in the developing mouse forebrain. This domain differentiates before rudiments of the forebrain subdivisions are seen and may be crucial to their formation. The ingrowth of axons from a specific source, the olfactory epithelium, further suggests that this regional specialization may be required for establishing initial axonal projections to the forebrain.

462.6

DIFFERENTIAL ADHESIVITY IN DISTINCT DOMAINS OF THE DEVELOPING FOREBRAIN. J.G. Whitesides* and A-S. LaMantia. Department of Neurobiology, Duke University, Durham, NC 27710.

Early in mouse forebrain development the ventrolateral wall of the telencephalon has unique cellular and molecular characteristics. Immunohistochemical staining shows that this region is reactive for the calcium-independent, homophilic cell adhesion molecule NCAM . This molecule is also present on axons projecting to this region from the olfactory epithelium. NCAM may act in the ventrolateral zone to confine a group of cells to a distinct region of the telencephalic vesicle as well as to allow for recognition of the incoming olfactory axons.

To begin to evaluate this hypothesis we used a rotation-based cell adhesion assay to determine whether the differential distribution of cell adhesion molecules in the ventrolateral zone is paralleled by differential adhesivity of cells in regions of the developing forebrain. Medial, ventral, and lateral regions of the E12 mouse telencephalic vesicle were isolated by microdissection, dissociated into single cells, and allowed to reaggregate in suspension cultures. After 24 hours videomicroscopic images were captured and the size and number of reaggregates from each region determined. Reaggregates from the lateral and ventral regions averaged 57% and 72% larger than medial reaggregates. Treatment of the cultures with the calcium chelators, EDTA and EGTA, did not disrupt the reaggregates, indicating that these early adhesive interactions are largely calcium independent. These findings suggest that distinct domains in the developing forebrain are differentially adhesive, perhaps due to the distribution of calcium-independent cell adhesion molecules such as NCAM.

462.7

ACTIVATED RETINOIC ACID RECEPTORS DEFINE A DISTINCT DOMAIN IN THE DEVELOPING MOUSE FOREBRAIN. A-S. LaMantia*, A.K. McAllister, J.G. Whitesides, M. Colbert, and E. Linney. Departments of Neurobiology and Microbiology and Immunology, Duke University, Durham NC 27710.

The acquisition of regional identity in embryos is orchestrated by transcription factors acting within distinct domains. In the vertebrate CNS these domains have been seen in the developing spinal cord and hindbrain; however, they have not been described for the forebrain. We have shown a unique zone of early neurogenesis, differential cell adhesion, and neurite ingrowth from the olfactory placode in the developing mouse forebrain. We now show that the emergence of this zone is temporally and spatially correlated with that of a transcription factor, the retinoic acid receptor, using a transgenic detector mouse (Balkan et al, 1991 PNAS 89:3347-3351). The transgene contains three copies of the retinoic acid response element, a basal promoter, and ß-galactosidase as the reporter gene.

At the prosencephalic stage, cells in the ventrolateral zone of the forebrain and the olfactory placode are transgene positive based upon anti-ß-gal immunostaining. At the early telencephalic stage, transgene activity is coextensive with neuron-specific markers. Neurons are seen in the outer 1/3 of the epithelium, while transgene activity is limited to proliferative cells in the inner 2/3. Similarly, transgene positive cells are segregated from neurons in the olfactory epithelium. Transgene activity decreases as more postmitotic neurons are observed in the ventrolateral zone and olfactory epithelium, and by the forebrain rudiment stage it is absent in both places. These observations support a role for region specific transcriptional regulation in the earliest subdivision, differentiation and innervation of the mammalian forebrain. *A-S. LaMantia is supported by a National Down Syndrome Society Science Scholar Award.*

462.8

TEMPORAL AND SPATIAL PATTERNS OF RETINOIC ACID SYNTHETIC PATHWAYS IN THE DEVELOPING RETINA. P. McCaffery* and U. C. Dräger, Harvard Medical School, Dept. Neurobiology, Boston MA 02115

Previously we identified an aldehyde dehydrogenase present at high levels in the dorsal retina of the embryonic and adult mouse as the basic isoform AHD-2 known to oxidize retinaldehyde to retinoic acid (RA). Comparative estimates of RA levels with a reporter cell line placed the retina among the richest organs in the early embryo; levels in ventral retina, however, exceeded dorsal levels. A zymography-bioassay, the test of charge-separated protein fractions for RA synthetic activity with the reporter cells, revealed a different, acidic dehydrogenase in ventral retina. Affinity purification shows a 60 kD protein whose presence and amount parallels the ventral enzyme activity. Per protein amounts, this putative novel enzyme is several hundred-fold more powerful in RA synthesis than AHD-2.

The relative contributions of the two enzymes to total RA synthesis in the eye region vary with developmental age. The acidic dehydrogenase precedes AHD-2: alone it mediates the very active RA synthesis we see in the optic groove and surrounding tissue at E8.5. At E10 the acidic enzyme accounts for ~90% of synthesis in the eye region, at E14 for ~70% of synthesis in the retina, and at P5 for ~50%. In the adult retina the acidic enzyme is no longer detectable and all RA synthesis is mediated by AHD-2. The patterns in the expression of the enzymes suggest (1) that the acid dehydrogenase plays a role in the determination of the eye as an organ, and (2) that a particular constellation, created by the use of different dehydrogenases, is specifically targeted at the biochemical basis of positional information.

462.9

EFFECTS OF INHIBITORS OF RETINOIC ACID SYNTHESIS IN VERTEBRATE EMBRYOS. N.R. Marsh-Armstrong, P. McCaffery, J.E. Dowling, W. Gilbert and U.C. Dräger*. Dept. Cellular and Developmental Biology, Harvard Univ., Cambridge MA 02138 and Dept. Neurobiology, Harvard Medical School, Boston MA 02115

Retinoic acid (RA) in the embryo is generated from retinaldehyde by a series of dehydrogenases that differ in spatial localization and enzymatic characteristics including inhibitor susceptibility. In the early mouse embryo the retina and the spinal cord have the highest RA concentrations. Synthesis in the retina of zebrafish, chick and rodents is mediated by different dehydrogenases, one in the dorsal, the other in the ventral retina.

In tests of dehydrogenase inhibitors on RA-synthesizing enzymes *in vitro* and for teratogenic effects in *vivo*, we found several reagents with relatively selective effects. In the retina, disulfiram suppressed preferentially dorsal RA synthesis in all species tested. The ventral enzyme was much less susceptible to disulfiram; in the mouse it was preferentially inhibited by p-hydroxymercuribenzoate. When disulfiram was tested on early neurulation-stage zebrafish embryos, the most striking effect was in the trunk region: after very low concentrations of the drug the trunk was shortened and the notochord appeared swollen and wavy; higher concentrations caused in addition other gross defects including visible eye abnormalities. Inhibition of RA synthesis may be involved in malformations linked to disulfiram (=Antabuse®) in experimental animals and humans.

462.10

ANALYSIS OF THE *En-2*^{hd} CEREBELLAR FOLIATION PHENOTYPE AND IDENTIFICATION OF INTERACTING GENTIC PATHWAYS K.J. Millen, K. Herrup* and A.L. Joyner Samuel Lunenfeld Res. Inst., Toronto, and Alzheimer Res. Lab. Case Western Reserve, Cleveland.

Members of the *engrailed* (*en*) gene family encode highly conserved transcription factors, containing five conserved protein domains, including a homeodomain. The murine *en* genes, *En-1* and *En-2*, are initially co-expressed in a spatially restricted pattern across the presumptive mid/hindbrain junction. Later, the genes are expressed in restricted groups of neurons important for motor control. To examine *En-2* function, a mutation which deletes the homeobox of the *En-2* gene was made by homologous recombination in embryonic stem cells. Mice homozygous for this mutation, *En-2*^{hd}, exhibit a reduced cerebellar size and a distinct patterning defect of the cerebellar folia. Examination of the developmental profile of foliation has revealed that at the midline, the secondary fissure forms in a more anterior position, causing a fusion of the pyramis and the uvula. As well, laterally, the ansoparamedial fissure fails to form causing a fusion of CrusII with the paramedial lobe. Analysis of a lobe-specific *lacZ* transgene marker also indicates evidence for lobe transformation within the mutant cerebellum. In an attempt to identify genes that interact with *En-2*, we have begun making *En-2*^{hd} compound mutants with other mutants such as *weaver* and *meandertail* that affect the cerebellum. In addition, we are testing whether the mouse homologs of genes such as *wingless* (*wg*) interact with *En*. In Drosophila, *wingless* (*wg*) activity is required for *en* expression. In mouse, *Wnt-1*, a homolog of wg, is expressed in a similar domain as the *En* genes during early development. Mice homozygous for a *Wnt-1* targeted mutation exhibit a deletion of the mid-hindbrain region of the developing brain. We have preliminary genetic and morphological evidence suggesting that *Wnt-1* interacts with *En-2* in the patterning of cerebellar foliation. We are currently examining the expression patterns of *Wnt-1*, *En-1* and *En-2* genes during cerebellar foliation to correlate their expression with the observed phenotypes.

462.11

WHY DO WHISKER-RELATED PATTERNS FAIL TO DEVELOP IN THE BRAINSTEM AFTER FETAL NGF INJECTION?

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We (Henderson et al., Neurosci. Abstr. 17, '91) have reported that rats given 20 or 30 μg of NGF systemically on embryonic day (E) 15 and again on E18 failed to develop whisker-related cytochrome oxidase patterns in trigeminal (V) brainstem nuclei by the time of birth. Controls indicated that this effect was not due to surgical trauma, reduced body weight, or reduced metabolic activity in the brainstem. Similarly treated rats given NGF daily for up to 3 postnatal days also failed to develop brainstem whisker patterns. A series of experiments were designed to test 3 hypotheses: 1) naturally occurring V ganglion cell death is ameliorated by NGF injections; 2) elevated NGF levels alters ganglion cell projections to the whiskerpad and/or 3) to the brainstem. Stereological analysis indicated that V ganglion cell numbers were higher than normal at birth after NGF injections at E15 and again at E18 (125 ± 12% of control, $p < .05$) and brainstem whisker patterns did not develop. One injection at E15 did not alter cell numbers at birth (97 ± 12% of control, $p > .05$) and brainstem whisker patterns did develop. In cases where patterns failed to develop, DiI- and DiA-labeled infraorbital nerve fibers had normal fasciculation patterns and trajectories. Axonal projections to the whiskerpad were not unusual. Yet, DiI-labeled V primary afferent projections to the brainstem did not exhibit whisker-related patches. Thus, naturally occurring ganglion cell death may be important for the patterning of central primary afferent projections. DE07734, DE07662, NS24679.

462.12

DOES ACTIVITY-BASED COMPETITION PLAY A ROLE IN BARREL DEVELOPMENT IN HAMSTER?

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Prior studies in rat have failed to implicate activity-dependent competitive interactions in postnatal barrel development (eg. Henderson et al, Dev. Br. Res. 66, '92). To determine if such interactions occur earlier in development, deprivation studies were carried out in the newborn hamster, whose gestation period is 5 days less than that of the rat. A-E row whiskers were trimmed daily for 13 days from birth ($N=11$) and cytochrome oxidase (CO) patterns in the barrelfield were normal. In another litter, trimming all whiskers except those in the C-row also failed to alter barrel patterns or whisker patch sizes (vs corresponding areas on the control side: C-row 101±11%; B-row 97±8; D-row, 104±10; $N=4$). Yet, after C-row cautery at birth, C-row patches were absent in 7 of 9 animals and B+D-row patches were significantly larger than control patches (B: 127±18%; D: 121±18; $p < .01$; $N=5$). To test if cautery-induced enlargement of the B+D rows reflects an activity-based competitive advantage over C-row, C-row cautery was combined with daily trimming of the remaining whiskers. B+D row patches were again significantly larger than controls (B: 122±10%; D: 156±46; $p < .01$; $N=4$); however, the extent of the B+D row patch enlargement did not differ from that seen in the cautery alone condition ($F=1.62$; $p > .05$). Thus, uniform or selective sensory deprivation does not affect barrel formation in the hamster. Moreover, while competitive interactions can be demonstrated in the hamster barrel cortex by follicle cautery at birth, such plasticity would not appear to be activity-based. Support: NIH DE07734, DE07662, NS17763.

NERVE GROWTH FACTOR VI

463.1

Expression and regulation of the transgene in the CNS of c-*fos*-NGF transgenic mice.

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A transgenic mice line has been obtained which carries the *prepro-NGF* gene under a c-*fos* promoter. The expression of the transgene in cortical astrocytes and neurons has been assessed *in vitro*, in correlation with ELISA estimations of the amount of NGF synthesized and secreted by both cell types. In transgenic astrocytes, basal intra- or extra-cellular amounts of NGF were not significantly different from normal despite the presence of high amounts of mRNA specific for the transgene. Stimulation of the promoter with phorbol esters (TPA) produced a 60-fold increase in the secretion of NGF in both normal and transgenic astrocytes. Stimulation of the cAMP pathway by dibutyryl-cAMP or forskolin induced a 2- to 3-fold increase in the secretion of NGF.

In transgenic neurons, intra- and extracellular basal levels of the protein were respectively 4- and 2-times above normal ones. Fetal calf serum, which increased by 4 the levels of NGF secreted by normal neurons had no effect on transgenic ones. TPA, which increased by 10 the amount of NGF secreted by normal neurons had a slighter effect on transgenic neurons while dibutyryl-cAMP and forskolin increased the secretion of NGF by 10 in transgenic neurons and by 3 in normal ones.

The possibility to selectively regulate the production of NGF in transgenic embryonic tissue offers new insights to the *in vitro* studies of the factor and to *in vivo* investigations by transplantation.

463.2

MICE LACKING THE P75 LOW AFFINITY NGF RECEPTOR.

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We have generated mice carrying a mutation of the low-affinity NGF receptor $p75^{NGFR}$ gene by targeted mutation in embryonic stem (ES) cells. Mice homozygous for the mutation were viable and fertile. Immunohistochemical analyses of the footpad skin of mutant mice revealed markedly decreased sensory innervation by calcitonin gene related peptide (CGRP) and substance P (SP) immunoreactive fibers. The defective innervation was correlated with loss of heat sensitivity and associated with the development of ulcers in the distal extremities. Complicated by secondary bacterial infection, the ulcers progressed to toenail and hair loss. Crossing a human $p75^{NGFR}$ transgene into the mutant animals rescued the decreased heat sensitivity and the occurrence of skin ulcers and increased the density of neuropeptide-immunoreactive sensory innervation of footpad skin. Surprisingly, the mutation in the $p75^{NGFR}$ gene did not decrease the size of sympathetic ganglia or the density of sympathetic innervation of the iris or salivary gland. Our results suggest that the low affinity NGF receptor $p75^{NGFR}$ has an important role in the development and function of sensory neurons. This line of mouse should provide an opportunity to dissect the effects of different neurotrophins on cell differentiation, target innervation, neural plasticity, and nerve regeneration.

463.3

DIFFERENTIAL INFLUENCE OF NERVE GROWTH FACTOR ON NEUROPEPTIDE EXPRESSION IN VIVO - A NOVEL ROLE IN PEPTIDE SUPPRESSION.

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In normal adult rats 40-50% of lumbar DRG neurons have high-affinity receptors for NGF. A role for NGF in these neurons appears to be maintenance of differentiated phenotype as demonstrated by the ability of exogenous NGF to counteract loss of SP and CGRP following injury. To examine further at a cellular level possible mechanisms underlying the role of NGF in regulation of peptide expression in intact and injured sensory neurons in vivo, the right sciatic nerve was transected two weeks prior to sacrifice. In other animals, NGF was infused intrathecally (125ng/hr) for 8 days following the two week transection period. Cryostat sections were processed for receptor radioautography with radioiodinated NGF and for in situ hybridization with ^{35}S-labelled oligonucleotide probes to detect α-CGRP, β-CGRP, SP, SOM, VIP, CCK, NPY and galanin (GAL) mRNAs. In normal neurons α-CGRP, β-CGRP, SP, and SOM are abundantly and heterogenously expressed, whereas few neurons express VIP, CCK, NPY or GAL. Following injury, expresssion of α-CGRP, β-CGRP, SP and SOM decrease dramatically to background grain density levels in all but a few neurons. In contrast, the expression of VIP, CCK, NPY and GAL are upregulated and heterogenously expressed. Delayed infusion of NGF counteracted the decrease in α-CGRP, β-CGRP, and SP mRNA expression, but with the exception of a few neurons appeared not to influence the SOM population. The exogenous NGF also mitigated the injury-induced increases in VIP, CCK, NPY and GAL mRNAs. These results implicate NGF in positive and negative regulation of the synthesis of several peptides in intact sensory neurons. We suggest that NGF may be part of the signal that allows reversion to normal of responses to axotomy when axons regenerate into the distal sciatic nerve stump, a rich source of NGF. Supported by Canadian & Swedish MRCs.

463.4

NGF REVERSES NEURONAL ATROPHY IN A MOUSE MODEL OF GENETICALLY PROGRAMMED NEURODEGENERATION

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Mouse trisomy 16 (Ts 16) is an animal model of Down syndrome (DS). We have previously shown that fetal Ts 16 basal forebrain transplants undergo time-dependent cholinergic neuronal atrophy similar to that seen in DS and Alzheimer's disease (AD). We now asked whether the transplants contained NGF receptors and would respond to NGF. We transplanted cell suspensions of fetal Ts 16 or control basal forebrain into the hippocampus of young adult mice. Six months later, mice received continous intraventricular infusion of NGF or vehicle for 2 weeks. Grafts were examined by immunohistochemistry or *in-situ* hybridization to characterize neuronal morphometry and gene expression. In both Ts 16 and control grafts, $p75^{NGFR}$ and $p140^{trk}$ mRNA were expressed in cholinergic neurons and not in the host mouse hippocampus. Similar to prior findings, Ts 16 cholinergic neurons appeared atrophic and were significantly smaller than controls in vehicle treated animals (126 vs. 156 μ^2, $p < 0.001$). NGF markedly increased the size of all cholinergic neurons (Ts 16=201 μ^2; C=206 μ^2). There was no evidence of ß/A4 deposition. NGF reversed neuronal atrophy in an animal model of spontaneous, age-related neurodegeneration. We speculate that NGF may be reversing a neuronal death program and that expression of candidate cell death genes can be examined in this model.

463.5

NGF RECEPTOR ACTIVATION AND INTERNALIZATION IN PC12 CELLS.

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Neurotrophins interact with receptors on neurites to cause a response in the distant cell body. To understand how the signal is carried to the cell body, we have studied early events in the endocytosis of NGF in mechanically permeabilized PC12 cells. Radiolabeled NGF was crosslinked to 75 and 135 kD proteins on the plasma membrane. After warming to 37°C for 10 min, NGF was found in large heterogeneous membrane structures that escaped permeabilized cells. When permeabilized cells were warmed to 37°C in the presence of an ATP regenerating system, an increasing fraction of NGF was present in released small vesicles. Production of small vesicles was dependent on warming the cells prior to permeabilization. Radiolabeled transferrin exhibits a similar distribution into large, loosely attached membranes whose release is ATP-independent and small vesicles whose release is ATP-dependent. We conclude that NGF, like transferrin, is internalized into early endosomes, and that transport vesicles arising from early endosomes accumulate in vitro. Preliminary experiments suggest that both p75 (NGFR) and trk are present in the small vesicles. Experiments are underway to determine the phosphorylation state and enzymatic activity of trk kinase in these vesicles.

463.6

SYMPATHETIC NEURON DEATH AFTER NGF DEPRIVATION ENTAILS DNA FRAGMENTATION INTO NUCLEOSOME-SIZED SEGMENTS (OR APOPTOSIS). A. M. Tolkovsky, S. N. Edwards and A. E. Buckmaster (SPON: Brain Research Association). Dept. of Human Anatomy, University of Oxford, Oxford OX1 3QX, UK.

We have examined whether NGF-deprived sympathetic neurons undergo programmed cell death by apoptosis, the hallmark of which is activation of endonuclease(s) that cleave DNA into nucleosomal-sized fragments of ~180-200 bp (thus forming a DNA 'ladder' on agarose gels). After NGF withdrawal, established seven-day cultures (Edwards et al., 1991, J. Neurochem. 57:1240) and newly-isolated sympathetic neurons both contain DNA 'ladders' typical of apoptosis, an activity which peaks ~20 hours after NGF withdrawal in newly-isolated cells. Agents that promote survival in the absence of NGF, including cycloheximide and actinomycin D, prevent the ladder from appearing. Laddering can also be induced with similar kinetics in the presence of NGF by the cytotoxin cytosine arabinoside, which promotes cell death over 20-30 h. Unlike apoptosis in thymocytes, neuronal endonuclease activity does not appear to require elevation of Ca^{2+} nor to be inhibited by Zn^{2+} or phorbol esters, and calmodulin inhibitors actually promote cell death without laddering in the presence of NGF. Thus, we have identified novel endonuclease(s) which appear to be important mediators of the cell death program in sympathetic neurons. These may constitute the molecular targets whose functional (and transcriptional) suppression by NGF leads to neuronal survival.

This work is supported by the Wellcome Trust and Action Research.

463.7

NERVE GROWTH FACTOR HAS NEUROTROPIC EFFECTS ON ADULT RAT SEPTAL CHOLINERGIC AXONS IN VIVO S. Varon, J. van Minnen* and T. Hagg, Dept. of Biology, Univ. California San Diego, La Jolla, CA 92093;
* Department of Biology, Vrije Universiteit, Amsterdam, The Netherlands.

Nerve growth factor (NGF) induces sprouting of injured adult rat septal cholinergic neurons and promotes their regeneration into nerve grafts and hippocampal formation. We have investigated the potential neurotropic or chemotactic effect of NGF in the adult rat cholinergic septohippocampal model. i) Animals received bilateral sciatic nerve grafts between the disconnected septum and hippocampal formation. A 4-week NGF infusion into the rostral lateral ventricle caused sprouting of cholinergic fibers in the dorso-lateral septum with a gradient toward the ventricle. However, the number of cholinergic axons in the nerve was only one third of those in control animals, i.e. NGF had diverted regrowing axons away from the nerve toward the ventricle. ii) In animals with nerve grafts and 2-week infusions with NGF into the fornix (proximal to the lesion and grafts), axons sprouted in the medio-dorsal septum, i.e. oriented toward the infused fornix. However, no fibers were seen in the nerve, i.e. regrowing axons had remained near the NGF source. iii) NGF infusion into the contralateral ventricle of animals with a unilateral fimbria-fornix transection but no graft, induced sprouting toward the midline of the septum but not in the normal side. iv) Infusion of a low NGF dose into the lesioned septum induced sprouting only around the infusion site. Thus, the location of an NGF source (the location of the highest NGF concentration) can control the orientation of outgrowing cholinergic axons, indicating that NGF has neurotropic effects in the adult animal, as it has for developing peripheral neurons in vitro and in vivo. Support: NINCDS grant NS-16349 and 27047.

463.8

NGF dependency of functional sympathetic recovery after 6-OHDA lesions in adult rats. A. Gloster* and J. Diamond. Department of Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

Axotomy evokes a cell body reaction (CBR), which is greater the closer the lesion is to the cell body. Our new findings indicate that the intensity of the CBR in adult sympathetic neurons is inversely related to the trophic dependency of the ensuing axonal outgrowth. After crush, sympathetic nerves regenerate quite normally during an anti-NGF treatment which totally blocks the collateral sprouting of the undamaged axons (Soc. Neurosc. Abstr. 1989. 15:333). Surprisingly, this regeneration extends readily into skin well beyond the original field, i.e. into territory whose invasion by collateral sprouting is prevented by anti-NGF treatment. In an attempt to throw light on this apparent paradox, we have examined the NGF dependency of sympathetic regrowth after 6-OHDA treatment. This treatment destroys only the terminals of the sympathetic axons, and not surprisingly, there is a minimal CBR.

A single remaining cutaneous nerve was stimulated to reveal its sympathetic pilomotor field in the otherwise totally denervated skin. Pilomotor function was then abolished by 6-OHDA treatment. Function gradually returned: by 20 days the original pilomotor field area was restored, and by 70d the field had tripled its size. When daily anti-NGF administration was used to "challenge" this functional recovery, the expansion of the field developed normally up to approximately 60% of the original field size; however, expansion then ceased, resuming upon cessation of treatment.

We suggest that the CBR is normally associated with the "regeneration mode" of these neurons. However, the minimal CBR to 6-OHDA treatment is only sufficient to support a limited amount of NGF independent regenerative recovery. At about 60% field size, the neurons revert to their normal status. In the continued presence of adjacent denervated skin, however, the "sprouting mode" emerges, the further outgrowth then being the normal NGF dependent collateral sprouting.

(Supported by MRC Canada).

463.9

EFFECTS OF NGF AND ANTI-NGF ON WEIGHT GAIN AND FEMALE SEXUAL BEHAVIORS IN THE RAT. R.B. GIBBS*, M. M. McCARTHY, & D.W. PFAFF. Laboratory of Neurobiology & Behavior, The Rockefeller University, N.Y., N.Y. 10021.

Expression of nerve growth factor (NGF) by hypothalamic neurons has been reported; however, few studies have examined NGF effects on hypothalamic function. In the present study, adult, ovariectomized, Sprague Dawley rats received chronic ICV infusions of mouse 7S NGF (50 µg/ml; n=15) or rabbit anti-NGF (Collaborative Research; n=8). Control animals received rat cytochrome-C (50 µg/ml; n=9), or normal rabbit serum (n=3). Drugs were infused for nine days via a 28 g cannula implanted into the third ventricle and attached to an Alzet mini-osmotic pump. On the day of implantation, each animal was weighed and then administered the first of six daily injections of estradiol benzoate (1 µg in 0.1 cc oil s.c.). Two days later, daily testing for sexual receptivity began. Females were placed into arenas with vigorous males and lordosis, rejection behavior, and audible vocalization were measured. On the fifth day of testing, animals received progesterone (P; 0.5 mg in 0.05 cc oil s.c.) 4-6 hr. prior to testing. Two days later animals were weighed and perfused with paraformaldehyde and the brains processed for histochemical analysis.

NGF had a significant ($p<0.01$) effect on weight gain -- over the nine day period, average body weight decreased 7.0 ± 2.5% among NGF-treated animals and increased 3.6 ± 3.0% and 5.2 ± 1.7% among anti-NGF-treated and control animals respectively. All animals showed a steady, daily increase in lordosis quotient and average lordosis score. Neither NGF nor anti-NGF had any significant effect on lordosis behavior. Control animals also showed a daily increase in vocalization which reached significance ($p<0.05$) on day 4 of testing. This effect was both accelerated ($p<0.01$ on day 2 of testing) and increased ($p<0.05$) by NGF, and blocked by anti-NGF. Following P-treatment, vocalization in both NGF-treated and control animals was reduced to comparable levels. NGF also produced a significant increase ($p<0.006$) in rejection behavior (anti-NGF had no effect). These data suggest that hypothalamic NGF can influence behavior. Histochemical changes associated with NGF treatment will be discussed. Supported by NIH grant # NS28896.

463.10

NEUROTROPHINS ARE REQUIRED FOR FOLLICULAR FORMATION IN THE MAMMALIAN OVARY. G.A. Dissen,[1]* S. Malamed,[2] J.A. Gibney,[2] A.N. Hirshfield,[3] M.E. Costa[1] and S.R. Ojeda.[1] [1]OR Reg. Prim. Res. Ctr., Beaverton OR 97006; [2]Dept. Neurosci. and Cell Biol., UMDNJ-New Jersey, Piscataway, NJ 08854; [3]Dept of Anat., Univ. Maryland, Baltimore MD 21201.

The neurotrophin family of growth factors has several members, including nerve growth factor (NGF), all of which promote neuronal survival and differentiation. The biological actions of neurotrophins are exerted via activation of tyrosine kinase receptors. In addition, all neurotrophins are recognized by a different receptor molecule, known as p75, which is required for NGF signal transduction and is expressed in neurons as well as in some non-neuronal tissues. In the prepubertal ovary, non-neuronal p75 expression is localized to thecal cells of developing follicles. Immunohistochemical identification of p75 in embryonic rat ovaries using a specific monoclonal antibody (IgG 192) revealed that the receptor is expressed in mesenchymal cells. By gestational day 18, these cells begin to form stromal "pockets" which, as gestation proceeds, separate the presumptive pre-granulosa cells into discrete groups surrounding individual oocytes. This enclosure continues postnatally resulting in an abrupt formation of primordial follicles between 24 and 48h after birth. *In vitro* exposure of neonatal ovaries in organ culture to affinity purified NGF antibodies (2.4 µg/ml), resulted in a striking reduction (>80%) in follicular formation quantitated 48h later. Unexpectedly, this was accompanied by widespread cell death confined to p75-positive stromal cells. The results suggest that NGF and/or other members of the neurotrophin family are essential components of the differentiation program that governs follicular formation. (Supported by NIH grants HD24870, HD07438, HD18185, RR00163)

463.11

MOLECULAR CLONING AND DEVELOPMENTAL ANALYSIS OF FIVE trk GENES IN ZEBRAFISH. S.C. Martin*, J.W. Wu and G. Heinrich University Hospital and Boston University School of Medicine, Boston, MA 02118

The trk A, trk B and trk C genes have been shown to encode a class of receptors for nerve growth factor, brain derived neurotrophic factor, neurotrophin 3 and neurotrophin 4 . We have sought to study the role of these tyrosine kinase receptors in a vertebrate model of neurodevelopment, the zebrafish Brachydanio rerio.

Nucleotide sequence analysis of 120 clones derived from PCR have revealed five distinct representatives homologous to trk A, trk B and trk C. A lambda ZAP cDNA library has been constructed from day 12 embryo RNA and cDNA clones have been isolated. Southern blot hybridization has been used to show that each of these trk sequences represent a unique zebrafish gene. Northen blot analysis reveals that these zebrafish trk genes are expressed early in embryonic development. Some of the novel trk members identified in zebrafish may be the receptors for previously uncharacterized neurotrophic factors. These results suggest that one or two trk genes have not yet been described in mammals.

463.12

IMMUNO-ULTRASTRUCTURAL LOCALIZATION OF LOW- AND HIGH-AFFINITY NGF RECEPTORS ON NEURAL CELLS. P. Spoerri*, L. Petrelli, R. Dal Toso and S.D. Skaper. Fidia Research Laboratories, Abano Terme, Italy.

Responsiveness of neural cells to NGF appears to require expression and ligand binding to both the low-affinity NGF receptor (LNGFR) and the protooncogene product trk, the latter being a receptor tyrosine kinase. We recently described the immunolocalization of LNGFR on PC12 pheochromocytoma and C6 glioma cells using immunogold electron microscopy. We now demonstrate the immunolocalization of the LNGFR and the high-affinity component of the NGF receptor, trk (HNGFR), on the former cells and cultured neonatal rat dorsal root ganglia neurons using a double labeling technique. Receptor-specific antibodies were utilized in conjunction with immunoglobulin conjugated to colloidal gold particles of different sizes. NGF-treated cells displayed considerable colocalization of LNGFR-HNGFR-immunoreactivity (IR). Gold particles associated with LNGFR were by far the more numerous, being frequently seen near 2-3 (or more) gold particles delineating the HNGFR. Positive trk-IR thus seems to colocalize with LNGFRs in at least these neural cells.

463.13

LOCAL RESPONSE TO INTRACEREBRAL GRAFTS OF NGF-SECRETING FIBROBLASTS: INDUCTION OF A PEROXIDATIVE ENZYME. D.M. Frim, I. Schumacher*, M.P. Short, X.O. Breakefield, and O. Isacson. Neuroregeneration Laboratory, Mclean Hospital, Belmont, MA.; Molecular Neurogenetics Unit, Neurology and Neurosurgery Services, Massachusetts General Hospital, Boston, MA.

Intracerebral implantation of cells genetically altered to secrete high levels of NGF has profound effects on local neuronal survival after excitotoxic or knife-cut lesions. The effects of such biological NGF delivery systems, specifically the induction of genes encoding protective enzymes or stress proteins in surrounding neurons or glia, has not been well studied. We chose to investigate the effects of an implanted NGF-secreting fibroblast cell-line on local levels of acetylcholinesterase (AChE), the stress proteins c-fos, c-myc, heat shock protein-72 kDa (hsp72), and ubiquitin (Ub), and the peroxidative enzyme catalase. Seven days after unilateral corpus callosum implantation of either a genetically altered NGF-producing (NGF[+]) or control (NGF[-]) fibroblast cell line, we found no specific immunostaining in tissues surrounding either the NGF[+] or NGF[-] grafts using antibodies raised against c-fos, c-myc, or Ub, though both types of grafts stained faintly for c-fos. Both NGF[+] and NGF[-] grafts elicited a halo of hsp72 immunoreactivity in the tissue immediately surrounding the grafts. AChE staining was unchanged in areas adjacent to the grafts, and though there was no fibroblast AChE staining, multiple AChE positive fibers appeared to be growing into or traversing the NGF[+] grafts. In contrast to the above, NGF[+] cells caused increased catalase staining in astroglial cells adjacent to the grafts. These catalase positive astroglial cells were not seen in areas distant from the NGF[+] grafts, nor were they seen in brains implanted with NGF[-] cells. These results suggest that the local protective effects of NGF[+] fibroblast grafts may be mediated through changes in peroxidative metabolism due to increased levels of catalase. If so, NGF-mediated protection and neurotropism in the adult brain may be caused in part by an increase in cell resistance to lipid peroxidation.

VISUAL CORTEX: MOTION PROCESSING II

464.1

REVERSIBLE ABOLITION OF VISUAL MOTION PROCESSING MECHANISMS IN THE LATERAL SUPRASYLVIAN CORTEX OF THE BEHAVING CAT. S. G. Lomber*, P. Cornwell, J. S. Sun, M. A. MacNeil and B. R. Payne, Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA 02118.

The purpose of the present study was to test the hypothesis that the lateral suprasylvian (LS) visual cortex of the cat is critical for the identification of a stationary pattern partially obscured by a moving mask. Two cats were trained with food reward to discriminate outline "I" and "O" shapes which were partially obscured by a moving mask of regular intersecting diagonal stripes of the same thickness as the outline shapes. One cat (S) was trained binocularly and the second cat (V) was trained monocularly after transection of the optic chiasm and corpus callosum. Bilateral cooling probes were implanted over LS cortex of Cat S and a unilateral probe was implanted over LS cortex of Cat V. Prior to and after cooling inactivation of LS cortex, both cats could discriminate the "I" from the "O" in ≥80% of the test trials. Cooling of LS cortex reduced performance to almost chance levels (mean = 55%). Virtually identical results were obtained with a moving irregular mask. When the regular or irregular masks were held stationary over the "I" and "O", and LS cortex was operational, the performance levels were indistinguishable from those when the mask was in motion. However, cooling blockade of LS cortex did not influence the cats ability to discriminate the "I" in the presence of either regular or irregular static masks. Reversible cooling blockade of LS cortex was verified electrophysiologically. These results show that operational LS cortex is critical for the extraction of stationary patterns from a field filled with contours in motion; in the absence of LS cortical activity this ability is abolished. (Supported by NIMH #44647 and the Cornwell Family).

464.2

GLOBAL MOTION COMPUTATION AND THE BEHAVIOR OF SINGLE CELLS - A NEURAL NETWORK APPROACH Nava Rubin and Shaul Hochstein*, Neurobiology Dept. & Center for Neural Computation, Hebrew University, Jerusalem, Israel.

It has been suggested that the visual system, after extracting information about the motion of 1-D components of an image, uses an intersection-of-constraints (IOC) procedure to compute the 2-D direction of motion, and thus overcome the aperture problem (Adelson & Movshon, '82). To study the implications of the algorithmic solution on the implementation level, we studied a neural network model based on a physiologically plausible architecture. We found that the output units exhibit response inseparability to speed and direction of motion already for 1-D stimuli (gratings). In particular, the tuning curve to direction of motion is always bi-lobed for stimuli moving at sub-optimal speeds. This predicted behavior contrasts with that observed for MT cells, which exhibit remarkable separability of their response to speed and direction (Rodman & Albright, '87). Thus, a feed-forward network cannot both perform the IOC computation and exhibit physiologically-observed characteristics. The only way to mask this coupling pattern induced by the input layer may be by elaborate lateral connections among nonlinear output units, connections which themselves couple the speed and direction dimensions. An alternative model is proposed whereby the visual system computes global velocity not by interactions in velocity space but rather in real space, using information regarding the spatial organization of the stimulus components. Such a model accounts both for MT cell behavior and psychophysical results (Ferrera & Wilson, '90; Rubin & Hochstein, '92).

464.3

INTEGRATING VISUAL MOTION RESPONSES FROM NEURONS IN CORTICAL AREA MT BY ADAPTIVE FILTER SELECTION. S. J. Nowlan* and T. J. Sejnowski, The Salk Institute, La Jolla, CA, 92037.

A moving object excites many motion-sensitive neurons in the visual cortex. How could this distributed representation of motion be used to estimate the velocity of the object for eye tracking? The integration must be a dynamic process, dependent on properties of the visual stimulus such as contrast, spatial frequency, binocular disparity, color, and transparency or occlusion. We have developed a model for estimating the velocity of an object in visual area MT that is based on adaptive filter selection. The model assumes two sets of units with local receptive fields. One set of units computes local estimates of motion (using the motion energy model of Adelson and Bergen with physiologically determined parameters). The second set of units computes the relevance or reliability of each local motion estimate based on the estimate itself and additional information from the image. Outputs from this second pool of units can "gate" the outputs from the first pool of units through a gain control mechanism. This gating occurs before the local motion estimates are integrated to form more global estimates. The proposed mechanism of gain control is consistent with measured responses of MT cells under conditions of interfering transparent motions. The active process for selecting only a subset of visual motion responses for integration distinguishes our model from previous models of velocity estimation. The model yields accurate velocity estimates from synthetic images of moving targets of varying size, luminance, and spatial frequency profile. In addition, the sensitivity of the output of the model to both the acceleration and the velocity of moving targets is qualitatively similar to that observed in primate smooth pursuit tracking experiments. (Supported by the Howard Hughes Medical Institute).

464.4

CORRELATED ACTIVITY OF NEURONS IN AREA MT. E. Zohary,* M. N. Shadlen, and W. T. Newsome, Dept. of Neurobiology, Stanford University, Stanford, CA 94305.

We have previously reported that the mean sensitivity of MT neurons to weak motion signals is similar to the mean psychophysical sensitivity of rhesus monkeys. If single neurons are so informative, it is natural to wonder why monkeys do not perform even better by averaging signals from many neurons. The benefits of averaging, however, are attenuated if the neuronal responses contributing to the pool, are partially correlated. We therefore calculated the correlation between spike counts recorded simultaneously from pairs of MT neurons. Data were obtained using a spike sorter that operated on the basis of template matching; extra care was taken to verify that the two templates were highly discriminable both from the noise level and from each other. Neural activity was recorded (n = 12 pairs) while the monkey performed a direction discrimination task or a fixation task.

For each pair of neurons, the correlation coefficient (**r**) between the spike counts was computed for every stimulus condition. The value of **r** did not vary systematically with the direction or strength of the motion signal, nor was it affected by the behavioral relevance of the stimulus (i.e. "discrimination" or "fixation" condition). Among the 12 neuronal pairs, **r** ranged from -0.14 to 0.47; the mean value of 0.15 was significantly greater than 0 (t-test, $p < 0.01$). Somewhat surprisingly, Monte Carlo simulations indicate that modest correlations of this magnitude can limit significantly the beneficial effects of pooling.

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464.5

PREDICTING PSYCHOPHYSICAL PERFORMANCE FROM POOLED NEURONAL RESPONSES. M.N. Shadlen*, W.T. Newsome, K.H. Britten, E. Zohary and J.A. Movshon†, Dept. of Neurobiology, Stanford Univ., Stanford, CA 94305; †Howard Hughes Medical Institute and Center for Neural Science, New York Univ., New York, NY 10003.

We have calculated expected psychophysical thresholds on a motion discrimination task from the pooled responses of neurons recorded from MT. We compared simulated values to mean psychophysical thresholds produced by our monkeys during recording experiments. Since the manner in which neural signals are actually pooled is unknown, we modelled various assumptions about the way neural signals add, the precision by which pooled signals are compared, and the degree of correlation among neurons comprising such pools.

Under most model assumptions, simulated performance rapidly exceeds the sensitivity of our monkeys as neurons are added to the pool. Interestingly, this beneficial effect of pooling is sharply limited if neurons are partially correlated in their response; arbitrarily large pools fail to lower expected threshold beyond some asymptotic bound. Still, the exquisite sensitivity of single neurons precludes pooling unless additional sources of noise, beyond the measured variability of our neurons, is assumed. Under these conditions, modest correlation (r = .1 to .3) between large numbers of neurons can account for psychophysical performance. In agreement with experimental data, moreover, simulated psychophysical decisions covary weakly with the responses of individual neurons on a trial-by-trial basis. Thus partial correlation provides a basis for reconciling the existence of large pools of sensitive neurons in the motion pathway with the observed psychophysical sensitivity of our monkeys, permitting the observer to base behavior on thousands of neurons while appearing to utilize but a few.

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464.6

RESPONSES OF NEURONS IN AREA MST DURING DIRECTION DISCRIMINATION PERFORMANCE: A COMPARISON OF NEURONAL AND PSYCHOPHYSICAL SENSITIVITY. Simona Celebrini and William T. Newsome*. Dept. of Neurobiology, Stanford University., Stanford, CA 94305.

This laboratory has previously reported that direction discrimination thresholds of neurons in area MT are remarkably similar to psychophysical thresholds measured simultaneously in alert monkeys. In addition, there frequently exists a trial-by-trial covariation between the response of an MT neuron and directional judgements made by the monkey. As a first step in extending this analysis to other visual areas on the cortical motion pathway, we performed similar experiments in MST, a higher visual area that receives direct inputs from MT.

We recorded, in one monkey, from a subset of MST neurons that responded well to the variable-strength random dot stimuli employed in our previous studies. Due to the very large size of MST receptive fields (about 6,000 deg^2 on average), our visual stimuli usually covered only a small fraction of the receptive field. Despite this limitation, neurometric functions computed from the responses of MST neurons were indistinguishable from psychometric functions generated by the animal in 55% of the experiments (N = 40). The ratio of neuronal to psychophysical threshold was near 1 (geometric mean = 1.2), as was the ratio of neurometric and psychometric function slopes (geometric mean = 1.14). In addition, the trial-by-trial covariation of neuronal response and psychophysical decision previously observed in MT was also present in MST. Thus MST neurons, like those in MT, encode motion signals with sufficient sensitivity to mediate psychophysical performance on our direction discrimination task. The pools of sensory neurons contributing to performance on our task may therefore include cells from MST as well.

Supported by the National Eye Institute (05603) and by CNRS

464.7

MT RESPONSES TO TRANSPARENT AND NON-TRANSPARENT MOTION. Ning Qian*, Richard Andersen and Edward Adelson, Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

We reported previously that patterns with well-balanced motion signals in opposite directions are perceptually non-transparent. This is exemplified by the paired random dot patterns composed of many randomly located pairs of dots. The two dots in each pair move across each other in opposite directions over a certain distance and then jump to a new random location. We found that when the distance the two dots in each pair traveled across each other is small, no transparent motion was observed. When the dots were simply unpaired, however, a perception of transparency emerged. We have recorded 42 MT cells from behaving monkeys using these paired and unpaired random dot patterns. The responses of most cells to these patterns were much weaker than their responses to a single set of dots moving in their preferred directions alone, indicating directional suppression. 17 cells also showed statistically significant differences in their responses to the paired (non-transparent) and unpaired (transparent) dot patterns. Among them, 11 cells responded to the transparent patterns better than to the non-transparent ones and 6 cells showed the opposite behavior. We further found that cells with the opposite behavior responded much better to noise patterns made of flickering random dots and therefore were less selective for motion. In addition, a strong negative correlation exists between the degrees of directional suppression of cells and their responses to the flickering noise patterns. This suggests that a major functional role of directional suppression in MT is noise reduction. These results, together with our previous findings that V1 cells could not distinguish between transparent and non-transparent patterns and that most of them showed no or weak directional suppression, support a two stage model for the transparent motion perception we proposed earlier. In the first stage, motion signals in each direction are extracted and in the second stage, the signals from opposite directions suppress each other. For patterns with well-balanced motion signals the suppression is more complete resulting in weaker responses to these patterns, which could account for their perceptual non-transparency.

464.8

RELATIVE DEPTH FROM MOTION AND STEREO CUES. George J. Carman[1]*, Leslie Welch[2], and Thomas D. Albright[1]. Salk Institute[1] and University of California[2], San Diego, CA.

While it is well-known that motion and stereo cues convey information about depth, how these two cues are integrated to form a single percept of depth is not understood. Based on theoretical consideration of viewing geometry, we predicted that differential motion parallax and differential stereo parallax should both have the same dependence on relative depth (GJC, *Soc. Neurosci. Abstr.*, **17**(1): 847, 1991). If true, then the matching of depth from stereo cues to depth from motion cues should be characterized by a linear relationship. Here we report findings of psychophysical experiments which confirm this hypothesis.

Human observers viewed two computer-generated stimuli presenting either monocular motion cues or static stereo cues, and adjusted the relative depth of one stimulus to match that of the other. Stimuli consisted of surfaces which presented a gaussian profile in depth, characterized by an absolute surround distance (SD) of the stimulus from the observer, and a relative center-surround distance (CSD) of the center from the surround. Stimuli were presented at SDs of 28.65, 57.3, and 114.6 cm, covering a range of CSDs scaled by SD so as to control for monocular size cues. Stimuli were rendered using dynamic random dots of limited lifetime so as to control for texture density cues. Stimuli were viewed under perspective projection via a mirror stereoscope in three conditions: (1) natural viewing, which simulated viewing of real objects; (2) isovergence, in which the vergence angle of the eyes was held constant, while otherwise simulating natural viewing; and (3) isoincidence, in which the retinal disparity cues were held constant, while otherwise simulating natural viewing.

Data obtained thus far indicate that: (1) relative depth based on motion cues is matched linearly, but nonveridically, to relative depth based on stereo cues; (2) the slope of this linear relationship varies with absolute depth of the stimulus; and (3) this variation in slope is due to changes in retinal cues which covary with vergence, but are not due to vergence per se. Our findings suggest that perception of the three-dimensional visual environment is mediated by a common representation of relative depth supported by linear combination of two-dimensional retinal cues arising from motion and stereo parallax. (NIH NRSA EY06179 & McDonnell-Pew Foundation.)

464.9

COMPUTING DIRECTION OF HEADING FROM AFFINE FLOW. Jack M. H. Beusmans.* Center for Neural Science, New York University, 4 Washington Place, New York, NY 10003.

Observers moving through a 3D environment can use optic flow to determine their direction of heading. The algorithms proposed so far for computing heading all use cartesian flow fields in which image flow is the change in the projection of identifiable points in the environment over time. I propose an algorithm that uses affine flow instead; in contrast to cartesian flow, affine flow makes explicit the change in vantage point between the observer and points in the environment.

To compute the affine flow associated with a point P in the environment, first compute the affine (i.e., linear) transformation $\mathcal{A}$ that transforms the neighborhood of p_1 (the image of P in the first view) into that of p_2; then apply $\mathcal{A}$ to p_1, to predict the location of P in the second view; affine flow is defined as $q = p_2 - \mathcal{A}p_1$ (Koenderink & van Doorn, JOSA A 8, 377, 1991). Modeling the observer's instantaneous motion by a translation and a rotation about an axis through its eye, affine flow is tangent to the translational field lines, which form a radial flow field on the observer's viewing sphere. The direction of heading is the center of this flow field. Note that q represents the change in vantage point of P. Translational field lines can also be approximated through differential cartesian motion (Rieger & Lawton, JOSA A 2, 354, 1985), although this approximation requires considerable differences in depth to be acceptable. Affine flow does not depend on such differences.

Affine flow involves simple, local computations that could be performed in a cortical area such as V1; affine flow vectors could be combined in areas such as MST or PG whose receptive fields cover a substantial fraction of the visual field. (Supported by NIH 5F32EY06319.)

464.10

TRANSCRANIAL MAGNETIC STIMULATION (TMS) OF PARIETAL-LATERAL OCCIPITAL CORTEX DEGRADES HUMAN MOTION PERCEPTION. JR Hotson*, D Braun, W Herzberg, D Boman, Santa Clara Valley Med Ctr, Stanford Univ Sch Med, San Jose and Stanford, CA 94305.

Extrastriate visual areas are involved in motion vision. TMS of striate cortex degrades spatial visual acuity for letter trigrams and Landolt-C stimuli. TMS of extrastriate cortex may also degrade motion perception of random dot stimuli. Subjects identified the direction that correlated dots moved through a dynamic random dot background located 10 deg to the left or right of fixation. Psychometric functions for this four-direction, forced- choice task were derived, and the percentage of correlated dots required to achieve 80-90% correct responses was determined. The test was then repeated with additional randomly-interspersed trials at the determined percentage of correlated dots during which TMS was applied 50-250 msec after the onset of the 50 msec motion stimulus. TMS was applied with a 9 cm coil over the lateral posterior hemisphere at an intensity of 1.8 Tesla.

In five subjects, TMS degraded the motion perception when applied 100-150 msec after stimulus onset. TMS produced a 36% mean decrease (range 33% to 44%) in correct responses when applied 125 msec after motion stimulus onset. In three subjects, little or no decrease was found when TMS was applied 50-75 msec or 175-250 msec after stimulus onset. Therefore, TMS applied over human extrastriate cortex transiently degrades motion perception and may be valuable in tracing pathways of visual information processing. (Supported by NEI EY03387 and The Valley Foundation).

464.11

TRANSITORY DEFECTS OF VISUAL PERCEPTION INDUCED BY CORTICAL STIMULATION IN MAN G. Beckers* and V. Hömberg Neurological Therapy Centre NTC Heinrich Heine University Düsseldorf (F.R.G.) Hohensandweg 37, W4000 Düsseldorf 13 (SPON: European Neuroscience Association)

Transcranial magnetic stimulation (TMS) in healthy human subjects (N=42) can produce *specific reversible defects of visual perception*. Single TMS pulses were applied systematically at various locations over the occipital, parietal, temporal, central and frontal cortex using a Cadwell MES-10 magnetic stimulator device with a maximum output of 2 Tesla peak flux density. A number of different visual tasks have been employed, including literal trigram identification, Landolt "C" gap detection, visual short term memory scanning, motion direction detection and color identification. The effects of varying TMS intensity, the delay between visual stimulus onset and TMS, and type of magnetic coil have all been tested. In the motion perception task, for instance, it was found that TMS applied *unilaterally* (1.4T, biphasic pulse duration 800usec) to either the left or the right area V5 (area MT) produced an instantaneous dramatic defect in the perception of the direction of motion, which lasted for about 40msec, whereas the effect of TMS of area V1 was less marked and occurred only at TMS-delays of 70-80msec post motion onset. TMS of area V5 (about 5cm left or right of the midsaggital plane) *did not impair* the perception of randomly varied colored Land-Mondrian stimuli. Further examination of the effects of visual stimulus eccentricity (4 to 9deg, size 2.2 x 2.2deg) and visual field stimulation, motion velocity (2.2 to 26.9deg/s) and exposure time (28 to 210msec) revealed that (A) TMS of the left area V5 produced a more marked impairment than TMS of the right area V5, (B) it is the corresponding contralateral visual hemifield only that is mapped in either the left or the right area V5 in man, (C) area V5 in man occupies a region of roughly $1cm^2$ at the temporo-parieto-occipital pit, (D) perception of motion direction varies with stimulus eccentricity and also with (E) stimulus velocity. The TMS induced transitory akinetopsia *was not* contaminated by eye-movements, since TMS is incapable of inducing eye-movements during fixation, whether applied to frontal, temporal, parietal or occipital cortex. The combined psychophysical-TMS method can be fruitfully used to study functional specialization and localization in human visual cortex with reasonable spatial acuity. In contrast to the PET technique, TMS reveals more about the *temporal order of arrival of signals* in various functionally specialized cortical areas, and thus has additional relevance for the comparison of functional specialization in monkey and man.

464.12

ELECTROPHYSIOLOGICAL CONCOMITANTS OF APPARENT MOVEMENT IN MAN. R. Pierantoni, W.G.Sannita*. Center for Neuroactive Drugs, Dept. of Motion Sciences, Univ. Med. School; Inst. of Cybernetics and Biophysics, CNR; Genova, 16132 Italy.

A Wertheimer's paradigm to induce a visual percept of apparent motion was approximated on a VENUS Neuroscientific system by producing a bidimensional 6x5.9 deg image of alternating black and white, 70% contrast sinusoidal vertical bars counterphasing at 0.5-8.0 Hz. Stimulation was at 0.4 or 5.0 c/deg spatial frequency, consistent with parameters used to produce (foveal) retinal and cortical evoked potentials in man. The signal was recorded (0.2-100.0 Hz) via dermal electrodes located on inferior eyelid (reference: contralateral eye) and occipital areas (reference: midfrontal), and processed offline (512 sample/s) by FFT. Eight healthy subjects were recorded; all agreed in reporting the expected compelling percept of horizontal movement with higher probability at 2.0 Hz or 5.0-8.0 Hz temporal frequencies when spatial frequency was 0.4 and 5.0 c/deg respectively. Significantly higher amplitude values of the retinal and cortical 2nd harmonic were observed at these temporal frequencies compared to higher or lower frequencies (including neighbouring frequencies) when the percept was not reported to be as evident and the 1st harmonic amplitude was higher than 2nd harmonic. The observation was consistent between- and within-subject, and suggests a potential concomitance of Wertheimer's phenomenon with the spatial/temporal frequency-dependent functions of the visual system concurring in the generation of retinal/cortical visual evoked potentials.

NEUROTOXICITY

465.1

^{1}H AND ^{31}P *IN VITRO* NMR STUDIES OF CHRONIC HYPOXIA IN FISCHER 344 RATS. K. Panchalingam*, J.W. Pettegrew, Lab. of Neurophysics, Univ. of Pittsburgh, Pittsburgh, PA 15261

Recent biochemical and clinical observations suggest that repeated, brief, mild energetic stress could, in some individuals, trigger molecular and metabolic mechanisms that result in the biochemical findings in the brains of Alzheimer's disease (AD) patients. In this study, we investigated the biochemical responses of Fischer 344 rat brains by subjecting them to repeated hypoxia for 30 seconds for a period of two weeks. Three months and 10 days old animals were used to investigate age dependent biochemical changes under hypoxia. *In vitro* ^{1}H NMR studies of the freeze clamped brains of controls and affected animals at the end of the two weeks indicate: (1) a nonsignificant increase in the level of the excitatory amino acid aspartate and glutamate in 3 month old animals. In the 10 days old animals, aspartate level was unchanged, whereas a 5% elevation ($p=0.07$) of glutamate was measured; (2) the inhibitory amino acid GABA is increased by 20% in 3 month old animals ($p=0.04$) and unchanged in 10 days old animals; (3) myo-inositol and alanine levels are decreased by about 9% in 3 month old animals ($p=0.02$) and by about 2-4% in 10 days old animals. These preliminary results indicate that aged animals are more susceptible to energetic stress than the young animals. Our ^{1}H NMR results indicate a permanent alteration in some amino acid levels but not in others. However, ^{31}P NMR results show no significant alteration in energy metabolism or membrane phospholipid metabolism indicating recovery of the brain from these energetic stress.

465.2

GLUTATHIONE DEPLETION INDUCES HEME OXYGENASE-1 (HSP32) mRNA AND PROTEIN IN RAT BRAIN. J.F. Ewing* and M.D. Maines. Dept. of Biophysics, Univ. Roch. Sch. of Med., Rochester, NY 14642.

In mammals heme oxygenase isozymes, HO-1 and HO-2, cleave heme to produce bile pigments. HO-1 is a stress protein (HSP32) induced by chemicals in systemic organs. This study demonstrates the sensitivity of brain HO-1 mRNA and protein to cellular glutathione (GSH) levels and provides the first evidence of reciprocal regulation of two antioxidant pools: GSH and bile pigments in the brain. Specifically, treatment of adult rats with diethyl maleate (DEM) (4.7 mmoles/kg,ip) caused a pronounced decrease in brain GSH within 1 h. GSH levels remained depressed for at least 24 h post-injection. Northern blot analysis of brain poly $(A)^+$ mRNA following DEM treatment revealed an increase in HO-1 1.8 Kb mRNA level up to 10-fold that of controls in a manner reciprocal to that of GSH. Similarly, treatment of neonatal rats with buthionine-SR-sulfoximine (BSO) (3 mmoles/k,ip; twice daily, 2-4 days of life) caused a marked depression in total brain GSH and a concomitant 10-fold increase in brain 1.8 Kb HO-1 mRNA above control level as evidenced by Northern blot analysis. In contrast, the level of two homologous HO-2 transcripts (1.3 and 1.9 Kb) did not increase in response to either DEM or BSO treatment. Analysis of brain HO-1 immunoreactive protein following 9 h DEM treatment indicated induction of HO-1 protein in only select non-neuronal cell populations. Notably, the ependymal cells lining ventricles of brain, Bergmann glia of cerebellum, leptomeninges lining brain and glia throughout brain responded to treatment by increasing HO-1-like immunoreactive elements.

We suggest that when GSH is depleted, an increase in HO-1 protein, hence increased capacity to form bile pigments, may be vital to those brain cells which normally depend on the tripeptide for antioxidant defense. Supported by NIH Grants R37 ES04391, ES03968 and ES01247.

465.3

CYTOCHROME P450IID1 IN IMMORTALIZED CELL LINES.

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Catalytic, pharmacological and molecular studies have recently identified the genetically variable cytochrome P450IID1 in mammalian brain. P450IID1 (debrisoquine hydroxylase) is responsible for the metabolism of many drugs including neuroleptics, codeine and antidepressants. In addition, significant overlap was observed between substrates / inhibitors for the dopamine transporter and for the P450IID1, for example, (-)cocaine, MPTP, methylphenidate and d-amphetamine. In order to study the CNS P450IID1, we used PCR to detect the presence of P450IID1 and cell marker RNA in immortalized cell lines. 10 of 15 centrally-derived cell lines were positive for the P450IID1 RNA. Variation of RNA levels in 8 rat brain regions was also studied. In addition, 2 of 3 pancreatic cell lines and a fibroblast cell line also contained the P450IID1 RNA. Studies of regulation, inducibility and enzyme activity are underway. Cell lines containing P450IID1 should enable us to further our understanding of the role of the P450IID1 enzyme in the CNS. (RFT is supported by the MRC of Canada)

465.4

RELATIONSHIP BETWEEN THE EFFECTS OF MPTP ON ENERGY METABOLISM AND LEVELS OF L-ASPARTATE AND L-GLUTAMATE IN THE MOUSE BRAIN.

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The effects of the neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on the levels of L-glutamate and L-aspartate were studied in different areas of the mouse brain and correlated with changes in energy metabolism. C57BL/6 mice (7-8 weeks old) were injected with either saline or MPTP (40 mg/kg, s.c.) and sacrificed 4 hours later. MPTP caused a decrease in the levels of both L-aspartate and L-glutamate. This decrease appeared to be selective for the nigrostriatal pathway because it occurred in the striatum and ventral mesencephalon, but not in the cerebellar cortex. Furthermore, this toxic effect of MPTP was probably due to its conversion to the pyridinium metabolite MPP^+ since it was prevented by pretreatment of mice with the MAO B inhibitor deprenyl (10 mg/kg). In order to evaluate the possible role of changes in energy metabolism in the decrease of L-aspartate and L-glutamate, we injected mice with 2-deoxyglucose (2-DG, 1 g/kg, i.p.) prior to MPTP administration. 2-DG is an inhibitor of glucose uptake and utilization and has been shown to enhance the loss of ATP caused by MPTP in the mouse brain. The decrease in striatal levels of L-aspartate and L-glutamate was significantly potentiated by 2-DG. Data strongly suggest that impairment of energy metabolism caused by MPTP may affect the biodisposition of excitatory amino acids. Excitotoxins may then be involved in MPTP neurotoxicity.

465.5

EFFECT OF NEUROLEPTICS ON THE MITOCHONDRIAL ELECTRON TRANSPORT CHAIN.

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The etiology of tardive dyskinesia (TD), a frequent and usually irreversible side effect of the chronic use of neuroletics, remains unknown. Because available pathological data do not support present biochemical hypotheses and impairments in the mitochondrial electron transport chain have recently been documented in several movement disorders, we thought it important to test the possibility that neuroleptics may affect mitochondrial respiration. Accordingly, adult rats received fluphenazine (FLU; 4 mg/kg s.c.) in saline daily for 30 days and were sacrificed 1, 4 and 7 days after the last injection. Control animals received vehicle only. Chronic FLU administration caused significant changes in complex I, II, III and IV activities in both caudate-putamen and nucleus accumbens compared to controls. The effect of FLU on mitochondrial respiration was partially reversed as striatal complex I activity improved 7 days after the last injection, while the other complexes still exhibited some alterations. In addition, brain mitochondria prepared from naive rats and incubated with different classes of neuroleptics showed that complex I activity was impaired as follows: phenothiazines > butyrophenones > thiothixenes > diphenylbutylpiperidines > indolics; while atypicals had no effect even at the highest dose (10 mM). Our study, demonstrates that neuroleptics can affect mitochondrial electron transport chain activity both in vivo and in vitro. Because deficits in mitochondrial respiration have been implicated in neurodegenerative processes, our findings with neuroleptics in rats may be relevant to the underlying pathophysiological mechanisms of TD.

465.6

EFFECT OF L-DOPA ON THE MITOCHONDRIAL ELECTRON TRANSPORT CHAIN.

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Reduction in complex I activity found in parkinsonian brains has been suggested to be implicated in the development of this illness. Because this reduction was also found in tissues such as muscle and platelets, which apparently are not affected by the disease, we thought it important to test the possibility that factors such as anti-parkinsonian treatment may play a role in the unexpected widespread alteration in the mitochondrial electron transport chain activity. Accordingly, adult rats received 50 mg/kg L-DOPA + 12.5 mg/kg benserazide in saline i.p. twice daily for 40 days and were sacrificed 1, 3 and 7 days after the last injection. Control animals received vehicle only. Chronic L-DOPA administration caused a significant reduction in complex I activity in rat brain compared to controls which seems to parallel dopaminergic innervation: striatum ≈ ventral mesencephalon > frontal cortex, no significant changes in the cerebellum. In contrast, no significant changes were observed in complex II, III and IV activities. The effect of L-DOPA on complex I activity was found to be reversible since striatal complex I activity returned to normal 7 days after the last injection. In addition, brain mitochondria prepared from naive rats and incubated with L-DOPA or dopamine showed significant reductions in complex I activity, while dopamine metabolites such as HVA, DOPAC or 3-O-methy-dopamine did not cause any significant changes. Our study demonstrates that chronic L-DOPA administration causes a reduction in brain complex I activity in rats. This finding supports the view that the deficit in complex I seen in muscle and platelets of parkinsonian patients may be due to the chronic use of L-DOPA-containing drugs. In addition, because reduction in complex I activity may play a role in the degenerative process in Parkinson's disease, the use of drugs such as L-DOPA that can increase this enzymatic deficit should therefore probably be postponed as long as possible.

465.7

KAINATE RECEPTOR EXPRESSION INDUCES TOXICITY IN FIBROBLASTS AND HIPPOCAMPAL SLICE CULTURES.

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The CA3 region of the hippocampus is prone to damage by the excitotoxin kainate (KA) through potential mechanisms involving either recurrent excitation and/or the high expression of KA receptors. In order to develop a gene transfer system to study KA toxicity, a cDNA encoding the KA receptor subunit, gluR6, (provided by S. Heineman) was cloned into a defective HSV-1 viral vector (HSVgluR6), and used to transfer and express KA receptors in N1H3T3 fibroblasts and organotypic slice cultures. Fibroblasts infected with HSVGluR6 became sensitive to KA toxicity; whereas infection with HSVlac, which expressed ß-galactosidase, did not. This suggested that HSVgluR6 efficiently transfered and directed expression of KA receptors, resulting in toxicity after exposure to KA. Slice cultures were infected by a 250nl microapplication of virus to the stratum pyramidale of CA1 or CA3. Infection with HSVlac resulted in expression of the reporter gene in 51 ± 3 cells centered on stratum pyamidale with no overt damage to the slice culture. In contrast, microapplication of HSVGluR6 to CA1 and CA3 produced toxicity. While the cytotoxic effect in CA1 was limited to the site of injection, most CA3 pyramidal cells were killed, suggesting that recurrent excitation in CA3 may be an important transynaptic excitotoxic mechanism.

465.8

EXCITOTOXIC LESIONS IN ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES

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Slow neuronal excitotoxicity is believed to underlie many chronic neurodegenerative diseases. A 36 hour exposure of organotypic hippocampal slice cultures to low concentrations of glutamate agonists or GABA-A antagonists results in loss of specific populations of both principal cells and interneurons three days after the drugs are removed. The loss of principal cells is detected by methylene blue staining, while subpopulations of interneurons are detected with antisera directed against GABA as well as calbindin D28K, parvalbumin, neuropeptide Y, somatostatin, and VIP. A complete loss of dentate granule cells and most hilar neurons is seen after treatment with 50µM picrotoxin. This treatment has no effect on CA1 or CA3. Application of concentrations of picrotoxin up to 500µM does not appreciably increase the lesion. Exposure to 5µM kainate kills only CA3 neurons and hilar cells, and 10µM NMDA kills only CA1 neurons. Exposure to 1µM of either agonist has no effect. Co-application of both 50µM picrotoxin and 1µM kainate, in contrast destroys CA3, the hilus and the dentate gyrus. This suggests that specific patterns of delayed excitotoxicity can result from the synergistic action of both increased excitation and decreased inhibition.

465.9

CALPAIN INHIBITORS PREVENT CAPSAICIN-DEPENDENT NEUROTOXICITY IN DORSAL ROOT GANGLION NEURONS.

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Capsaicin (8-methyl-N-vanillyl-6-nonemamide) exerts a specific excitatory action on nociceptive sensory neurons resulting in elevated $[Ca^{2+}]_i$ and subsequent neuronal degeneration. A possible intermediary connecting elevated $[Ca^{2+}]_i$ with cell death is the Ca^{2+}-dependent thiol-protease, calpain. In the present study we used cultured dorsal root ganglion (DRG) neurons to examine whether calpain inhibitors can prevent capsaicin-mediated neurotoxicity. DRG neurons were isolated from 3-5 day old rat pups and grown in culture for up to 35 days. Two functional assays were employed to determine the number of capsaicin sensitive neurons in the culture: (1) Capsaicin stimulated Co^{2+}-uptake; after exposure to 1-100μM capsaicin for 20 minutes, in the presence of 5mM Co^{2+}, silver staining revealed that approximately 30-40% of neurons examined were capsaicin sensitive. (2) Fura-2 based microfluorimetry; bath application of capsaicin (1-100μM for 30-60 seconds) resulted in increases in $[Ca^{2+}]_i$ in 30-45% of DRG neurons examined. The capsaicin responses in both (1) and (2) were prevented by applying the capsaicin receptor antagonist ruthenium red (1μM), but were unaffected by the thiol-protease inhibitor E64 (10μM) or the calpain inhibitor MDL-18170 (10μM). Capsaicin-mediated neurotoxicity was assessed by adding capsaicin (1-100μM) to DRG cultures for 20 minutes in 5mM or 10mM extracellular Ca^{2+} ($[Ca^{2+}]_o$) and after 17-24 hours using propidium iodide/fluorescein diacetate fluorescence to determine the percentage cell survival. Capsaicin treatment (30-100μM) in 10mM $[Ca^{2+}]_o$, produced cell death in up to 30% of the neurons. However, when either E64 or MDL-18170 (both at 10μM) were added to the media, the death produced by capsaicin treatment was completely prevented in 5mM $[Ca^{2+}]_o$ ($p<0.001$, n=4) and partially prevented in 10mM $[Ca^{2+}]_o$ ($p<0.001$, n=9). Removal of extracellular Ca^{2+}, preincubation with the Ca^{2+}-chelator BAPTA-AM (50μM) or addition of ruthenium red (1μM) also prevented capsaicin-dependent cell death. These experiments provide strong evidence that capsaicin neurotoxicity is mediated via activation of the Ca^{2+}-activated protease, calpain.

465.10

DEFICIENT HEAT SHOCK RESPONSE IN CULTURED CNS AND PNS NEURONS. C.J. Marcuccilli, L.E. Fox and R.J. Miller. Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637.

The induction of heat shock proteins (HSP) has been suggested to play a role in neuroprotective mechanisms. In this study, we examined the effect of heat stress on cultured central neurons and acutely dissociated sympathetic neurons on the expression of the 70 kD HSP (HSP70). Rat hippocampal neurons cultured from day E17 embryos (HIP) and acutely dissociated neurons from the superior cervical ganglia from adult rats (SCG) were heat stressed for 60 min at 42.5°C. The neurons were allowed to recover for 5 hrs, after which they were assayed for HSP70 by immunocytochemistry using a monoclonal antibody. Neither HIP nor SCG neurons expressed the inducible form of HSP70 in detectable amounts. Similar results were observed with various temperatures (39 to 42.5°C). In other experiments, it was also observed that cultured cerebellar granule cells did not respond to heat stress. Western blot analysis of the HIP neurons confirmed the lack of HSP70 induction 3, 6 and 12 hrs following a 60 min heat stress period at 42.5°C. Unlike the HIP and the SCG neurons, astrocytes (AST) heat stressed for 30 min at 42.5°C did induce HSP70 immunoreactivity 2.5 hrs after the heat treatment. In addition, HIP neurons grown on secondary AST were heat stressed for 30 min at 42.5°C and 12 hrs following the treatment, immunocytochemistry revealed that only the AST responded. These results suggest that HIP and SCG neurons do not express the inducible form of HSP 70 following acute heat treatment.

465.11

PERIPHERAL NEUROPATHY IN A RABBIT MODEL OF DIDEOXYCYTIDINE (ddC) NEUROTOXICITY. G.H. DeVries*, J.T. DeVries, R.O. Calderon, T.D. Anderson, A. Davidovich, D. Feldman, T.J. Sprinkle, J. Arezzo, and C.Brosnan. Dept. Biochemistry Med. Coll. Va., Richmond, Va., Neurotech, Richmond, Va., Dept. Toxicol. Path., Hoffman-La Roche, Inc. Nutley, N.J., Dept. Neurol./Cell Mol. Biol., Med. Coll. Ga., Augusta, Ga., and Dept. Neurosci. and Path., Albert Einstien Coll. Med. Bronx, N.Y.

Forty rabbits were given either vehicle or ddC, by oral intubation , at a dose of 35 mg/kg/day for 24 weeks. The sciatic nerves were examined at 4 week intervals beginning at 8 weeks. Myelin splitting at the interperiod line and intramyelinic edema were first evident at 16 weeks and closely correlated with enlarged mitochondria with abnormal ultrastructure. Analysis of a myelin fraction isolated from the nerves showed that ddC treatment did not affect protein distribution, lipid distribution or CNPase activity. No abnormalities were noted in neurons of dorsal root ganglia. We conclude that the primary effect of ddC is on mitochondrial function in Schwann cells with subsequent myelin degeneration.

TRANSMITTERS IN INVERTEBRATES III

466.1

MODULATION OF PEPTIDE CO-TRANSMITTER RELEASE FROM B15 NEUROMUSCULAR JUNCTIONS OF *APLYSIA*. F.S. Vilim*[1], D.A. Price[3], I. Kupfermann[1], and K.R. Weiss[2]. [1]Cntr. Neurobiol. & Behav., Columbia Univ., NYS Psych Inst., NY, NY, [2]Dept. Physiol. & Biophys., Fishberg Res. Ctr. in Neurobiol., Mt. Sinai School of Med., NY, NY, and [3]Whitney Labs St Aug. FL.

The accessory radula closer muscle (ARC) and its innervation provide a model system for studying the role of neuromodulation and co-transmission. The ARC is innervated by two cholinergic motorneurons, B15 and B16, and a serotonergic modulatory neuron, the MCC. The motorneurons also contain modulatory peptide co-transmitters falling into 4 families. B15 contains the SCPs, buccalins, and FRPs, and B16 contains the buccalins and myomodulins. We have developed a method using RIA to directly measure SCP and buccalin release from the ARC following intracellular stimulation of a single motorneuron. We previously showed that SCP and buccalin are co-stored in the same dense core vesicles, and co-released in response to physiologically relevant patterns of B15 stimulation. Here, we report the effects of some of the modulators on peptide release from B15. Buccalin A, at $5x10^{-6}$M, decreased SCP release from B15, suggesting that buccalin released from B15 terminals can inhibit its own release, since SCP and buccalin are co-released. Furthermore, buccalin released from B16 may decrease peptide release from B15 because stimulation of the two motorneurons together produced less SCP release from B15 than stimulating B15 by itself. SCP_B, at 10^{-6}M, had no effect on buccalin release from B15, suggesting that SCP released from B15 does not affect on its own release. Serotonin, at $5x10^{-7}$M produced an increase in SCP release from B15, as did stimulation of the MCC. These results suggest that the extrinsic serotonergic modulatory system can affect the intrinsic peptidergic modulatory system and that the intrinsic modulatory system can affect itself.

466.2

CONVERGENT PEPTIDERGIC PHOSPHORYLATION OF PROTEINS IN THE ARC MUSCLE OF *APLYSIA*. W.C. Probst, E.C. Cropper*, S.L. Hooper, I. Kupfermann† & K.R. Weiss. Dept. Phys. & Biophys., Mt. Sinai Sch. of Med., NY, NY 10029; †Cntr. Neurobiol. & Behav., Columbia Univ. and NYS Psych. Inst., NY, NY 10032

The peptides SCP and myomodulin (MM) are contained in the ARC motor neurons B15 and B16 and increase ARC contraction amplitude and relaxation rate. The mechanism by which these changes occur was investigated by SDS-PAGE and protein phosphorylation and phosphatase assays. We previously reported that synthetic peptide application to intact ARC muscles induced phosphorylation of a >500 KDa protein.

B15 neurons were stimulated intracellularly with individual current pulses at physiological rates for 10 min. Stimulated and non-stimulated muscle homogenates were phosphorylated using a back-phosphorylation paradigm with γ-^{32}P-ATP in the presence and absence of cAMP. Autoradiography after SDS-PAGE showed that physiological stimulation prevents cAMP induced incorporation of ^{32}P into a >500 KDa protein in ARC muscles. Stimulation of B16 at physiological rates for 10 min also prevents cAMP induced incorporation of ^{32}P into this protein. These results indicate that either B15 or B16 stimulation induces phosphorylation of this band. This phosphorylation could be due to either increased kinase activity or decreased phosphatase activity. Measurements of phosphatase activity following MM application show that phosphatase activity is not changed. Direct evidence implicating PKA in the phosphorylation of this band was provided by forward phosphorylation experiments in which muscle homogenates containing either SCP or MM were treated with a specific peptide PKA inhibitor (PKI). PKI inhibited the phosphorylation of the >500 KDa protein in a dose dependent manner. These results suggest that SCP and MM converge on the same substrate protein and utilize the same second messenger pathway.

466.3

EFFECTS OF NEURON B16 STIMULATION AND MYOMODULIN APPLICATION ON cAMP AND PKA LEVELS IN ARC MUSCLE OF *APLYSIA*. S.L. Hooper*, E.C. Cropper, W.C. Probst, I. Kupfermann† & K.R. Weiss. Dept. Phys. & Biophys., Mt. Sinai Sch. Med., NY, NY 10029; †Cntr. Neurobiol. & Behav., Columbia U and NYS Psych. Inst., NY, NY 10032

The SCPs and myomodulins (MMs), modulatory neuropeptides present in the innervation of the ARC, exert similar actions on ARC contraction amplitude and relaxation rate. Previous work demonstrated that the SCPs act *via* the cAMP/PKA signal transduction pathway. Our protein phosphorylation data (Probst et al., this volume) suggest that MM may also utilize cAMP and PKA as a second messenger system.

We now report that MM application to ARC muscles causes dose dependent cAMP increases, but to maximal levels only approximately one tenth that induced by SCP application. The maximum increases of cAMP levels following physiologically relevant stimulations of neurons B15 (SCP) and B16 (MM) are approximately the same. However, unlike SCP application or B15 stimulation, MM application or B16 stimulation show desensitization, with cAMP levels decreasing rapidly with continued B16 stimulation or MM application.

Unlike SCP, MM application to intact ARC muscles induces only small increases of active PKA in low speed supernatants of centrifuged muscle homogenates. However, MM induces large increases (though still small compared to those induced by SCP) in active PKA in both uncentrifuged homogenates and in resuspensions of the pellet fraction, suggesting that MM induces both PKA activation and translocation to the pellet. Taken together, these data indicate MM's effects are likely mediated *via* the cAMP/PKA signal transduction pathway. The rapid dose dependent desensitization of MM induced cAMP augmentation may contribute to the depression of ARC contraction amplitude seen when high doses of MM act on the ARC.

466.4

A NEUROPEPTIDE Y HOMOLOG IN *APLYSIA* IS A POSSIBLE BAG CELL NEUROTRANSMITTER. D.F. Owens, S.M. Rajpara, D. Cumrine and E. Mayeri*. Department of Physiology, University of California, San Francisco, CA 94143-0444.

The neuroendocrine bag cells of *Aplysia* utilize four peptide neurotransmitters that are derived from a common precursor protein and contribute to the initiation and control of egg laying behavior. Each transmitter produces responses in abdominal ganglion neurons following a bag cell burst discharge (BCB). However, one response for which a transmitter has not been firmly established is the prolonged inhibition of neurons L3 and L6. Recently, we identified a peptide that mimics this prolonged inhibition. The peptide shares sequence homology with neuropeptide Y (NPY) and other members of the pancreatic polypeptide superfamily and has been designated *Aplysia* NPY (apNPY). Here we provide additional evidence that apNPY is a bag cell neurotransmitter. 1) Immunocytochemical analysis indicates that apNPY coexists in the bag cells with egg laying hormone, a previously identified bag cell neurotransmitter. 2) Arterial perfusion of apNPY produces prolonged inhibition of neurons L3, L6, and R2. It mimics the effect of a BCB on these cells at a concentration of 5 μM. 3) When apNPY is perfused at 100 μM for approximately 1 hr, subsequent BCB-induced inhibition of these cells is occluded. 4) ApNPY also produces short-term inhibition in neurons L2, L4, and L10, which is consistent with the responses seen in these cells following a BCB. It has no effect on many other ganglion neurons we surveyed, indicating that its effects are selective. These results suggest that the bag cells utilize peptide neurotransmitters that are derived from two distinct precursor proteins.

466.5

PDH-LIKE IMMUNOREACTIVITY IN IDENTIFIED SENSORY AND MOTOR NEURONS OF MEDICINAL LEECH EMBRYOS AND ADULTS. J.W. Bledsoe,* J. Jellies and M.P. Nusbaum. Neurobiology Research Center and Department of Physiology & Biophysics; Univ. of Alabama at Birmingham, Birmingham, AL, 35294.

Identified neurons in the medicinal leech, *Hirudo medicinalis*, are known to express several neuromodulators. Using an antiserum against a synthetic peptide, crustacean pigment dispersing hormone (PDH) (Dircksen *et al.*, Cell Tissue Res. 250:377, 1987), we have examined the leech nerve cord for immunoreactivity. In the adult, the anti-PDH labels a large number of neurons in both the ventral and dorsal aspects of each ganglion.

We used electrophysiological techniques and microinjection of the fluorescent dye Lucifer Yellow to identify some of the neurons and then double-labelled the preparations with anti-PDH, visualized with a rhodamine-conjugated secondary antiserum. Of particular interest was the double-labelling of two well studied neurons, including a primary sensory neuron known as the lateral nociceptive (N) cell and the heart excitor (HE) motor neuron. There has been no previous data to suggest possible neurotransmitters or modulators for any of the primary sensory neurons. In contrast, the HE was previously shown to be cholinergic and to also express a FMRFamide epitope (Kuhlman *et al.*, J. Neurosci. 5:2301, 1985). Certain other FMRFamide labelled neurons, including the dorsal excitor motor neuron 3, showed no staining with anti-PDH. Other double labelled neurons include the nut-neuron and the inhibitory motor neurons 1 and 2. In embryonic *Hirudo*, the N-cell appears to be the first neuron labelled by anti-PDH, being distinguished by embryonic day 10 (E10). The HEs are identifiable using this antibody by E12, which is 3-4 days prior to the earliest FMRFamide labelling. Additionally, a cell that has been identified as a possible HE homolog in ganglion 2 labelled at the same stage of development as definitive HEs. This staining pattern and temporal progression argue that the PDH-epitope is carried by a different antigen than that which presents a FMRFamide epitope. Supported by NS28603(JJ) and NS29436(MPN).

466.6

OCTOPAMINE-IMMUNOREACTIVE NEURONS IN THE LOBSTER CNS, *H.Schneider, B.A.Trimmer, J.Rapus*+, *M.Eckert*+ *& E.A.Kravitz**, *Harvard Medical School, Neurobiology Dept., Boston, MA 02115, and* +*Universität Jena, Tierphysiologie, O-6900 Jena, FRG.*

With an antibody directed against an octopamine-glutaraldehyde-thyroglobulin complex we detected about 86 immunoreactive neurons within the entire CNS of 4th stage larvae of the American lobster, *Homarus americanus*. The cells are distributed as follows: brain - 12, circumoesophageal ganglia - 2, suboesophageal ganglia - ~38, thoracic ganglia - 6 each, and 4th and 5th abdominal ganglia - 2 each. All the octopamine-immunoreactive cells are paired and located along the dorsal or ventral midline. Of the 86 neurons, 24 have been identified as neurosecretory cells, and 32 are intersegmental ascending thoracic, ascending abdominal, or descending interneurons. The neurosecretory system is arranged segmentally and located entirely within the thoracic and suboesophageal neuromeres. The projections of these neurons elaborate extensive varicose fibres along the proximal regions of 2nd thoracic and suboesophageal roots. These neurons are complementary to 2 pairs of large serotonin-containing neurosecretory neurons found in the fifth thoracic and first abdominal ganglia. The sets of neurosecretory neurons are arranged differently: the serotonin cells are intersegmental while the octopamine cells are segmental. Using a biochemical assay, the cell bodies of octopamine-immunoreactive neurosecretory cells in the thoracic segment of the nerve cord were found to contain 40-100 fmol of octopamine,while control neurons had none. Supported by NIH and DFG Schn 368/1-1.

466.7

IDENTIFICATION OF A *DROSOPHILA* HISTIDINE DECARBOXYLASE GENE REQUIRED FOR PHOTORECEPTOR SYNAPTIC TRANSMISSION. **M.G. Burg*, P.V. Sarthy+, and W.L. Pak, Dept. of Biol. Sci.,Purdue Univ., West Lafayette, IN and +Dept. of Ophthalmology, Northwestern Univ. Med. School, Chicago, IL.**

Histamine has been proposed to be the synaptic transmitter used by invertebrate photoreceptors, including *Drosophila*. Using genetic and molecular cloning approaches, we have identified a *Drosophila* gene encoding histidine decarboxylase (HDC), the enzyme which converts histidine to histamine. *Drosophila* mutants from a single complementation group with defective on-/off-transients of the electroretinogram (ERG) were found to be deficient in histidine decarboxylase (HDC) activity in a gene-dosage dependent manner, suggesting that this gene (*hdc*) may be a HDC structural gene in *Drosophila*. A rat HDC cDNA (Joseph et al., PNAS 87:733) was used to isolate a *Drosophila* cDNA homolog which encodes a protein with ~60% identity to mammalian HDCs. The *Drosophila* cDNA hybridized to the 46F region of the polytene chromosomes, to which the *hdc* gene was mapped using mutants. Northern blot and tissue *in situ* hybridizations demonstrate that the *Drosophila* cDNA detects 4 transcripts, expressed primarily in photoreceptors and small regions of the central nervous system. These transcripts are severely reduced in *hdc* mutants. The above results show that the *Drosophila* cDNA homolog corresponds to the *hdc* gene, and that mutations in this gene disrupt photoreceptor synaptic transmission. Supported by EY06214, EY03664, and EY00033.

466.8

EXPRESSION OF TDVDHVFLRFamide, A *DROSOPHILA* NEURAL PEPTIDE.

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We have generated affinity-purified antisera to a unique portion of TDVDHVFLRFamide, a novel neural peptide from *Drosophila*. TDVDHVFLRFamide immunoreactivity is expressed in *Drosophila* brain. Two medial neurosecretory cells bilaterally symmetrical to the midline send projections to the brain lobes and projections down the ventral ganglion. Two anterior neurosecretory cells send projections along the midline to the brain lobes. The medial neurosecretory cells arise earlier in development than the more anterior cells. Double-labeling studies indicate that the neurosecretory cells expressing TDVDHVFLRFamide are distinct from those expressing DSK peptides. TDVDHVFLRFamide DNA has been amplified from adult *Drosophila* RNA.

466.9

STRUCTURAL BASIS FOR PROCESSING SITE USE AND MISUSE IN AN INSECT PROHORMONE. R.C. Rayne, T. J. Horne§, A. Linacre, I. D. Campbell§, A. Drake†, and M. O'Shea*, Centre for Neuroscience, Univ. of Sussex, Brighton BN1 9QG, UK

Neuropeptides are produced by specific and limited proteolysis of precursor polypeptides. Although pairs of basic amino acids (Lys or Arg) can precede cleavage sites, such sequences are not invariably processing signals. For example, in the prohormone for locust adipokinetic hormone (proAKH), there are two dibasic sites, only one of which is recognised during processing *in vivo*.

Using computer-aided structure prediction, circular dichroism (CD) spectroscopy and ^{1}H 2D NMR, we are studying the solution structure of a complete synthetic proAKH. CD and NMR indicate that the unused processing site is located within a region of α-helix, whereas the used processing site is not. The used site appears to be associated with a 7 residue Ω-loop in which Lys and Arg define its C-terminal neck. The importance of Lys and Arg and their positions have been studied experimentally by replacing them with their analogues thialysine and canavanine. Replacement of Lys (within the loop) but not Arg (adjacent) prevents correct cleavage C-terminal to the dibasic site. Thus, although normal cleavage is C-terminal to Arg, it does not depend on the presence of Arg, but on higher order structural features N-terminal to this site. To study how these features may interact with the enzyme, we are now cloning the cDNA of the proAKH processing endopeptidase.

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466.10

IN VIVO RECORDINGS OF INDIVIDUAL INSECT PEPTIDERGIC NEURONS REVEAL BEHAVIOR-SPECIFIC DIFFERENCES IN FIRING PATTERNS. N.J. Tublitz*, Inst. of Neurosci., Univ. Oregon, Eugene, OR 97403.

The cardioacceleratory peptides (CAPs) are a family of myoregulatory neuropeptides found in the CNS of the tobacco hawkmoth, *Manduca sexta*. The CAPs are released several times throughout the life cycle of *Manduca* with each release resulting in a functionally distinct response. My interest is in understanding the mechanisms underlying this stage-specific functional plasticity.

This study's focus was in adult moths, where the CAPs act as cardioregulatory neurohormones at least twice. The CAPs are released once to expedite wing inflation (WI) in the newly-emerged moth and again during flight to facilitate nutrient transfer between abdomen and thorax. Although the heart is the primary CAP target in both cases, the time course of the CAP-induced cardioexcitation is very different in each instance. *In vivo* heart recordings show that heart rate rises rapidly during WI while heart activity escalates much more slowly during flight. Measurements of CAP blood titers during WI and flight demonstrate that the differences in heart activity are due to variations in the kinetics of the CAP titer, with a pulsatile appearance of the CAPs during WI whereas a more gradual peptide rise is detected during flight. This result suggested that the temporal differences in CAP titers at WI and flight may be due to variations in the firing pattern of the 4 pairs of CAP-secreting neurons found in each ganglion in the adult CNS. To address this issue, activity of individual CAP neurons was recorded *in vivo* during the performance of WI and flight. During WI all 8 CAP cells fire in unison immediately prior to the appearance of the CAPs in the blood. In contrast, the same 8 CAP cells exhibit a distinctly different firing pattern during flight, with one pair discharging during flight onset, a second pair firing a few minutes later, and the remaining pairs becoming active 20 minutes into flight. These results indicate that differential peptide effects can be accounted for by changes in the firing patterns of peptidergic neurons.

466.11

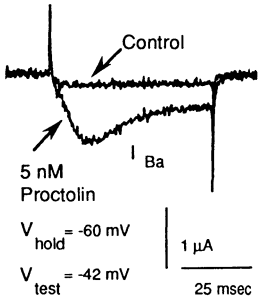
DAKH GENE STRUCTURE AND REGULATION OF PEPTIDE LEVEL. M.H. Schaffer* and B.E. Noyes. Dept. of Psychiatry, Univ. of Texas Southwestern Medical Center, Dallas, TX 75235-9070.

DAKH, a member of the RPCH/AKH family of neuropeptides, is found in some diptera including **Drosophila melanogaster.** The **Drosophila** gene encoding this peptide's precursor was identified using an oligonucleotide probe based on the known peptide sequence. This cloned DNA identifies a 530 nucleotide message on RNA blots which is consistent with the expected processing of this two exon gene and the message size of other family members. The general organization of the predicted DAKH precursor is identical to that of other family members: a signal peptide followed immediately by the DAKH peptide, a gly-lys-arg processing sequence, and a carboxy terminal peptide. In contrast to the DAKH peptide, the carboxyl peptide is larger (46 a.a.) and quite different in sequence from the homologous **Manduca** and grasshopper peptides, suggesting that the carboxyl peptides serve some role other than binding to a receptor. The DAKH gene has been localized on salivary gland polytene chromosomes to the region 64A10-64B1,2. Others have identified fly strains which carry duplications or deletions of this area. Adult flies from these strains, carrying one or three copies of the DAKH gene rather than the normal two, have near wild type levels of the peptide suggesting that DAKH levels are tightly regulated.

466.12

PROCTOLIN-INDUCED EXCITABILITY IS ASSOCIATED WITH MODULATION OF TWO POSTSYNAPTIC ION CONDUCTANCES AT AN INSECT NEUROMUSCULAR JUNCTION. David N. Mbungu* and Michael E. Adams. Depts. of Entomology and Neuroscience, Univ. of California, Riverside, CA 92521.

Proctolin release from a subset of motor neurons at house fly neuromuscular junctions produces increased postsynaptic excitability. Repetitive motor neuron stimulation or perfusion with nanomolar proctolin concentrations causes appearance of Ca^{2+} action potentials and enhancement of twitch contraction in single muscle cell targets. The proctolin effect is mimicked by the Ca^{2+} channel agonist, Bay K 8644 (1 μM), and blocked in a reversible manner by nifedipine (2 μM), verapamil (2 μM), and diltiazem (100 μM), suggesting the involvement of L-type Ca^{2+} channels. Increased excitability is correlated with a shift in the voltage activation of inward Ba^{2+} currents to more negative potentials (see inset). Proctolin exposure also causes slow depolarization of the muscle resting potential that is correlated with as much as a 50% increase in muscle input resistance. This appears to result from reduction in a resting K^+ channel conductance. Consistent with this, depolarizations caused by brief exposures to elevated $[K^+]_o$ are reduced during exposure to proctolin. We conclude that the cotransmitter actions of proctolin include modulation of postsynaptic Ca^{2+} and K^+ channels.



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466.13

PROCTOLIN MODULATION OF INSECT MUSCLE EXCITABILITY IS MEDIATED BY PROTEIN KINASE C. L.D. Acevedo* and M.E. Adams. Entomology Dept, Univ of California, Riverside, CA 92521.

Proctolin is one of the transmitters at a dual-transmitter motor neuron innervating the longitudinal ventrolateral muscles (6A and 7A) of the larval housefly, *Musca domestica*. 6% of preparations display a muscle action potential after nerve shock or current injection into the muscle. The remaining 94% of preparations are inexcitable. In the presence of proctolin, however, these muscles display action potentials and also a change in input resistance (see also Mbungu and Adams, this meeting).

We examined the signal transduction mechanisms underlying this change in muscle excitability. Both the endogenous and the proctolin-induced action potentials were blocked by 4-bromophenacyl bromide, a broad spectrum phospholipase inhibitor, indicating that hydrolysis of membrane phospholipids is involved. The phorbol ester PMA, which mimics diacylglycerol in its activation of protein kinase C, evoked an action potential similar to the endogenous and proctolin-evoked potentials. PMA, however, did not induce a consistent change in the muscle input resistance. This response was reversible and was not reproduced by the inactive 4α analog of PMA. Intracellular injection of inositol trisphosphate, generated during hydrolysis of certain membrane phospholipids, did not evoke muscle action potentials. Two kinase inhibitors, H-7 and staurosporine, suppressed both proctolin-induced and endogenous action potentials. However, membrane permeable analogs of cAMP and cGMP did not evoke action potentials. These results are consistent with activation of PKC as a signal transduction pathway for the proctolin-mediated change in muscle excitability.

467.1

APPEARANCE OF K^+, Na^+ and Ca^{2+} CURRENTS IN RAT CEREBELLAR GRANULE NEURONS REMOVED DIRECTLY FROM THE EXTERNAL GERMINAL LAYER AND MAINTAINED IN PRIMARY CELL CULTURE. R.R. Stewart*, J.-L. Bossu, J.-L. Dupont and A. Feltz. C.N.R.S., Lab. d'Etude des Régulations Physiologiques, Strasbourg, France, F-67087

Differentiation of cerebellar granule neurons was assessed by recording voltage-dependent ionic currents using the whole-cell patch clamp method on cells maintained in medium consisting of DMEM, 10% horse serum and 10^{-7} M insulin. Single precursor cells and aggregates were removed directly from the external germinal layer (EGL). During the first 3 to 10 hours in culture (day 0), only outward currents were present: a small, rapidly activating and inactivating I_A and a large, slowly inactivat-ing sustained current that partly obscured I_A and was very sensitive to TEA. By day 5, the aggregates had spread out on the poly-ornithine substrate; I_{Ba} and I_{Na} had appeared in granule neurons; I_A had increased in amplitude to dominate the sustained K current; and a second sustained outward current had appeared that was insensitive to TEA. These experiments were undertaken to provide a baseline for assessing factors that influence the differentiation and survival of granule neurons.

467.2

ION CHANNEL EXPRESSION BY EGF-RESPONSIVE STEM CELLS ISOLATED FROM MAMMALIAN CNS. D.D. Fraser*, B.A. Reynolds, S. Weiss, and B.A. MacVicar. Neuroscience Research Group, Univ. of Calgary, AB, Canada.

Stem cells isolated from the embryonic mouse striatum were induced to proliferate *in vitro* by the mitogen EGF (Reynolds and Weiss, Science 255:1707, 1992). Under appropriate conditions, these cells differentiate into neurons or glia with phenotypes that parallel adult striatum *in vivo*.

Under whole-cell patch-clamp in the current-clamp configuration, injection of depolarizing current induced a single regenerative potential followed by small amplitude membrane oscillations (n=11). Stem cells had an input resistance of 2.02 ± 0.3 GΩ. Voltage-clamp experiments revealed whole-cell capacitance values of 6.66 ± 0.4 pF and both inward and outward current components (n=117). The activation threshold for the inward current was -40 mV with a mean peak amplitude of 320 pA at -10 mV. The current was depressed by removal of external Na^+ or 1 μM TTX and reversed at the Na^+ equilibrium potential. Steady-state inactivation was observed positive to -100 mV, half-inactivation at -76 mV, and complete inactivation at -40 mV. The time constant of inactivation ranged from 3.7 ms at -30 mV to 0.7 ms at 20 mV. Recovery from inactivation occurred within 50 msec. Inward currents carried by Ca^{2+} were never observed. Outward currents consisted of both a transient and delayed component that were depressed by 5 mM 4-AP and 10 mM TEA, respectively. Both currents reversed at the K^+ equilibrium potential. The activation threshold for the transient current was -40 mV. Steady-state inactivation was observed positive to -100 mV, half-inactivation at -64 mV, and complete inactivation at -30 mV. Recovery from inactivation occurred in 900 msec. The activation threshold for the delayed current was -30 mV. Stem cell ion channels have slow kinetics and a negative voltage dependence and therefore are more similar to immature glia channels than neuronal. Ion channel expression may contribute to stem cell function *in vivo*. Supported by the MRC(Canada) and Savoy Foundation.

467.3

EGF-RESPONSIVE PROGENITOR CELLS IN THE EMBRYONIC HUMAN CENTRAL NERVOUS SYSTEM. B.A. Reynolds*[1], C. Lundberg[2], A. Bjorklund[2], P. Brundin[3], O. Lindvall[3], P. Odin[3], R. Hasham[1], R.G. Lee[1], N.B. Rewcastle[1], O. Suchowersky[1], W.G. Tetzlaff[1], T.W.J. Watson[1] and S. Weiss[1]. [1]Neuroscience Research Group, University of Calgary, Calgary, Canada and [2]Dept. of Medical Cell Research, University of Lund, [3]Department of Neurology, University Hospital, Lund, Sweden.

The presence of EGF-responsive progenitor cells in the embryonic through to adult murine CNS (Reynolds and Weiss, *Science* 255:1707, 1992) prompted us to examine whether a similar cell exists in the human CNS. Embryonic human CNS tissue (cortex, striatum and cerebellum) was mechanically dissociated and plated onto untreated tissue culture flasks in serum-free culture medium containing 20 ng/ml of EGF. Dividing cells were observed within 7 days *in vitro*, and over the following 21 days the dividing cells formed spheres that detached from the substrate. When floating spheres were dissociated and replated at low density as single cells, proliferation was re-initiated and over a 2-4 week period new spheres were formed. When spheres were plated onto poly-L-ornithine-coated glass coverslips, cells migrated from the central core, adopting the morphology of neurons and astrocytes. The presence of neurons was confirmed with antisera directed against human neuron-specific enolase. The similarity of these findings to those we reported for the murine CNS suggest that EGF-responsive stem cells are present in the human CNS.

467.4

AN EGF-DEPENDENT STEM CELL LINE DERIVED FROM THE EMBRYONIC MOUSE CNS PRODUCES NEURONS, ASTROCYTES AND OLIGODENDROCYTES. S. Weiss*, R. Hasham and B.A. Reynolds. Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

We have isolated a stem cell from the embryonic to adult mouse striatum (Reynolds and Weiss, *Science* 255:1707, 1992) which proliferates *in vitro* in response to EGF, forming a cluster of undifferentiated cells (neurospheres) which detach from the substrate and float in suspension. Neurospheres can be mechanically dissociated into single cells and a large percentage will proliferate forming new neurospheres. The spheres can be perpetuated weekly, resulting in a logarithmic growth in the number of undifferentiated cells. EGF-generated neurospheres were differentiated by mechanical dissociation and plating at high density, in the absence of EGF. After 7-14 days *in vitro* the three major cell types of the CNS were observed. Astrocytes, immunoreactive for GFAP, had a stellate morphology. Neurons were identified with antibodies recognizing MAP-2, neuron-specific enolase or neurofilament (168 kDa). Oligodendrocytes were immunoreactive for the cell surface antigens O4 and GC. All three cell types were present in differentiated EGF-generated progenitor cells that had been passaged for eight months (42 passages). A growth factor-dependent CNS stem cell line may provide a continuous source of cells for intracerebral grafting.

Supported by the Medical Research Council of Canada.

467.5

EGF-GENERATED MOUSE STRIATAL NEUROSPHERES EXPRESS THE *trk*B NEUROTROPHIN RECEPTOR. J.S. Williams*[1], A. Vescovi[1], B.A. Reynolds[1] J.P. Hammang[2], E.E. Baetge[2] and S. Weiss[1]. [1]Neuroscience Research Group, University of Calgary, Calgary, AB, Canada T2N 4N1 and [2]Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492.

We have identified an EGF-responsive stem cell in the embryonic and adult mouse striatum (Reynolds and Weiss, *Science* 255:1707, 1992), which will proliferate *in vitro* into a sphere of undifferentiated cells (neurospheres) that detach from the substrate after 5-7 days *in vitro*. The expression of the *trk* family of neurotrophin receptors in EGF-generated neurospheres was examined by northern blot analysis. Total mRNA was isolated from mouse and rat striatal EGF-generated neurospheres. Both rat and mouse neurospheres expressed high levels of *trk*B receptor mRNA, but did not express *trk* nor *trk*C mRNA. In preliminary experiments, single EGF-generated mouse neurospheres were plated on poly-L-ornithine coated glass coverslips and cultured in the absence or presence of 10ng/ml of BDNF. When examined after 14-28 days *in vitro*, neurospheres plated in the presence of BDNF contained NSE-immunoreactive cells with extensive and highly branched processes; well-developed NSE-IR cells were not observed in the absence of BDNF. Activation of the *trk*B receptor on EGF-generated neurospheres may enhance survival of and/or neurite outgrowth from newly generated neurons.

Supported by the Medical Research Council of Canada.

467.6

bFGF SUPPORTS THE SURVIVAL OF EGF-RESPONSIVE STRIATAL STEM CELLS. A.L. Vescovi*[1], E. Parati[1], A. Gritti[1], B.A. Reynolds[2] and S. Weiss[2]. [1]National Neurological Institute, Milan, Italy and [2]Neuroscience Research Group, University of Calgary, Calgary, AB, Canada T2N 4N1.

We have identified a stem cell, isolated from the embryonic through to adult striatum (Reynolds and Weiss, *Science* 255:1707, 1992), which in response to EGF will reproduce itself and generate cells that differentiate into neurons, astrocytes and oligodendrocytes. In previous studies, we found that when plated at low density (2500 cells/cm^2), addition of EGF up to 7 days *in vitro* (DIV) could initiate proliferation of the stem cell, but not if applied after 7 DIV. In the present study, we sought to determine if bFGF could prolong the survival of the EGF-responsive stem cell. Striatal cells (E14, 2500 cell/cm^2) were plated in the absence or presence of 20 ng/ml of bFGF. After 11 DIV, cultures were washed and media containing 20 ng/ml of EGF was added. After 4-5 DIV, in cultures that were primed with bFGF, >70% of the wells examined contained clusters of proliferating cells that developed into colonies with the morphologic and antigenic properties of the EGF-generated cells we have previously described. Cultures that had not been primed with bFGF showed no EGF-responsive proliferation. These findings suggest that the EGF-responsive striatal stem cell may possess bFGF receptors that regulate it's long term survival.

Supported by the Medical Research Council of Canada.

467.7

GAP-43 IS DEVELOPMENTALLY REGULATED IN GLIAL CELLS DERIVED FROM EGF-RESPONSIVE CNS STEM CELLS.

J.P. Hammang*[1], B.A. Reynolds[2], E.E. Baetge[1], and S. Weiss[2]. [1]Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT. 06492, and [2]Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

GAP-43 is a nervous system-specific membrane phosphoprotein which is down-regulated during development. Originally, GAP-43 was thought to be neuron-specific, however, recent reports indicate that this protein may be at least transiently expressed during development in some astrocytes, oligodendrocytes and in Schwann cells. At present, the role of GAP-43 in macroglia is not known. We have begun to investigate the transient expression of GAP-43 in glial cells generated from the EGF-responsive stem cells derived from the embryonic and adult murine striatum (Reynolds and Weiss, *Science* **255** p. 1707 1992). Astrocyte differentiation was induced by plating the stem cells in a medium containing 1% FBS with no EGF. The cells were then probed with specific antibodies for GAP-43 and GFAP using dual-label immunofluorescence. Initially, GAP-43 and GFAP expression is low in all cells, but by 3-4 days, high levels of GAP-43 and GFAP is co-expressed in a large number of cells. By one week however, the large majority of GFAP-expressing astrocytes no longer express GAP-43. The EGF-responsive stem cells may represent a useful model system for the study of the role of GAP-43 in glial and neuronal development (supported by the MRC Canada).

467.8

IGF-I STIMULATES CHICK SYMPATHETIC NEURON PROLIFERATION BOTH IN VITRO AND IN VIVO AND IS PRODUCED IN DEVELOPING SYMPATHETIC GANGLIA.

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The ability of immature neurons from chick lumbosacral sympathetic ganglia to proliferate *in vitro* was used to identify factors that affect neurogenesis. We found that neuron proliferation, but not survival, is dependent on the presence of serum. Under serum-free culture conditions, IGF-I, insulin or PDGF caused an increase in both the cell number and proportion of cells that incorporated ^{3}H-thymidine. This effect was not seen when bFGF, aFGF or TGFα was used to replace serum. Application of IGF-I onto the chorioallantoic membrane of chick or quail embryos also stimulated the incorporation of 3H-thymidine in sympathetic ganglion neurons *in vivo*. IGF-I mRNA was detectable in E7 sympathetic ganglia by PCR. These results show that sympathetic neuron proliferation is subject to extrinsic control both *in vitro* and *in vivo* and suggest an autocrine/paracrine action of IGF-I in the generation of avian sympathetic ganglion neurons.

467.9

CNS GLIAL CELLS SUPPORT SURVIVAL, DIVISION AND DIFFERENTIATION OF OLFACTORY NEURONAL PRECURSOR CELLS IN VITRO.

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In the adult mammal, new olfactory receptor neurons (ORNs) are produced from neuronal progenitor cells retained in the olfactory neuroepithelium. To determine whether the progenitor cells could divide and differentiate in culture, we have disaggregated nasal mucosal cells from newborn Sprague Dawley rats and plated them on either a polylysine coating or a monolayer of neonatal rat astrocytes. On polylysine, both mature and immature ORNs died within 5-6 days. On the monolayer of CNS astrocytes, mature ORNs (OMP+) again died rapidly (by 5-7 days), while immature ORNs (positive for neuron-specific tubulin, NST) appeared to multiply and formed large aggregates of neurons. OMP+ neurons re-appeared 10 days after plating and were very prominent in 15 day cutlures. Three different CNS area astrocytes were equally effective in promoting survival and differentiation. Pulse labeling with tritiated thymidine was done over 15 days in culture. Uptake of isotope in neurons was detected by combined autoradiography and immunocytochemistry. Neuronal progenitor cells both divided and differentiated in culture. Both OMP+ and NST+ neurons were generated by cell divisions that took place during restricted times after plating. Aspects of the data suggest that OMP expression is an innate property of ORNs and that expression may rely more on survival than target-specific regulatory factors. The neuronal progenitor cell appears to be negative for NST. Astrocytes (but not conditioned medium) allow survival, division and subsequent differentiation. Support: Amer Paralysis Assoc PBI-8803-1 + NIH DC00347.

467.10

DIFFERENTIAL EFFECTS OF CNTF, LIF AND IL-6 ON OLIGODENDROCYTE-TYPE 2-ASTROCYTE PROGENITOR CELLS.

J.C. Louis*, E. Magal, S. Takayama and S. Varon, Department of Biology 0601, University of California, San Diego, La Jolla CA 92093.

The proliferation and development of oligodendrocyte-type 2-astrocyte progenitor (O-2A) cells have been shown to be regulated by a number of peptide growth factors, including PDGF, FGF and IGFs. We report here that three members of the newly-defined family of neuropoietic cytokines (Bazan, *Neuron* 1991, 7:197-208) - ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF) and interleukin-6 (IL-6) - exert different influences on the CG4 cell line (Louis et al., *J. Neurosci. Res.* 1992, 30:193-204) of O-2A cells. Both LIF and IL-6 are potent mitogens for CG4 cells (cell cycle: 36-48 hr). In their presence, CG4 cells become multipolar, lose the A2B5 antigen and acquire the O4 antigen, but do not differentiate to mature oligodendrocytes or type-2 astrocytes. When given together with PDGF, LIF as well as IL-6 promote the continuous proliferation of CG4 cells as A2B5$^+$/O4$^-$ progenitors (cell cycle: 20 hr), while in PDGF alone, they would cease their proliferation and differentiate to oligodendrocytes within 5-7 days. In sharp contrast, CNTF is not mitogenic for CG4 cells. In the presence of CNTF alone, the CG4 cells express transiently the astrocyte marker GFAP (for 1-2 days), after which they differentiate to mature oligodendrocytes. CNTF, however, reduces dramatically the growth rate of CG4 cells maintained in PDGF: after 7 days in CNTF and PDGF, the length of the cell cycle is increased from 20 hr to >60 hr. This change in the growth rate is accompanied by the appearance after 7 days of a population of CG4 cells (about 40%) that displays the morphological (unipolar cells) and antigenic (O4$^+$/A2B5$^-$/galC$^-$/vimentin$^-$/GFAP$^-$) properties of *adult* O-2A progenitor cells. These results indicate that, in addition to their actions on CNS and PNS neurons, the neuropoietic cytokines, alone or in combination with other factors, are capable of regulating the proliferation and differentiation of O-2A cells. In particular, CNTF may be a major instructive factor for the generation of adult O-2A progenitors from the perinatal ones. Supported by NINDS grant NS-16349.

467.11

BETA-2 INTEGRINS ARE CONSTITUTIVELY EXPRESSED ON MICROGLIA IN THE NORMAL HUMAN FETAL BRAIN.

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To further characterize microglia in the normal fetus, serial sections from 16 wk to 23 wk abortuses of HIV seronegative mothers were examined immunocytochemically with antibodies to Beta-2 integrins. Integrins are part of a group of cell adhesion molecules that are involved in cell-cell and cell-matrix interactions. As transmembrane glycoproteins, integrins link the intracellular cytoskeleton to either extracellular matrix proteins or other cellular receptors. Beta-2 integrins are heterodimers that are characterized by a Mr 95,000 Beta-2 subunit (CD18) and alpha subunits of either Mr 180,000 (LFA-1; CD11a), 170,000 (MAC-1; CD11b) and 150,000 (P150,95; CD11c). Monoclonal antibodies to these alpha chains included LFA-1 (CD11a), Bear-1/C3bir (CD11b) and Leu-M5 (CD11c). Preliminary data suggests that these markers are constitutively expressed on microglia in normal fetal human brain at 16 wks. These antigens were expressed on both amoeboid and more ramified forms of microglia in the cortical layers and in the subcortical grey and white matter. Microglia were also found to constitutively express leucocyte common antigen, in addition to HLA-DR (LN-3, HLA-DR), a major histocompatibility complex class II antigen and FCgammaRI as previously reported (Soc Neurosci Abstr 17:734:1991). The role of Beta-2 integrins, as expressed on fetal microglia, in normal gliogenesis and brain development is not clear. On leucocytes, Beta-2 integrins are involved in mediation of phagocytosis, adhesion and migration in the immune system. The presence of adhesion molecule receptors on microglia suggest that they may participate in cell-cell interactions, in the regulation of cell migration and differentiation during brain development. Since microglia are productively infected by HIV-1 in the CNS, these receptors may also be involved in the mediation of CNS pathogenesis.

467.12

BASIC FIBROBLAST GROWTH FACTOR AND NERVE GROWTH FACTOR INCREASE NEUROBLASTS PROLIFERATION AND DIFFERENTIATION INTO NEURONS CONTAINING GLUTAMATE-LIKE IMMUNOREACTIVITY.

F.M. Vaccarino*, D. Hartigan and J. F. Leckman. Child Study Center, Yale Univ. Sch. of Med., New Haven , CT 06510.

The development of mammalian telencephalon involves a sequence of multiplication of stem cells, differentiation into neurons and glia, migration to target sites and synaptogenesis. Each phase is likely to involve the expression of cell type-specific genes in a spatially and temporally-restricted manner. In order to study transcription factors orchestrating such processes, we created an *in vitro* model simulating aspects of early telencephalic development in primary culture. The neuroepithelium of E13 rat telencephalon was grown in dissociated cell culture in serum-free medium. At the density of 80,000 cells/cm^2, the addition of growth factors (bFGF and NGF) increased the number cells immunoreactive for nestin (a marker for multipotential stem cells) and the proliferation rate (at 5 days in vitro, bromodeoxyuridine incorporation during 3 hour labelling was 9.1+/-2.2 and 14.2+/-1.6 without and with growth factors, respectively). Cells containing GABA- and glutamate-like immunoreactivity started differentiating at 2 days in vitro and their number and complexity of arborization increased with time. Growth factors increased the differentiation of cells containing glutamate-like immunoreactivity at 2 and 4 DIV, while the effect on GABA-containing neurons was modest. GFAP-positive astrocytes were entirely absent until day 6 but differentiated from nestin-positive stem cells afterwords. The sequence and timing of these developmental events closely matches the development of telencephalon *in situ*.

467.13

THE EFFECTS OF EGF AND TGF-β ON OLFACTORY NEUROGENESIS AND NEURON SURVIVAL *IN VITRO*. N. K. Mahanthappa* and G. A. Schwarting. E.K. Shriver Ctr., Waltham, MA 02254; and Prgrm. in Neurosci., Harvard Med. Sch., Boston, MA 02115.

Peptide growth factors that affect growth and differentiation of ectodermally-derived tissues were tested for neurogenic and neurotrophic activity in neonatal rat olfactory epithelium (OE) cell cultures. In dissociated OE cultures, EGF, TGF-β1, and TGF-β2 have no neurotrophic activity - few cells survive beyond 1 week. ^{3}H-thymidine (^{3}H-T) labeling, however, revealed that EGF promotes division of keratin$^+$ basal cells two-fold, while TGF-β2 inhibited this labeling completely. Though NCAM$^+$ neurons show no ^{3}H-T labeling in EGF or control conditions, TGF-β2 promotes labeling of approximately 10% of these cells. In partially dissociated OE cultures, cell survival is improved. In this condition EGF effects were as above; both TGF-β1 and TGF-β2 inhibited keratin$^+$ cell division while promoting ^{3}H-T labeling of 20-30% of NCAM$^+$ cells. Tracking identified cell clusters maintained in EGF for 4 d and then transfered to TGF-β2, suggests that EGF maintains basal cells while TGF-β2 promotes neurogenesis from this population. We are assaying the presence of endogeneous TGF-β2 production in these cultures, and are testing known neurotrophic factors for effects on OE neuron survival.

CELL LINEAGE AND DETERMINATION III

468.1

SELECTIVE ISOLATION OF NEURAL AND GLIAL PRECURSORS FROM THE DEVELOPING MURINE BOWEL WITH ANTIBODIES TO A 110 kDa CELL SURFACE LAMININ BINDING PROTEIN A. Chalazonitis,* Y.M. Tennyson, T.P. Rothman and M.D. Gershon. Dept. of Anat. & Cell Biol., Columbia Univ., College of P & S, New York, NY 10032.

A 110 kDa laminin binding protein has been shown to be expressed by enteric and other peripheral neurons. This protein is not expressed by premigratory or early migratory neural crest-derived precursor cells. Immunocytochemical observations have suggested that the 110 kDa protein might be acquired by crest-derived cells shortly after these cells colonize the developing gut of avians or mice. Experiments were carried out to test the hypothesis that the 110 kDa laminin binding protein is selectively expressed in the developing gut by the crest-derived precursors of enteric neurons and glia. The bowel of fetal mice (E16) was dissociated and the resulting single-cell suspension was exposed sequentially to rabbit antibodies to the 110 kDa protein (α-110) and magnetic beads coated with goat anti-rabbit secondary antibodies; α-110-immunoreactive cells were finally isolated with a magnet. α-110-selected and unselected (residual) cells were seeded in dishes coated with collagen and laminin at a density of 27 X 10^3 cells/dish and cultured for 8 days. Neurons were identified immunocytochemically with antibodies to neurofilament proteins or neuron specific enolase, while glia were identified with antibodies to the S-100 protein. Neurons and glia were also found to be immunostained by α-110. Significantly more neurons and glia developed in cultures of α-110-immunoselected cells than in cultures of unselected cells. The proportion of cells in the cultures of selected cells that developed as neurons was about 3-fold that found in cultures of unselected cells. Similarly, the number of S-100 immunoreactive cells in cultures of selected cells was about 4-fold that observed in the cultures of unselected cells. Immunoselection of cells from E16 fetal mice thus supports the hypothesis that neurons or glia preferentially develop in cultures of cells selected with the α-110 reagent. It has been suggested that the 110 kDa laminin binding protein is a receptor that mediates the response of enteric neural and glial precursors to laminin. The current observations support this idea. Supported by NIH grants NS26766, HD17736, HD20470, HD 21032 and NS15547.

468.2

PHENOTYPES EXPRESSED BY NEUROGENIC NEURAL CREST-DERIVED CELLS IMMUNOSELECTED FROM THE WALL OF THE DEVELOPING BOWEL OF AVIAN AND RAT EMBRYOS. T. P. Rothman,* A. Chalazonitis, H. D. Pomeranz, Y. M. Tennyson and M. D. Gershon. Dept. Anatomy & Cell Biology, Columbia University P&S, New York, NY 10032.

The enteric nervous system (ENS) is formed by cells that migrate to the bowel from vagal and sacral levels of the neural crest. Since the developmental potential of crest-derived cells that have entered the gut is less than that of premigratory or early migratory crest cells, the properties of at least some crest-derived cells change as they migrate to the gut. In order to study the characteristics of enteric crest-derived cells, it is necessary to isolate them from within the bowel. Gut from chick or quail embryos (E4-7) or fetal rats (E11-15) was dissociated with collagenase to yield a suspension of single cells, which were then subjected to immunoselection. The suspension was mixed with a murine monoclonal antibody (NC-1) that reacts with a surface epitope that is found, in the gut, only on crest-derived cells. NC-1-coated cells were then isolated with magnetic beads coated with goat anti-mouse antibodies, and selected with a magnet. NC-1$^+$ cells were cultured for up to 8 days, as were the cells remaining after the NC-1$^+$ population had been removed. Immunoselection was found to greatly enrich the population in NC-1$^+$ cells. Expression of neurofilament proteins and neurite extension were preferentially seen in cultures of immunoselected cells. Neurite outgrowth was promoted by laminin. Some neurons contained vasoactive intestinal peptide (VIP) or serotonin (5-HT); however, tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) which are not expressed by avian enteric neurons, *in situ*, and are expressed only transiently in developing rat bowel were present in immunoselected cells. A glial marker, glial fibrillary acidic protein (GFAP), was apparent in a subset of immunoselected cells. Non-selected cells proliferated rapidly and relatively few expressed a neuronal or glial phenotype. These experiments demonstrate that neurogenic crest-derived cells can be isolated from within the enteric mesenchyme of both developing rats and birds. At least some of the immunoselected cells appear to be capable of expressing phenotypes appropriate for the ENS. The appearance of TH in cells immunoselected from avian bowel supports the hypothesis that the enteric microenvironment acts to suppress catecholaminergic expression *in situ*. Supported by NIH grants HD20470, NS15547, NS26766, HD17736, and a fellowship from Lilly Res. Labs.

468.3

PHENOTYPIC PLASTICITY OF EMBRYONIC CILIARY NEURONS. J. Sechrist, J. Wolf, and M. Bronner-Fraser*. Developmental Biology Center, Univ. of Calif., Irvine, CA 92717.

Ciliary neurons from 6.5 day quail embryos can change their neurotransmitter phenotype from cholinergic to adrenergic after transplantation to the trunk of chick embryos. Here, we examine if there is a critical period during which this transition can occur and if the post-mitotic neurons re-enter the cell cycle. After dissociation, individual ciliary neurons (identified by phase bright cell bodies and axonal stubs) were isolated manually, labelled with DiI and injected into the trunk somites. An average of 400 neurons per experiment (157 - 711 range) were derived from 6.5 - 10 day quail embryos and injected into 2.5 day embryonic chick trunks. To verify that these cells were neurons, some were plated on laminin and shown to be purely neuronal. Catecholamine histofluorescence in DiI-labelled cells was analyzed 4 - 5 days post-injection as described previously (Sechrist *et al.*, J.Neur.Transplant 1:113, 1989). Some embryos were treated with 2 - 3 doses of ^{3}H-thymidine after injection. For ciliary neurons derived from 6.5 to 8.5 day old embryos, as many as 14% developed catecholamine histofluorescence in the host sympathetic ganglia, aortic plexuses and adrenal medulla. In contrast, neurons from 8.5 - 10 day embryos never became catecholamine positive, indicating a limited time-frame for phenotypic conversion. Interestingly, a substantial number of DiI labelled cells took up ^{3}H-thymidine, indicating that they re-entered the cell cycle after transplantation. Our results indicate that phenotypic plasticity of ciliary neurons is age dependent and may involve re-entry into the cell cycle.

468.4

INFLUENCES OF THE NOTOCHORD ON FORMATION OF THE NEURAL CREST AND ITS DERIVATIVES. Kristin B. Artinger* and Marianne Bronner-Fraser, Developmental Biology Center, University of California, Irvine, CA 92717

Grafting experiments have shown that the notochord affects dorsoventral polarity of the neural tube by inducing formation of motor neurons and floorplate cells in the ventral neural tube. Here, we examine if the notochord inhibits formation of dorsal structures by grafting a notochord adjacent to or on the dorsal neural tube. When notochords were grafted onto the dorsum of the neural tube, a dorsal floorplate was induced; however, differentiation of dorsal structures was not inhibited. Neural crest cells formed and emigrated from the dorsal neural tube in the presence of the ectopic notochord. In addition, neurons with the appearance of commissural neurons formed adjacent to the ectopic floorplate. In fact, numerous neuronal cell bodies and axonal processes were observed traversing the induced, but not the endogenous, floorplate. When analyzed at stages after ganglion formation, the dorsal root ganglia were reduced in size and shifted in position in embryos that had received a notochord graft. Supernumerary ventral roots were observed adjacent to the implanted notochord. The effects were similar whether or not the host notochord was ablated. These results suggest that the notochord cannot prevent the formation of dorsal structures such as the neural crest, but can alter the size and position of neural crest-derived dorsal root ganglia.(Supported by USPHS HD25138)

468.5

SPECIFICATION OF THE ROSTROCAUDAL AXIS OF THE PREMIGRATORY AVIAN NEURAL CREST. Gabrielle G. Leblanc*. Dept. of Biological Structure and Function, School of Dentistry, Oregon Health Sciences University, Portland, OR 97201.

Different rostrocaudal populations of premigratory neural crest cells differ in their developmental potentials, both *in vivo* and *in vitro*. *In vivo*, cranial neural crest gives rise to large amounts of cartilage and bone, whereas trunk neural crest cells lack chondrogenic potential. *In vitro*, cranial neural crest produces large numbers of fibronectin (FN)- and procollagen I (Col I)-immunoreactive cells, whereas trunk neural crest produces relatively few of these cells. These *in vitro* differences in protein expression by cranial and trunk neural crest cells can be used to explore the mechanisms that determine the rostrocaudal axis of the premigratory neural crest.

The rostrocaudal axis of the premigratory neural crest may be specified by factors arising from the underlying mesoderm. To test this possibility, I examined whether coculture with anterior mesoderm can induce trunk neural crest cells to express cranial-specific traits *in vitro*. Trunk neural crest cells were cocultured together with explants of early (stage 3) Hensen's node, which contains prospective anterior mesodermal cells. After five days of coculture, both FN and Col I immunoreactivities were seen in the neural crest cells surrounding the Hensen's node explant. The apparent FN- and Col I-stimulating activity of early Hensen's node is mimicked by transforming growth factor (TGF)-ß1.

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468.6

BASIC FIBROBLAST GROWTH FACTOR (bFGF) INFLUENCES NEURAL CREST CELL FATE VIA AN INTRACRINE MECHANISM. G. Ciment*, L. Sherman, K.M. Stocker and R.S. Morrison. Dept. Cell Biology & Anatomy, Oregon Health Sciences Univ., Portland, OR 97201.

In previous work, we found that neural crest (NC)-derived Schwann cell progenitors of early avian embryos underwent a transformation *in vivo* or in culture into another NC-derivative -- melanocytes -- following treatment with either the phorbol ester TPA or basic fibroblast growth factor (bFGF). These and other data suggest that bFGF may be involved in the commitment of bipotent intermediates in the NC cell lineage to either the melanocyte or Schwann cell fate.

In these studies, we show that TPA induces bFGF expression in these melanocyte/Schwann cell progenitors, but that bFGF need not be released in order to influence cell fate. Treatment of cultures of early embryonic peripheral nerves with TPA, for example, induced expression of bFGF mRNA and protein and caused melanogenesis in cells which would normally have given rise to Schwann cells. Addition of two different bFGF antisense oligonucleotides blocked these effects of TPA, but that sense oligonucleotides or scrambled oligonucleotides (i.e., with the same base content but with a different sequence) did not, suggesting that bFGF expression is part of signalling pathway by which TPA induces transformation into melanocytes. Addition to the culture medium of bFGF neutralizing antibodies or other agents known to inhibit bFGF-binding to its extracellular receptor had no effect on the TPA-induction of melanogenesis, indicating that bFGF does not need to be released in order to act. These data indicate that bFGF may act as an intracellular "intracrine" factor in the determination of NC cell fate.

468.7

INVESTIGATION OF THE ROLES OF TGFα/EGF AND FGF SIGNALING IN RETINAL DEVELOPMENT THROUGH ALTERATIONS IN RECEPTOR EXPRESSION. L. Lillien* and C. Cepko. Department of Genetics, Harvard Medical School, Boston MA. 02115.

In order to begin to identify the signaling systems that regulate the development of the rat retina, we used in vitro assays to screen for extracellular signaling molecules that affect proliferation and cell type choice. These functional assays showed that several peptide growth factors found in the developing retina, including TGFα and bFGF, enhanced proliferation and selectively inhibited rod photoreceptor development (Lillien and Cepko, Development 1992). The effects of TGFα and FGF on proliferation were found to be temporally regulated in vitro: cultures of younger retinal cells (< E20) were more responsive to FGF while cultures of older retinal cells (>E20) were more responsive to TGFα. To determine the roles of these signaling systems in normal retinal development, we are using retrovirus vectors to express the human EGF receptor and mouse FGF receptor-1. To analyze the function of endogenous EGF and FGF receptors, we are introducing truncated forms of the receptors to block function in a dominant-negative manner. To determine whether limiting levels of receptor expression underlie the temporal changes in responsiveness to TGFα and FGF observed in vitro, we are introducing full length forms of the receptors. In order to label cells expressing these constructs, FGF R-1 viral constructs also contain the gene encoding the histochemical marker alkaline phosphatase. Cells infected with the EGF receptor virus can be distinguished with an antibody that selectively labels the human form of the receptor. Clones of infected cells will be examined.

468.8

RETINAL LINEAGE OF THE CLEAVAGE STAGE PROGENITOR IN XENOPUS IS DEPENDENT ON POSITION. S. Huang* and S.A. Moody Dept. Anatomy & Cell Biology, Univ. Virginia, Charlottesville, VA 22908.

Our previous studies (Neurosci. Abstr., 17,924, 1991) demonstrated that the retinal lineages of cleavage stage blastomeres change after the ablation of the major retinal progenitor and the changes are different for dorsal and ventral blastomeres. To determine whether the retinal lineage changes are due to the position of the remaining blastomeres two sets of experiments were carried out. First, several different sets of blastomeres were ablated at 32-cell stage embryo to demonstrate whether the change in position of the remaining blastomeres predicts their change in retinal fate. Along the dorsal midline when a blastomere was ablated its animal neighbor took over its retinal fate and produced an amount of retinal cells typical for the ablated vegetal neighbor. When more ventral blastomeres were removed, the dorsal blastomeres did not adopt the retinal fates of their ventral/animal neighbors. These results demonstrate that the blastomeres shift fate in a dorsal/vegetal direction but not in a ventral/animal direction. In order to directly test whether blastomere position determines retinal fate, reciprocal transplantations were done between a dorsal midline blastomere, the major retinal contributor, and a ventral blastomere, which normally only gives rise to caudal trunk structures. The transplanted blastomeres adopted a new fate according to their new position. The ventral blastomere became a major retinal contributor when it was placed in the dorsal animal pole, while the clone of the major retinal progenitor was restricted to the tail region of the embryo. These results indicate that the position of a blastomere prior to gastrulation is the important determinant of whether retinal cells are among its descendants.

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468.9

TEMPORAL RELATIONSHIP BETWEEN MITOSIS AND RETINAL GANGLION CELL DIFFERENTIATION. D. K. Waid*, S. C. McLoon. Dept. of Cell Biol. and Neuroanatomy, Univ. of Minnesota, Minneapolis, MN 55455.

The mature retina consists of many different cell types that develop from a seemingly homogeneous population of neuroepithelial cells. Previous studies have shown that all retinal cells arise from common precursors. It is unclear when during the life history of a retinal cell, it becomes committed to a particular phenotype. This issue was addressed in the present study. A monoclonal antibody, RA4, recognizes retinal ganglion cells in the chick retina. In embryonic chick retina, $RA4^+$ cells are found in the mitotic layer of the retina with a spatial and temporal distribution that correlates with ganglion cell birth. Dividing cells, however, were not observed to express the RA4 antigen. This early differentiation of ganglion cells shows that they are committed to this phenotype before they migrate from the mitotic cell layer. Double labeling with BrdU and RA4 antibodies allowed the temporal relationship between mitosis and ganglion cell differentiation to be established. To accomplish this, BrdU was injected into chick embryos. At various post-injection times, immunohistochemistry was used to identify $BrdU^+$ mitotic figures. The first BrdU labeled mitotic figures were seen one hour following BrdU injection. By three hours post-injection, all mitotic figures were BrdU positive. The first $BrdU^+/RA4^+$ cells appeared in the mitotic layer within minutes of mitosis. The average time between mitosis and RA4 expression for cells in E3 retina was 1.5hrs, and in E9 retina was 7hrs. This data was most consistant with there being two populations of ganglion cell differentiation: an early, fast differentiating population, and a late, slow differentiating population. The rapid differentiation of some ganglion cells is most consistent with determination taking place prior to mitosis, while the slower differentiation of other cells may indicate determination taking place after mitosis. In conclusion, these results suggest that determination of a specific phenotype may occur independent of mitosis.

468.10

AN ANALYSIS OF OLFACTORY RECEPTOR NEURON LINEAGE USING A REPLICATION INCOMPETENT RETROVIRUS ENCODING ALKALINE PHOSPHATASE. M. E. Caggiano, D. D. Hunter,* J. S. Kauer. Neuroscience Program, Tufts/New England Medical Center, Boston, MA. 02111.

Olfactory receptor neurons develop from progenitor basal cells lying in the deep region of the olfactory epithelium (OE). Two major types of basal cells (globose and horizontal) have been characterized on the basis of their response to ablation of their target, the olfactory bulb. Under these conditions, the more superficial global basal cells appear to be the major source of new cells during olfactory receptor neuron renewal. Although both types of basal cells have been partially characterized, precisely how basal cells differentiate to form receptor cells is not known. We have employed a recombinant retrovirus (DAP) that encodes the human placental alkaline phosphatase gene to infect olfactory basal cells in order to study their lineage. Ca. 5ul of concentrated DAP was injected through the skull into a region near the dorsal recess and septum of the OE in 7-10 day old rats. Following an incubation period of 5-20 days, regions of the OE that included the turbinates and septum were analyzed in whole mount preparations for incorporation of the retrovirus into cells. Under these conditions, scattered groups of small rounded cells with darkly stained cytoplasm and unstained nuclei were seen in anterior and posterior septal regions. Five days after infection, these clusters consisted of groups of 2 to 8 cells. A similar distribution was seen 15 days after infection, although some cells displayed a more oval shape and possible projections. Using specific markers we will now begin to characterize the cell types in these clusters; this should help us define the cell lineage in the OE.

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468.11

CLONAL HETEROGENEITY IN THE GERMINAL ZONE OF THE DEVELOPING RAT TELENCEPHALON, S. E. Acklin* and D. van der Kooy, Dept. Anatomy, Univ. Toronto, M5S 1A8 Toronto, CANADA.

In order to characterize the proliferation characteristics of precursor cell lines in the mammalian telencephalic germinal zone, we have previously employed a simultaneous double labeling technique which combines retroviral identification of individual proliferating clones in the E17-19 developing rat forebrain and their double labeling with 3H-thymidine. After 48 hours survival we found the cortical (but no the striatal) germinal zone to be segregated into three spatially distinct horizontal bands (A, B and C), comprising clonal populations with distinctive cell cycle times, incidences of cell death and modes (symmetric vs. asymmetric) of proliferation (Acklin & van der Kooy; soc. Neurosci. Abstr. 1479, 1991). It is important to ask whether these distinct cortical germinal zone populations predict the mature phenotypic (e.g. neurons vs. glia) or spatial identity (e.g. deep vs. superficial cortical layers) of the cells they give rise to. In a first set of experiments we characterized the spatial distribution and proliferation characteristics of cortical precursor populations (using our double labeling method) after only 17 (instead of 48) hours survival. We found that although the overall thickness of the germinal zone was slightly smaller after 17 hours survival (E17-18), it was still segregated into heterogeneous clonal populations (A, B and C) with comparable proliferation characteristics to those seen at E19. We also found that with the longer survival of 48 hours (E17-19) compared to the shorter survival time of 17 hours (E17-18), the clonal population in the ventricular zone proper (A) and one (B) of two horizontal bands in the subventricular zone increased in thickness, whereas the second horizontal band (C) in the subventricular zone decreased substantially in thickness. Thus, clonal population C (perhaps giving rise to a subpopulation of neurons) may have become mostly postmitotic by E19. We are presently asking which of the different clonal populations in the germinal zone remains perinatally, when neurogenesis has largely ceased but glia still are being produced. The identification of this remaining population (presumably giving rise to glia) by its proliferation characteristics may suggest an isomorphism between it and one of the earlier germinal zone populations.

468.12

SEPARATE PROGENITOR CELLS IN THE VENTRICULAR ZONE GIVE RISE TO CORTICAL PYRAMIDAL AND NONPYRAMIDAL NEURONS THROUGHOUT THE PERIOD OF NEUROGENESIS. J.G. Parnavelas*, C. Danevic, P. Boardman, M.C. Mione and M.E. Cavanagh (SPON: European Neuroscience Association). Department of Anatomy, University College London, London WC1E 6BT, U.K.

We reported recently that early in cortical neurogenesis (E15/E16), the ventricular zone in the rat contains separate progenitor cells for the principal neuronal cell classes, the pyramidal and nonpyramidal neurons. Here we sought to investigate whether these neuronal types originate from separate progenitor cells throughout the period of neurogenesis. For this purpose, recombinant retrovirus containing the reporter gene for E. coli β-galactosidase was injected into the telencephalic ventricles of rat embryos at E14/E21. Serially cut coronal sections of adult cortex were histochemically stained for β-galactosidase and processed for electron microscopy. Camera lucida drawings were made to map the position of labelled cells. Discrete clusters of closely spaced labelled cells were considered to be derived from the same precursor cell in the ventricular zone, i.e. to belong to the same clone. Clonally-related labelled neurons were examined with the electron microscope, and their phenotypes identified using ultrastructural criteria.

Clusters of clonally-related neurons examined from animals injected with retrovirus at various times during corticogenesis contained at all times either all pyramidal or all nonpyramidal neurons. These findings suggest that the ventricular zone contains separate progenitor cells for pyramidal and nonpyramidal neurons during the entire period of neurogenesis.

468.13

NEUROTRANSMITTER EXPRESSION IN CLONALLY RELATED NEURONS IN THE RAT CEREBRAL CORTEX. M.C.Mione*, C. Danevic*, M.E. Cavanagh*, P. Boardman and J.G. Parnavelas*(SPON: Brain Research Association). Department of Anatomy, University College London, London WC1E 6BT, U.K.

We examined the neurotransmitter content of clonally related neurons in the cerebral cortex of adult rats. Such neurons were marked with a retrovirus lineage tracer, containing the reporter gene for E. coli β- galactosidase, injected into the telencephalic ventricles of rat embryos at E15–E17 and subsequently detected histochemically. Discrete clusters of β- galactosidase positive cells, considered to be clonally related, were subjected to immunohistochemical analysis for the inhibitory neurotransmitter GABA and the excitatory amino acids Glu and Asp. This analysis was performed on Araldite embedded, 0.5 μm thick sections. Three consecutive sections through every neuron of 20 discrete clusters of neurons were processed each for one of the three neurotransmitter candidates, while thin sections were used to identify the phenotype of every cell with the electron microscope. Clusters contained between 2 and 8 neurons which were either confined to one layer or distributed over 2 or more layers.

Nineteen clusters contained neurons positive for only one transmitter candidate (9 GABA; 5 Glu; 5 Asp) and one group of 3 nonpyramidal neurons was immunonegative for all 3 amino acids. However, in the clusters positive for Asp or Glu a number of neurons contained both excitatory amino acids.

These findings support the hypothesis that genetic determinants play an important role in the expression of the principal neurotransmitters in neurons of the mammalian cerebral cortex.

468.14

PARALLEL DEVELOPMENT OF RADIAL GLIA AND MYELINATION IN HUMAN FETAL SPINAL CORD. K.M. Weidenheim, I. Epshteyn, W.K. Rashbaum, C.S. Raine* and W.D. Lyman. Albert Einstein College of Medicine, Bronx, NY 10461

Radial glia and differentiated myelin-forming oligodendrocytes are present in the human fetal spinal cord (HFSC) by 10 weeks of gestation (WOG). However, neuroanatomic correlation of the development of these two cell types has not been performed. In this study, immunohistochemical methods using antibodies to vimentin and glial fibrillary acidic protein (GFAP) were used to mark radial glia and an antibody to myelin basic protein (MBP) identified oligodendrocytes and myelin in vibratome sections of 37 HFSC ranging in age from 9-20 WOG. Radial glia were more numerous in the anterior and anterolateral funiculi than the dorsal funiculus; the posterolateral funiculus had very few radial processes. In addition, radial glia were always more numerous at the cervical level. Expression of MBP followed the same pattern. The results suggest that radial glia and oligodendrocytes follow anterior-to-posterior and superior-to-inferior developmental patterns. These gradients appear to be independent of the tracts of the developing spinal cord. Interaction between these cell types may be necessary for appropriate development of each. Further work is necessary to elucidate this interaction.

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468.15

THE TIMING OF MOTONEURON COMMITMENT IN THE DEVELOPING CHICK SPINAL CORD. M.P. Matise* and C. Lance-Jones, Dept.of Neurobiology, Anatomy & Cell Science, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

When 3-4 Lumbosacral (LS) spinal cord segments (+/- notochord) are reversed about the rostrocaudal (R-C) axis in stage 15-16 chick embryos, motoneuron axons which emerge from these reversed segments alter their peripheral course to project to their originally appropriate muscle targets. This finding suggests that there are differences along the R-C spinal cord axis at neural tube stages (stages 15-16) and that the motoneurons within LS segments are committed to a particular peripheral target fate when their axons grow out of the cord (stages 18+). How and when are motoneurons committed to specific target fates? We have begun to address this question by carrying out R-C cord reversals at earlier stages.

At stages 13-14, presumptive LS cord segments 1-3 were separated from the notochord and other surrounding tissues and reversed about the R-C axis. At stages 35-37, retrograde HRP labelling was used to define the R-C location of motoneurons innervating the sartorius and femorotibialis muscles. Motoneurons innervating these muscles are normally found in discrete positions within LS 1-3. In 70% of stage 13 cases (n=14) and 55% of stage 14 cases (n=9), motoneurons innervating these muscles were located in discrete positions in accord with their new R-C location. In 30% of stage 13 cases and 33% of stage 14 cases, motoneurons were dispersed throughout the reversed segments. In only one case (at stage 14) did motoneurons project in accord with their origin prior to the reversal. These findings suggest that R-C differences within the spinal cord that lead to motoneuron commitment are not present at stages 13 and 14. (Supported by NIH HD25676).

469.1

SYNAPTIC INPUT TO TRANSIENT SUBCORTICAL SYNAPTIC ZONE (TSZ) NEURONS: I. INFRAGRANULAR CORTICAL NEURONS PROVIDE DIRECT SYNAPTIC INPUT TO UNDERLYING TSZ.

A.F. Shering and P.R. Lowenstein*, Laboratory of Cellular and Developmental Neurobiology, Department of Anatomy and Physiology, University of Dundee, DD1 4HN, Scotland.

In the neonatal cat cerebral cortex antibodies raised against synapsin I and synaptophysin reveal a TSZ beneath the developing cortex. This synaptic neuropil disappears as the animal matures. We have examined the source of synaptic input to the TSZ by iontophoretically injecting the anterograde lectin Phaseolus vulgaris-leucoagglutinin (PHA-L) into the infragranular layers of the visual or somatosensory cortex. PHA-L was visualised using immunohistochemistry and sections were prepared for light and electron microscopy (EM). Terminal arborisations of labelled axons were predominantly located in the lateral geniculate nucleus. Fibres traversing the TSZ were seen to be varicose and when examined under the EM many varicosities were found to establish asymmetric synapses:

Age	Target structures: Spines	Shafts	Somata
<10 days	85%	15%	-
1 month	60%	36%	4%
Adult	-	100%	-

These results demonstrate that neurons located in the infragranular layers of the developing cortex provide direct synaptic input to subcortical TSZ neurons. The developmental transition of post-synaptic targets from spines to shafts, suggests that cortico-TSZ axo-spinous synapses are indeed a population of transient synapses. This constitutes, to our best knowledge, the first morphological identification of a population of transient synapses.

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469.2

NEOCORTICAL SYNAPTOGENESIS: LOW DENSITY PRIMARY CULTURES AND USE OF HSV-1 VECTORS FOR GENE TRANSFER INTO POSTMITOTIC NEURONS AND GLIAL CELLS.

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Neocortical synaptic organization is very specific in terms of the post-synaptic targets of individual neurons, and the synaptic input onto individual cells. Although cortical neurones have 5 different membrane domains on which they could potentially receive synapses, mainly dendrites and cell bodies do receive synaptic input. Also, excitatory cell bodies only receive inhibitory synaptic input, while inhibitory somata receive both excitatory and inhibitory input. We now wish to examine the cellular and molecular basis of this synaptic specificity. Thus, utilizing a very low density primary culture system of neocortical neurons, based on the work of Banker, Kosik, and Ramakers, we determined that neurons: (a) survive long term in low density, defined medium, and absence of astrocytic contact; (b) develop proper morphological polarity (e.g. dendrites and axon); (c) after 3 weeks in vitro MAP-2 is found only in dendrites, while tau is localized to all cellular processes; (d) immuno-localization of synaptophysin combined with confocal microscopy suggests that the pattern of synapses in vitro is comparable to the in vivo one; (e) HSV-1 tsK vectors can be used to transfer foreign genes into both postmitotic neurons and glial cells in primary culture. Thus, in spite of its intrinsic complexity in vivo, the cellular and molecular basis of neocortical synapse formation can now be examined using a simple in vitro model.

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469.3

DEVELOPMENT OF SYNAPTIC CONTACTS BETWEEN NEURONS OF BASAL FOREBRAIN AND CEREBRAL CORTEX IN ORGANOTYPIC TISSUE SLICE CULTURE

P.G. Distler & R.T. Robertson*, Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717

We have previously shown that AChE-positive neurons of rat basal forebrain project toward co-cultured cerebral cortex slices and, after entering the tissue, branch within the different cortical layers (Distler and Robertson, 1992). We are now investigating whether these projections also form synaptic contacts with neurons in the cortical target region.

Slices of neonatal basal forebrain tissue were co-cultured with 4 day old cerebral cortex tissue over a time period up to 4 weeks. Various developmental stages of the basal forebrain-cerebral cortex innervation were then investigated on the fine structural level. For identification of afferent projections, two different labeling techniques were applied: (i) placement of the lipophilic dye DiI on fixed basal forebrain tissue and subsequent DAB-photoconversion of stained fibers within cortical tissue, and (ii) AChE-histochemistry, a label which is known to be a reliable marker for cholinergic basal forebrain neurons.

The electron microscopical investigation revealed consistent results for both staining methods: First, basal forebrain afferent fibers form terminal synaptic contacts on dendritc shafts in the superficial cortical layers. Second, the observed synapses are of the symmetric type. Furthermore, both methods reveal the presence of clear spherical vesicles in the presynaptic profile. Within superficial cortical areas, stained synaptic contacts were evident as early as one week after beginning culturing.

In conclusion, the data reveal parallels to the cholinergic innervation in vivo, and provide further evidence about the significance of this model system for the study of the development of the cerebral cortex.

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469.4

INTERRUPTION OF AXONAL GROWTH BY TARGET NEURONS IS ENHANCED BY NMDA.

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Cultured cerebellar granule neurons interrupt the growth of their mossy fiber afferents originating from explants of basilar pontine nuclei (Baird et al. 1992 *J. Neurosci.*12:619-634). This "stop-growing signal" is afferent and target cell specific (Baird et al. *J. Neurobiol.* 1992 in press; Baptista et al, this volume). Action potentials and neurotransmitters play a role in the stop-growing signal as TTX, TTX + high magnesium, or kynurenic acid, an antagonist of glutamate receptors, all interfere with the stop-growing signal.

To determine which types of glutamate receptors might be involved in the stop-signal, explants of pontine nuclei were cultured on granule neurons for two days in the presence of glutamate agonists and antagonists specific for NMDA and non-NMDA receptors. The NMDA-specific antagonist D-AP5 interferes with the stop-signal, resulting in increased numbers of long pontine neurites extending over granule neurons compared to control cultures in ordinary medium. In the presence of 20μM NMDA the number of long neurites was reduced to less than 40% of controls. Pontine explants cultured without granule neurons but with NMDA showed a small increase in the number of long neurites produced. In contrast, explants co-cultured with granule neurons in 50μm AMPA (a non-NMDA agonist) showed little reduction in the number of long neurites produced. AMPA had no effect on the number of pontine neurites in the absence of granule cells.

These results suggest a role for NMDA receptors in regulating the growth of mossy fibers on target granule neurons and potentially in contributing to target cell specificity during the innervation of the cerebellum.

469.5

SPECIFICITY OF CEREBELLAR AXON-TARGET INTERACTIONS *IN VITRO*.

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Granule neurons *in vitro* present a stop-growing signal for their appropriate afferents, the mossy fibers. This signal is afferent-specific since neither retinal nor inferior olivary fiber growth is arrested by granule neurons (Baird et al, J. Neurosci. 1992 12:619-634; J. Neurobiol., in press).

With the ability to purify Purkinje cells (Baptista et al. 1991 Soc. Neurosci. Abstr. 17:38), we examined the capacity of this cell type to regulate afferent growth. To study the growth of one afferent to Purkinje cells, the parallel fibers axons from granule neurons, we co-cultured reaggregates of purified granule cells with purified Purkinje cells. Purkinje cells stimulate and maintain the growth of parallel fiber axons, in line with the mode of growth of parallel fibers across the dendritic fields of multiple adjacent Purkinje cells. In turn, granule neurons and their axons maintain and promote the differentiation of Purkinje cells. Thus, Purkinje cells and granule cells may have different modes of regulating afferent growth, in some cases stimulating growth.

Second, we examined how an inappropriate afferent, the pontine mossy fibers, interact with Purkinje cells. Mossy fiber growth is abundant on a number of substrates and is not affected by co-culture with Purkinje cells. However, when purified Purkinje cells are added to granule neurons, mossy fiber growth is increased three fold compared to growth on granule cells alone. While it is not known if Purkinje cells stimulate mossy fiber growth directly, or disrupt the stop-signal for mossy fiber growth by interacting with granule cells, these results suggest that Purkinje cells do not send a stop-signal to mossy fibers and that the signal is target cell-specific.

The above paradigms will allow the analysis of mechanisms involved in the specificity of axon growth regulation, its maintenance by target cells and the establishment of specific synaptic connections.

469.6

SPATIAL DISTRIBUTION OF EXCITATORY AND INHIBITORY SYNAPSES ON A PURKINJE CELL IN A RAT CEREBELLAR CULTURE.

T. Hirano*, K. Kasono, Department of Physiology, Faculty of Medicine, Kyoto University, Yoshida Konoe-chou, Sakyo-ku, Kyoto 606, Japan.

Spatial distribution of synapses on Purkinje cells formed in a dissociated cell culture of rat cerebellum, were studied by intracelluar fluorescent stainings of pre- and postsynaptic neurons and by immunocytochemical staining of presynaptic terminals. Simultaneous whole-cell recordings were performed both on a presynaptic small neuron and on a Purkinje cell, and the property of synaptic transmission was determined. Inward excitatory synaptic currents were suppressed by CNQX, and outward inhibitory synaptic currents were suppressed by bicuculline, respectively. A presynaptic neuron was stained with intracellularly injected lucifer yellow and a Purkinje cell was filled with texas red. Axonal varicosities presumed synaptic terminals of excitatory granule cells on a Purkinje cell, were exclusively localized along dendrites, and presumed synaptic terminals of inhibitory interneurons such as basket or stellate cells were fond both along dendrites and on the soma. After the electrophysiological recordings, neurons were fixed and stained with an anti-synaptophysin monoclonal antibody in order to visualize overall distribution of synaptic terminals on a Purkinje cell. The staining confirmed that the varicosities are presynaptic terminals and that a Purkinje cell receives synaptic inputs both on a soma and on dendrites. Thus, differential spatial distribution of excitatory and inhibitory synapses on a Purkinje cell in a simple culture system was demonstrated. Synapses were also observed with a scanning electron microscope.

469.7

EVIDENCE FOR THAT GANGLIOSIDES ARE ESSENTIAL FOR SYNAPSE FORMATION BETWEEN CEREBRAL CORTICAL NEURONS; ENDOGLYCOCERAMIDASE INHIBITS *IN VITRO* SYNAPTOGENESIS. Kazuyo Muramoto*[1,3], Kazuo Kobayashi[1], Masahiro Kawahara[1], Makoto Ito[2], Tatsuya Yamagata[2] and Yoichiro Kuroda[1], [1]Dept. of Mol.and Cell. Neurobiol. Tokyo Metropol. Inst. for Neurosci., Fuchu, Tokyo 183, [2]Lab. of Glycoconjugate Res., Mitsubishi-Kasei Life Science Inst., Machida, Tokyo 194, [3]Dept. of Pharmacol., Fac. of Med., Univ. of Tokyo, Tokyo 113, Japan.

Synapse formation between cultured cerebral cortical neurons was observed by multi-site Ca^{2+} fluorometry (Muramoto,K., *et al., Proc.Japan Acad.Ser.B*, **64**, 319, 1988). Most neurons in the culture showed synchronous oscillations of the fluorescence intensity which correspond to spontaneous synaptic activity. Continuous application of K-252b, a protein kinase inhibitor, blocked the synchronous firing and significantly decreased the number of synapses identified morphologically using electron microscopy (Kuroda,Y., *et al., Neurosci.Lett.*, **135**, 255, 1992). Since K-252b does not permeate the cell membrane, the data strongly suggest that phosphorylation of cell surface by an ecto-protein kinase (Ehrlich,Y.H., *et al., Nature*, **320**, 67, 1986) is inhibited by K-252b. Since it has been reported that an ecto-protein kinase activity is regulated by a specific type of gangliosides (Tsuji,S., *et al., J.Biochem.*, **104**, 498, 1988), we applied purified endoglycoceramidase (EGCase) which can remove all carbohydrate moieties from any ganglioside but not from proteins and others (Ito,M., *et al., J.Biol.Chem.*, **264**, 9510, 1989) to the cortical culture. Continuous presence of EGCase together with its activator protein inhibited the occurrence of synchronous oscillation after 8 days in culture, when control culture showed oscillation which represents a significant amount of synapse formation between cerebral cortical neurons. The inhibition was dose-dependent and without any significant morphological changes of neurons and their neurites which was observed by phase-contrast microscopy and immunostaining of MAP 2 and neurofilament.

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469.8

CHARACTERIZATION OF SYNAPSE FORMATION BETWEEN CNS NEURONS IN CULTURE: BINDING OF MONOCLONAL ANTIBODIES AND LECTINS TO LIVING HIPPOCAMPAL NEURONS. Kazuo Kobayashi*, Masumi Ichikawa, Masahiro Kawahara, Kazuyo Muramoto, Yoichiro Kuroda, Depts. of Molecular & Cellular Neurobiology, and of Anatomy & Embryology, Tokyo Metropol. Inst. for Neuroscience, Fuchu, Tokyo 183, Japan.

We have carried out quantitative analysis of synapse formation between cultured CNS neurons using multi-site Ca^{2+} fluorometry and electron microscopy (Kuroda et al. *Neurosci.Lett.* 133:255, 1992). Synaptophysin immunostaining using laser confocal microscopy demonstrated that neurons were connected by many synapses which matured during the culture (Ichikawa et al. *Neurosci. Res.* 12:452,1991). To investigate whether these synapses are formed between specific pairs of neurons in culture or not, we have attempted to identify different types of CNS neurons. We developed a library of monoclonal antibodies which bind to living PC12 cells differentiated by NGF treatment. At least 14 monoclonal antibodies in the library bound almost exclusively to living neurons from rat hippocampus in culture. We also screened a series of lectins to the living neurons. *Vicia villosa agglutinin* (VVA) binding appeared to be significant to multipolar neuron in the hippocampus after 2 weeks in culture. These neurons appear to be inhibitory, which identified in fixed hippocampal tissue(Drake et al. *Brain Res.* 554:176,1991).

469.9

DEVELOPMENT OF FUNCTIONAL SYNAPTIC TRANSMISSION BETWEEN CULTURED RAT HIPPOCAMPAL NEURONS. T.A. Basarsky, V. Parpura and P.G. Haydon*. Signal Transduction Training Group and Dept of Zoology and Genetics, Iowa State University, Ames, IA 50011.

The formation of a functional synapse requires the simultaneous expression of voltage-dependent calcium channels, calcium responsive secretory machinery, and post-synaptic receptors. In rat neonatal hippocampal cell cultures we find the presence of functional synapses is not detected until 5-8 days *in vitro*. Paired whole-cell recordings were used to assay the presence of evoked excitatory and inhibitory connections. To identify the rate-limiting element in synapse formation we have begun studying the developmental appearance of voltage-dependent calcium channels and postsynaptic receptors.

Internal calcium levels were monitored using the calcium indicator Fluo-3 AM (8 μM). Focal application of either 50 mM K^+ or 100 μM glutamate reliably elevated internal calcium in neurons 3 days *in vitro*, prior to the onset of functional synaptic transmission. The actions of high K^+ and glutamate were reduced in zero calcium saline.

These data indicate that calcium channels and glutamate receptors are present prior to the detection of functional synapses. However, further studies must examine the timing of appearance of specific channel types and their spatial relation to the synaptic terminal.

469.10

A PROTEASE INHIBITOR REDUCES ACTIVITY-DEPENDENT SYNAPSE ELIMINATION AT THE MOUSE NEUROMUSCULAR JUNCTION *IN VITRO*. Y.Liu*, R.D.Fields, S.Fitzgerald, W.Nyhus and P.G.Nelson, LDN, NICHD, NIH, Bethesda, MD 20892.

The possible involvement of proteolysis in the process of activity-dependent synapse elimination was studied in an *in vitro* model of the neuromuscular junction. Neurons of the superior cervical ganglion (SCG) and muscle cells were isolated from new born mice and cultured in a multicompartment system, the Campenot chamber. The SCG neurons were kept in the two side compartments; muscle cells in the center. Cholinergic synapses developed between neurons and muscle cells after 2-3 weeks. Functional synapses were monitored by the contraction of muscle cells in response to stimulating the afferent neurons.

The elimination of these synapses was activity-dependent. After 1-2 days of extracellular stimulation to the presynaptic axons, 52% of the synaptic connections were eliminated (n=275). When 50 μM leupeptin, a protease inhibitor, was added into the central compartment during the whole period of stimulation, only 35% of the synapses were lost (n=193). The difference between the effect of stimulation in the presence and absence of leupeptin was statistically significant ($p<0.001$). Synapse elimination in unstimulated controls was 8% (n=177). In the presence of 1 μM tetrodotoxin (TTX), stimulation did not cause synapse elimination (n=107).

In conclusion, the partial block of synapse elimination by this protease inhibitor suggests that proteolysis may play a role in the mechanism of activity-dependent synapse elimination.

469.11

A TROPHIC MODEL OF NEUROMUSCULAR SYNAPSE ELIMINATION: CORRELATION OF MOTOR UNIT SIZES WITH ACTIVITY. J.N.Carr and M.Morgan-Carr*, Dept. Of Biology, Bryn Mawr College, Bryn Mawr, PA. 19010

It has been proposed that neuromuscular synapse elimination is driven by pre-synaptic competition for a post-synaptically supplied trophic factor. This competition is activity dependent in that pre-synaptic uptake and/or post-synaptic supply of trophic factor requires activity in the respective element. Because pre-synaptic growth is tied to trophic factor uptake, such models predict that the most active motor neuron forms the largest motor unit, in direct contradiction with observation. This contradiction can be removed by postulating that each motor neuron desires to obtain the same total amount, or target value, of trophic factor from its axonal arbor.

Our model of synaptic dynamics is based on the biology of NGF. We assume that trophic factor is taken up pre-synaptically by a receptor mediated endocytotic mechanism; therefore, active motor neurons are capable of taking up more trophic factor than inactive counterparts. Growth or retraction of a given pre-synaptic element is based upon a net difference between factor taken up locally and a decay process that operates when a pre-synaptic element is active. Growth occurs when a given synapse obtains enough trophic factor to overcome the internal decay process. Internalized trophic factor is retrogradely transported to the soma where it regulates trophic factor receptor expression. If the soma concentration of trophic factor exceeds the target value, receptor expression is down-regulated; up-regulation occurs if the concentration is too low. Thus, a motor neuron that is obtaining too much trophic factor places all of its synapses at a competitive disadvantage by limiting the number of receptors each synapse has available.

Computer simulations demonstrate that this model correctly predicts both the time course of normal synapse elimination and the observation that less active motor neurons form larger motor units. The model also reproduces the effects of numerous experimental manipulations, including partial denervation and the effects of pre- or post-synaptic blockade. It is worth noting that trophic factor receptor regulation serves to produce motor units whose sizes display the observed dependence upon activity levels, but is not required for the removal of polyneuronal innervation.

469.12

SOMATOTOPIC ORGANIZATION OF OVERLAPPING MOTOR UNITS WITHIN A LARVAL FROG JAW MUSCLE. F.F. Omerza* and K.E. Alley Ohio State University, Columbus, Ohio 43210.

Individual myofibers within the larval jaw muscles of Rana pipiens are polyinnervated. This is due to the presence of multiple neuromuscular junctions (NMJs) as well as multiple axons within individual junctions. The purpose of this study was to determine whether this polyinnervation arises from more than one neuron and, if so, to investigate their central and peripheral organization. In order to examine the neuronal distribution at muscle, myofiber and NMJ levels, we employed a triple labeling technique utilizing standard AChE histochemistry and the anterograde fluorescent tracers DiI and DiA. This procedure allowed us to distinguish the distributions of two separate groups of axons to their respective NMJs. A second study used retrograde axonal transport of Fast Blue and DiI from injection sites at opposite ends of the muscle. This provided the location of motoneurons serving different regions of the same muscle. Results of the triple label study indicate that individual NMJs and myofibers may receive innervation from axons within different portions of the nerve root, and further, suggests a regional distribution for motor units. Results of the retrograde study demonstrate that neurons projecting to the rostral and caudal regions of the muscle are distinct. Our observations indicate that several neurons can project to the same myofiber or NMJ and that the resultant overlapping motor distribution is reflected in a somatotopic organization of motoneurons.

469.13

INITIAL EMBRYONIC EXPRESSION OF MOTONEURON TOPOGRAPHY IN THE RAT DIAPHRAGM MUSCLE. M. Laskowski* and Jesse Owens. WAMI Medical Program, University of Idaho, Moscow, ID 83843 and Program in Biomedical Science, University of Alaska, Anchorage, AK 99508, U.S.A.

In previous reports we have shown that motoneurons in the adult phrenic nucleus of the rat map across the surface of the diaphragm muscle in a distinct rostrocaudal pattern. In an effort to understand mechanisms responsible for the establishment of this rostrocaudal map, in this study we examined the pattern of innervation of the developing diaphragm. Focal extracellular end-plate currents (EPCs) were recorded from curarized embryonic diaphragm muscle while stimulating sequentially each ventral root (C_3, C_4, C_5 and C_6). This method offers high spatial resolution of synaptic activity without the biases introduced by intracellular recording. The first evidence of EPCs in the center of the diaphragm was observed at day E-15.5 when all roots (C_4, C_5 and C_6 and sometimes C_3) had synaptic input. By E-16 as the phrenic nerve grew into slightly rostral and caudal regions of the muscle, a distinct topographic preference could be detected. By E-18 when two-thirds of the muscle was innervated the topographic map was further sharpened. We conclude first, that topographic selectivity cannot be explained on the basis of a difference in arrival time of rostral vs caudal motoneurons. Second, a topographic bias is present soon after the earliest period of evoked synaptic activity suggesting that growing motoneurons respond to topographic cues on muscle targets at a very early stage of development. Supported by NIH grant NS 27024.

NUTRITIONAL AND PRENATAL FACTORS: ALCOHOL

470.1

EFFECT OF IN UTERO ETHANOL EXPOSURE ON THE MORPHOLOGICAL DEVELOPMENT OF THE OCULOMOTOR NUCLEUS IN THE RAT. R.C. Burrows; A.K. Shetty; D.E. Phillips*. Dept. of Biology Montana State University, Bozeman, MT 59717.

This study was designed to determine if exposures to ethanol can alter the development of brainstem nuclei controlling the extraocular eye muscles. Pregnant rats were fed throughout gestation with either an ethanol containing liquid diet, in which 37.5% of the total caloric content was ethanol derived, or with an iso-caloric diet. Male offspring were perfused on postnatal day 15 and the oculomotor nucleus was examined using plastic section light microscopy, Golgi-Cox staining, and stereologic analysis of electron micrographs. A decrease in the number of neurons as well as an increase in the number of astrocytes per unit area was found in the ethanol exposed animals, while the total number of cells per unit area remained relatively constant. There was an alcohol-induced decrease in the overall complexity of the dendritic ramifications, as well as in the total number of dendrites. The area of the neuronal soma was also decreased in the alcohol exposed animals, but there was no change in the area of the nucleus or nucleolus. These results may help explain some of the motor deficits associated with the visual system after developmental alcohol exposures.
Supported by NIAAA AA7042 and NSF EPSCoR RII-8921978.

470.2

TEMPORAL CONSTRAINTS ON ALCOHOL-INDUCED CORTICAL ASTROGLIOSIS DURING THE NEONATAL BRAIN GROWTH SPURT IN RATS. J.T. Leo, C.R. Goodlett* and J.R. West. Alcohol and Brain Research Laboratory, Dept. of Anatomy, University of Iowa, Iowa City, IA 52242.

It has previously been reported that alcohol exposure during the brain growth spurt (postnatal days [PD] 4-9), in a pattern yielding cyclic blood alcohol concentrations with high peaks and low troughs, produces a conspicuous reactive gliosis in the cerebral cortex. Radioimmunoassays have shown that the amount of GFAP is increased more than 300% above controls. Immunocytochemical studies indicated that much of the increased GFAP was associated with loci of reactive astrocytes surrounding some cortical blood vessels. These loci, scattered throughout the cortex, contained hypertrophied astroglia with thick fibrillary processes heavily labeled by GFAP.

The goal of this study was to determine if a similar pattern of alcohol exposure later in the neonatal brain growth spurt (PD 10-14) would stimulate astrogliosis comparable to exposure on days 4-8. Pups were gastrostomized either on PD 4 or PD 10 and were given either 4.5 g/kg of alcohol per day [delivered in 2 of 12 daily feeding as a 10.2% (v/v) solution in milk formula] or were given a calorically matched diet free of alcohol. Pups were perfused on postnatal day 9 or postnatal day 15 and 40-μm thick frozen coronal sections were processed for GFAP immunoreactivity using peroxidase-antiperoxidase immunocytochemistry. Matched sections from control and alcohol-treated pups were evaluated microscopically for reactive gliosis around cortical blood vessels.

In contrast to the prominent effect seen in pups exposed on PD 4-8, alcohol exposure on PD 10-14 did not result in the intense reactive astrogliosis surrounding cortical blood vessels. These results indicate the presence of a temporal window of vulnerability to alcohol-induced reactive gliosis elicited around cortical blood vessels. The critical period for this effect appears to end by postnatal day 10. Supported by grants #AA 07313 and #AA 05523)

470.3

DEFICITS IN BEHAVIORAL DEVELOPMENT AND OLIGODENDROCYTIC PERCENTAGES IN THE NEONATAL RAT DUE TO PRENATAL ALCOHOL EXPOSURE. C.A. Aquilino and K.M. Cianci*. Dept. of Psychology, Franklin & Marshall College, Lancaster, PA 17604.

Subjects were Sprague-Dawley pups of dams receiving a diet of either 35% ethanol-derived calories, 35% carbohydrate-derived calories (sucrose), or 100% lab chow during the 21 days of gestation. Twenty subjects were tested on reflex activity, stereotropic behavior, open-field activity, and wheelrunning on days 7-10, 11-14, 15-21, and six weeks of age, respectively. Significant differences were found between ethanol-exposed rats and controls in their development of righting reflex, suckling behavior, tactile stimulation, negative geotaxis, phototropism, stereotropic behavior, and rearing activity. Sucrose-exposed animals resembled the ethanol-exposed in behavioral delays. Twenty ethanol-exposed rats and 19 controls were sacrificed at postnatal day two to establish cultures of the hippocampi, cerebral cortexes, and brainstems. At five days in culture, relative percentages of oligodendrocytes and neurons were determined using the immuno-fluorescent labels anti-galactocerebroside and anti-neurofilament, respectively. Ethanol-exposed rats had significantly lower per-centages of oligodendrocytes and neurons than controls. They were significantly lower in the hippocampus, cerebral cortex, and brainstem. The ethanol group may have had a lower percentage of neurons in the cerebral cortex (approached significance). Research was supported by Franklin & Marshall College Committee on Grants.

470.4

ALTERED DEVELOPMENT OF DOPAMINERGIC NEURONS IN THE RAT SUBSTANTIA NIGRA FOLLOWING PRENATAL ETHANOL EXPOSURE. A.K. Shetty*, R.C. Burrows and D.E. Phillips. Dept. of Biology, Montana State University, Bozeman, MT 59717.

The development of dopaminergic neurons of substantia nigra pars compacta (SNPC) was investigated following prenatal ethanol exposure. Pregnant rats were either fed with an ethanol containing liquid diet (6.7% v/v) or were pair fed an isocaloric diet throughout gestation. Offspring were sacrificed on postnatal day 15 and the morphology of neurons was assessed by tyrosine hydroxylase (TH) immunocytochemistry and by Golgi-Cox staining. Compared to control offspring, ethanol exposed offspring had more closely packed and smaller TH positive cell bodies, thinner and fewer TH positive fibers, reduced numbers of second, third and fourth order dendrites, fewer total dendritic segments per cell, and an altered dendritic branching pattern. Some dysmorphic neurons with irregular cell body contours and fusiform and/or spheroidal enlargements in the dendrites were also encountered. These results suggest that prenatal ethanol exposure causes retardation of the development of SNPC neurons, especially of the growth and branching of dendrites. The underdevelopment of dendrites could result in altered development of neuronal circuitry which in turn could contribute to the abnormal motor functions reported after developmental alcohol exposures.
Supported by NSF EPSCoR RII-8921978 and NIAAA AA7042.

470.5

EFFECTS OF ALCOHOL EXPOSURE DURING DEVELOPMENT ON THE NEUROCHEMISTRY OF THE AMYGDALA. Sandra J. Kelly.* Department of Psychology, University of South Carolina, Columbia, SC, U.S.A. 29208.

Previous work from this laboratory has suggested that the amygdala may be affected by exposure to alcohol during the early postnatal period. This period is similar with respect to brain growth to the third trimester in humans. The alcohol exposure is given via an artificial rearing procedure in which the rats are exposed to either 5 or 3 g/kg/day of ethanol from postnatal days 4 to 10. Control groups consist of rats artificially reared but not exposed to alcohol and rats reared normally by dams. At 21 days of age, the rats were killed and their heads were immediately immersed in liquid nitrogen. The amygdala region was dissected free, sonicated in 0.2 M perchloric acid, and frozen until time of assay. At that time, the sonicated tissue was thawed and centrifuged for 15 min. The supernatant was injected on a C18 reverse phase column for HPLC with electrochemical detection. The mobile phase was 6.7% (v/v) methanol in 1 mM sodium monophosphate, .631 mM sodium octyl sulfate, 1.0 mM sodium EDTA and 2.49 mM triethylamine (pH 4.0). The supernatant was analyzed for noradrenaline, dopamine, DOPAC, HVA, 5-HT, and 5-HIAA content.

Exposure to alcohol during development alters the amygdala in 21 day old rats. The dopamine levels were altered in rats of both sexes and the 5-HT levels were increased in female rats. There were also a number of effects of artificial rearing on neurochemistry. (Supported by NIAAA Grant AA08080)

470.6

THE INFLUENCE OF CHRONIC PRENATAL ETHANOL EXPOSURE ON CHOLINERGIC DEVELOPMENT IN THE SEPTOHIPPOCAMPAL SYSTEM OF THE RAT D.J.Swanson, D.W. Walker, and M.B. Heaton*. University of Florida College of Medicine and V.A. Medical Center Gainesville, FL, 32610.

In animal models of Fetal Alcohol Syndrome (FAS) the hippocampus has been shown to be especially sensitive to the effects of prenatal ethanol exposure, exhibiting neuronal loss and alterations in neuritic process elaboration. We have begun to characterize the influence of chronic prenatal ethanol exposure on the development of the cholinergic neuronal population which projects to the hippocampus, the medial septal nucleus and nucleus of the diagonal band (MS/DB). On gestation days 1-22 pregnant dams were either fed an ethanol containing liquid diet, pair-fed a calorically equivalent sucrose-containing diet, or given rat chow *ad lib*. Preliminary evidence shows that chronic prenatal ethanol exposure produces a significant decrease in MS/DB ChAT activity at postnatal day 1 (P1; 18% reduction in ethanol exposed pups compared to pair-fed pups). Subsequently, at P7 ChAT activity expression in ethanol exposed pups approximately equaled that of pair-fed and chow control pups. Further experiments are in progress to examine additional developmental time points as well as the anatomical basis for this alteration in ChAT developmental expression. These studies will determine whether the early decline results from a developmental delay, or whether a reduction in cholinergic neurons coupled with up-regulation of ChAT expression may occur. Supported by AA05332, AA00200, a grant from the A.B.M.R.F., and the Dept. of Veterans Affairs.

470.7

EXPRESSION OF LHRH mRNA IN THE C57BL/6J MOUSE FOLLOWING ACUTE IN UTERO ETHANOL EXPOSURE. H.C. Scott*, R.T. Zoeller, P.K. Rudeen, Department of Anatomy and Neurobiology, University of Missouri School of Medicine, Columbia, MO 65212.

Previously we have shown that an acute dose of ethanol on gestational day 7 (G7) alters the number of neurons immunoreactive for LHRH peptide (Scott et al., Dev. Brain Res. 66:119, 1992). The effect of an acute dose of ethanol on G10 on the expression of LHRH mRNA was examined in this study. G10 corresponds to the day of development when LHRH neurons begin to differentiate as a single neuronal population in the medial olfactory placode of the mouse (Wray et al., Proc. Natl. Acad. Sci. USA, 86:8132, 1989). C57BL/6J mice were exposed to two doses of 25% ethanol (2.9 g/kg body weight) on G10. Control pups were intubated with water. Animals were sacrificed on G18 and frozen coronal sections cut at 12 μm. In situ hybridization histochemistry localized individual neurons containing LHRH mRNA. A Bioquant MEG IV imalge analysis system determined the area covered by grains for each neuron expressing LHRH mRNA in the diagonal band of Brocca/medial preoptic area. Fetal ethanol exposure does not appear to interfere with LHRH gene expression when given during an important period of LHRH neurogenesis. (Supported by NIAAA grants AA07458, AA05893 and AA00107)

470.8

ETHANOL EXPOSURE INCREASES GFAP mRNA AND PROTEIN IN DEVELOPING RAT CORTEX AND CULTURED CORTICAL ASTROCYTES. T.L. Fletcher* and W. Shain, School of Public Health, The University at Albany and Wadsworth Center, Albany, NY 12201.

Prenatal exposure to ethanol can result in Fetal Alcohol Syndrome (FAS). We have shown that brief ethanol exposure causes changes in expression of a limited number of genes in the developing rat CNS. We describe here ethanol's effect on glial fibrillary acidic protein (GFAP) expression. Artificially-reared rat pups received 3.3 g/kg/day in 2 doses from postnatal days 5-7 resulting in peak blood alcohol levels of 180 mg/dL Artificially-reared control pups received an isocaloric diet. Suckle control pups were reared by their dams. Two hours after the last dose of ethanol, mRNA was isolated from four brain regions of some pups. Other pups were fostered to control dams and mRNA was isolated at 14, 21, or 90 days of age. Northern and slot blot analyses demonstrated a transient 3-fold increase in GFAP, but not β-actin, mRNA in cortex. No changes in GFAP were observed in the hippocampus. Western blot analysis of 7 day cortex showed a comparable increase in GFAP protein. To determine if this increase was due to a direct action of ethanol on glial cells, primary cultures of cortical astrocytes were exposed to ethanol resulting in media concentrations equivalent to pup ethanol exposure. Ethanol exposure increased GFAP expression parallel to the *in vivo* observations. These results suggest that ethanol directly disrupts the regulation of specific genes in astrocytes during CNS development. (Supported by AA-07472)

470.9

FETAL ALCOHOL EXPOSURE (FAE) BLUNTS THE IMMUNOMODULATORY RESPONSE TO SWIM STRESS IN MALE RATS. M.L. Pilati, F. Chiappelli, D. Tio and A.N. Taylor*. Depts. Anat./Cell Biol. & Psychol., BRI, Psychoneuroimmunol., UCLA; and Brentwood Div., West LA VAMC, Los Angeles, CA 90024.

Maternal alcohol consumption (36% ethanol-derived calories during the last two weeks of gestation) produces long-lasting alterations in stress-induced neuroendocrine and neurobehavioral responses and in basal (non-stressed) neuroimmune responses in the offspring. In order to determine the effect of FAE on stress-induced immune responses, adult male Sprague-Dawley FAE and control rats were exposed to swim stress (5 periods of 3-min swim in 37°C water over 30 min, with tails weighted). One hr later thymuses were removed and their proliferative response to Concanavalin A was tested. The thymoproliferative response of swim-stressed control rats dropped by 69% compared to unstressed controls ($p<0.01$), while it decreased by only 41% in stressed FAE compared to unstressed FAE rats ($p>0.05$). The ontogenic and temporal profiles of this FAE-induced differential immunosuppressive response to stress are currently under investigation. (Supported by VA Medical Research Service.)

470.10

THE EFFECT OF EARLY POSTNATAL ETHANOL EXPOSURE ON LIGHT-DARK PREFERENCE IN PREWEANLING RATS. Janie H. Wilson* and Sandra J. Kelly. Department of Psychology, University of South Carolina, Columbia, SC 29208.

This experiment examined the influence of early postnatal ethanol exposure on light-dark preference and the effect of home-cage shavings versus clean shavings on this preference. Rats were artificially reared and exposed to either 3 or 5 g/kg/day of ethanol from postnatal days 4 through 10. Control groups consisted of rats artificially reared but not exposed to alcohol and rats normally reared by dams. All rats were reared by dams from postnatal day 12 to the time of testing on postnatal day 18. The testing apparatus consisted of two chambers: one was made of black Plexiglas and completely enclosed, and the other was made of white Plexiglas and open to bright lighting. A small door connected the two chambers. Each animal was placed in the white chamber facing away from the dark chamber. Latency to enter the dark chamber and total time spent there were recorded.

The presence of home-cage shavings resulted in more time spent in the white chamber. It is plausible that the presence of home-cage shavings reduced the stress associated with a novel environment such that the animal leaves the shelter of the black chamber. There was a distinct sex effect in that males spent more time in the dark chamber than females. High-dose and low-dose animals spent less time in the dark chamber than animals reared by dams. Both control groups spent the same amount of time in the dark chamber. Early postnatal exposure to ethanol may cause a decrease in the adaptive unconditioned fear response when placed in a novel environment. An alternate explanation is that there was an increase in exploratory behavior due to ethanol during development. (Supported by NIAAA Grant AA08080 to S.J.K.)

470.11

AUDITORY BRAINSTEM RESPONSES (ABR) IN RATS PRENATALLY EXPOSED TO ALCOHOL: EFFECTS OF CRITICAL PERIODS. M.W. Church*, G.W. Overbeck, P. Holmes, J. Tilak. Fetal Alcohol Research Center, Dept. Ob/Gyn, Wayne State University School of Medicine, Detroit, MI 48201.

Prenatal alcohol exposure can cause sensory disorders and other nervous system morbidities. Our interest concerns the auditory system. For example, children with the Fetal Alcohol Syndrome (FAS) have a variety of hearing disorders (Church & Gerkin, Pediatrics 82:147-154, 1988; Church & Eldis, Alc. Clin. Exp. Res. 16:380, 1992). Using an animal model, we have also observed congenital hearing loss (Church, Alcohol 4:231-239, 1987) and developmental delays in auditory maturation (Church & Holloway, Alc. Clin. Exp. Res. 8:258-263, 1984) as evidenced by the ABR. The degree and type of morbidities caused by prenatal drug exposure depends, in part, on the gestational age at the time of exposure. To study the effects of "critical periods" of exposure, Sprague-Dawley rats were prenatally exposed to alcohol by administering liquid diets containing either 0% or 35% ethanol-derived calories to pregnant dams from gestation day 7-14 or 15-22. Untreated control groups were also used. The earlier (organogenesis) period of exposure proved more critical in producing ABR maturational delays in the offspring than the latter (histogenesis) period. Supported by NIAAA Grant AA07606.

GLIA AND OTHER NON-NEURONAL CELLS IV

471.1

ASTROCYTE DEVELOPMENT AND CHONDROITIN-4-SULFATE EXPRESSION IN FETAL HUMAN SPINAL CORD F.J. Liuzzi[1]*, T. Bass[2] and M. Bergevin[3]. Departs. of Anatomy and Neurobiology[1,2], Pediatrics[2] and Pathology[3], Eastern Virginia Med. Sch., Norfolk, VA 23501.

Cervical spinal cords from premature infants were obtained at autopsy and immersion fixed in Zamboni's fixative. Transverse 60μm vibratome sections were cut and stained with antibodies to the glycosaminoglycan, chondroitin-4-sulfate (CS-4), vimentin (VIM) or glial fibrillary acidic protein (GFAP) using a biotin-avidin-peroxidase protocol.

In developing white matter, radially-oriented astrocytes are both GFAP and VIM positive before 33 weeks of gestation. Few stellate astrocytes are evident in the white matter prior to that time. Later, the predominant white matter astrocytic type is stellate and appears to express GFAP only. The distribution and density of these cells correlate with the degree of myelination. GFAP-positive astrocytes are evident in the gray matter at 28 weeks, but are more numerous and more ramified by 33 weeks.

CS-4 is distributed throughout white and gray matters at the gestational ages examined. In the regions of the unmyelinated corticospinal tracts, CS-4 immunoreactivity is associated with septae. These septae are also GFAP and VIM positive prior to 33 weeks of gestation, but only GFAP positive at later times.

471.2

DEVELOPMENTAL REGULATION OF A NON-SPECIFIC TRANSPORTER BY ASTROCYTES IN CULTURE R.E. Petroski*, V.Quiñones-Jenab, J.A. Connor[†], and H.M. Geller. Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854 and [†]Department of Neuroscience, Roche Institute of Molecular Biology, Nutley, NJ 07110.

We have previously demonstrated that embryonic neurons growing on a substrate of rat cerebral cortical astrocytes were selectively labelled with the vital dye 5(6)-carboxy fluorescein diacetate (CFDA). Neurons displayed brilliant green somata as well as intensely labelled processes while the glial monolayer showed only a dim level of fluorescence. Using optical recording methods, we demonstrated that both neurons and astrocytes hydrolyze CFDA to the fluorescent free acid. However, once CFDA is removed from the medium, the astrocytes rapidly expel the dye with a time course of minutes as opposed to neurons which retain the dye for several hours. This has allowed the selective labelling of neurons with CFDA. When astrocytes are rinsed at 4°C, they retain the dye for several hours, suggesting that an active pump is responsible for the extrusion of the dye. The extrusion of the dye is also inhibited by reserpine in a concentration dependent fashion. Immature astrocytes were not able to expel the dye. However, the ability of embryonic astrocytes to pump out fluorescent dyes develops during the first week *in vitro*. Moreover, the immortalized V1 cell line from embryonic mouse hypothalamus, which expresses several mature astroglial properties, is able to expel the dye. This cell line will be used to investigate the properties of this membrane transporter. Supported by NIH NS 24168.

471.3

NEURONS FAIL TO INDUCE BRAINSTEM ASTROGLIAL CELL DIFFERENTIATION *IN VITRO* D.L. Cooper and M.E. Hatten* Department of Pathology and Center for Neurobiology and Behavior, Columbia University, New York, NY 10032

Tumors derived from cells of astrocytic lineage are the most common and devastating of the mammalian brain. Previous *in vitro* analyses, in which cerebellar astroglia and granule neurons were purified from early postnatal cerebellum and recombined, demonstrated that neurons provide a control mechanism for astroglial growth, inducing cerebellar astroglial glial differentiation via contact-mediated proteolytic activation of TGFβ1. In the present study, we have examined whether astroglial cells from the brainstem, a more primitive brain region where neuronal layers do not form, respond to neuronal regulatory signals and TGFβ1-induction of glial differentiation.

When neurons, purified from either brainstem or cerebellum, are co-cultured with brainstem astroglial cells, the neurons bind poorly to the astroglial cells and do not induce glial differentiation. Although neurons did not induce brainstem astroglial differentiation, brainstem glia had an augmented response to the TGFβs. In the absence of neurons or TGFβ, brainstem astroglial cell DNA synthesis was 2-4 times higher than that of cerebellar astroglial DNA synthesis. Addition of TGFβ1 or β2 (1-10 ng/ml) resulted in a 47-94% reduction in DNA synthesis in brainstem glia as compared with the 26-61% reduction seen with cerebellar astrocytes. At similar concentrations, both TGFβs induced glial differentiation, measured by Northern blot analysis of GFAP expression and the extension of glial processes. These experiments demonstrate the ability of TGFβ, but not neurons, to induce differentiation of brainstem glia, and suggest that the failure of neurons to induce differentiation may be related to neuron-glial binding.

471.4

DEVELOPMENT OF OLFACTORY NERVE GLIA. R. B. Norgren*, N. Ratner and R. Brackenbury. Depts. of Psychiatry and Anatomy and Cell Biology, Univ. Cincinnati Coll. Med., Cincinnati, OH 45267

The studies reported here were aimed at establishing an antigenic marker specific for olfactory nerve glia throughout development. We have recently developed a monoclonal antibody (1E8) that recognizes both myelinating and non-myelinating Schwann cells in the chick (Bhattacharyya, et al. 1991) but does not recognize astrocytes, oliogodendrocytes or neurons. In the current study, we used the 1E8 antibody and the avidin-biotin-HRP technique (Vectastain) on tissue sections of embyronic and adult chickens. 1E8 immunostained olfactory nerve glia both in early embryos and in adults indicating that olfactory nerve glia may be more closely related to non-myelinating Schwann cells than other glial cell types. Further, the pattern of immunostaining we obtained is consistent with the idea that most, if not all, progenitors of olfactory glia are derived from the olfactory placode rather than the neural crest. We also observed that immunostained glia were initially distributed around the perimeter of the olfactory nerve (late E4). By the end of E5, 1E8-immunostaining was found throughout the nerve. Finally, glia appear to enter the olfactory bulb with the olfactory nerve. In the adult, a thin rim of 1E8 immunopositive cells can be observed around the edge of the olfactory nerve layer. This work was supported by NIH grants NS30047 (RBN) and NS27227 (RB and NR).

471.5

GLIAL DEVELOPMENT IN PRIMARY CULTURES ESTABLISHED FROM X-IRRADIATED NEONATAL SPINAL CORD. D.L. Davies*, T.J. Sims, and S.A. Gilmore. Dept. of Anatomy, Univ. of Arkansas for Med. Sciences, Little Rock, AR 72205

X-radiation is a useful tool for manipulating the cellular composition of an organ and exploring the developmental consequences. Exposure of the spinal cord in 3-day-old rats to x-rays induces a profound yet incomplete depletion of astrocytes and oligodendrocytes (J. Neuropath. Exp. Neurol., 22:294-301, 1963; Exp. Brain Res. 75:513-522, 1989). This *in vitro* study addresses issues related to the vulnerability of specific cell phenotypes and maturational status. Lumbosacral spinal cords of rats were irradiated and removed within several hours to establish the cultures. Non-irradiated spinal cords from littermates provided the control cultures. Cells were cultivated for 8 days *in vitro* and a battery of immunocytochemical markers was used to characterize the cultures. In cultures derived from x-irradiated tissue, growth was markedly diminished and a significant portion of the surviving cells were GFAP-positive astrocytes. These cells were either stellate or epithelioid, and vimentin was colocalized in the majority of the epithelioid cells. Galactocerebroside, a marker for mature oligodendrocytes, was immunolocalized in an abundant population within control cultures but was virtually absent in cultures from x-irradiated rats. However, a small population within cultures from x-irradiated rats exhibited immunolocalization of A2B5 and 2',3'-CNP, markers of early oligodendrocytic lineage. This preliminary study demonstrated reduced survival and viability of glia in cultures derived from x-irradiated spinal cord. Moreover, the absence of mature oligodendrocytes suggested a specific vulnerability of a stage in this cell's lineage.

Supported by NIH Grant NS 04761.

471.6

IMMUNOCYTOCHEMICAL AND ULTRASTRUCTURAL CHARACTERIZATION OF GLIAL CELLS IN LONG-TERM CO-CULTURES. C. Andersson*, J. Brunso-Bechtold, and M. Tytell. Bowman Gray School of Medicine, Wake Forest University, Winston-Salem,NC 27101.

We examined a mixed glial culture containing type 1 astrocytes and O-2A lineage cells in fetal calf serum at 5 days in vitro (DIV), 12 DIV, and 30 DIV, using cell-specific immunocytochemical markers and electron microscopy. The flat type 1 astrocytes were polygonal, and GFAP+, GalC-, and A2B5- (specific markers for mature astrocytes and oligodendrocytes, and O-2A cells) at all three time points. From 5 to 30 DIV, the type 1 astrocytes increased in cell size. Ultrastructurally, the cytoskeleton changed dramatically over time, with the numbers of glial filaments increasing and microtubules decreasing.

At 5, 12, and 30 DIV, the O-2A lineage cells were multipolar, and A2B5+, GFAP-, and GalC-. Ultrastructurally, the O-2A lineage cells could not be regarded as either astrocytes or oligodendrocytes. These cells had a dense cytoplasm, a very small number of intermediate filaments, and a large number of vacuoles and dense bodies.

At no time were the differentiated type 1 astrocytes immmoreactive for HNK-1 and NCAM. O-2A lineage cells were HNK-1+ and NCAM+, further suggesting cellular immaturity.

The cytoskeleton of cultured type 1 astrocytes seems to develop comparable to astrocytes in vivo. Under these culture conditions, O-2A lineage cells were multipolar, but immature, and appear unable to differentiate.

471.7

EXPRESSION OF CD44 ANTIGEN ON HUMAN FETAL AND ADULT NEURAL CELLS IN CULTURE. G. Moretto* and S.U. Kim. Dept. of Neurology, Univ. of British Columbia, Vancouver, Canada.

CD44 is a 90 kDa lymphocyte glycoprotein that belongs to the family of cell adhesion molecules and therefore is likely to be involved in cell-cell and cell-matrix interactions. Its broad distribution in different tissues includes the fibrous astrocytes of normal white matter and reactive astrocytes in multiple sclerosis lesion. In this study, expression of CD44 antigen in primary neural cell cultures derived from fetal and adult human brains was investigated. By double staining with antibodies to human CD44 and to GFAP, we found that 70-75% of fetal and 90-95% of adult astrocytes expressed the CD44 antigen on the cell bodies and processes. Unlike in vivo where only fibrous astrocytes are CD44-positive, both flat and process-bearing astrocytes expressed CD44 in culture. CD44 was also detected on more than 50% of galactocerebroside-positive oligodendrocytes that were isolated from adult brains. Small fetal neurons, A2B5-positive and MAP2-positive cells, did not express CD44 at all. These results indicate that CD44 glycoprotein is restricted to a glial cell population in the human nervous system.

471.8

INSULIN-LIKE GROWTH FACTOR I (IGF-I) INDUCES ASTROCYTES TO ACQUIRE A RADIAL GLIAL-LIKE MORPHOLOGY *IN VITRO*. A. Casey and B.H.J. Juurlink*. Department of Anatomy, University of Saskatchewan, Saskatoon, SK, Canada S7N 0W0.

Insulin and IGF-I are known to have neuronotrophic effects; however, they can also affect astrocyte function. For example Dringen and Hamprecht have shown that they both affect glycogen metabolism (1992. J. Neurochem. 58:511) and we have shown that they affect astrocyte neuronotrophic activity (Ang et al. 1992. J. Neurol. Sci. in press). Since astrocyte morphology is intimately related to astrocyte function, our objectives were to determine whether insulin, and in particular IGF-I affected astrocyte morphology. Rat type-1 astrocytes were isolated using the McCarthy and de Vellis method. After two weeks in a serum-containing medium, the cultures were maintained on Bottenstein's G5 medium minus the insulin. On this medium the astrocytes maintained their flat fibroblast-like appearance. Addition of insulin (1-5μg/ml) or IGF-1 (10-100 ng/ml) resulted in a dramatic change in astrocyte morphology. In contrast, IGF-II (10-100 ng/ml) had no effect on astrocyte morphology. The insulin and IGF-I induced morphology consisted of a distinct soma from which emerged one long prominant and sometimes several long processes of narrower calibre. The processes were tightly packed with GFAP-containing intermediate filaments, whereas the somas had a lower density of intermediate filaments. This morphology is very reminiscent of that of radial glial cells. In the CNS, IGF-I mRNA as well as IGF-I receptors are expressed by both neurons and astrocytes in a stage-specific manner, i.e. the highest level of IGF-I expression occurring in embryonic and fetal development. It is likely, therefore, that IGF-I can act in a paracrine as well as in an autocrine fashion in the brain. We postulate that possibly IGF-I plays a role in the differentiation of radial glial cells. Furthermore, these paracrine and/or autocrine effects of IGF-I may be involved in the relationship of developing neurons and radial glia, which affects both neuronal migration and the differentiation of neurons and astroglia. *We thank Dr. B. Bhaumick for the gift of IGF-I and IGF-II.*

471.9

S100 AND ONTOGENESIS OF RADIAL GLIA-LIKE CELLS AND ASTROCYTES DURING DEVELOPMENT OF RAT BRAIN. S. Bledsoe* and F. C. Zhou. Indiana Univ. Sch. Med., Dept. Anatomy, Indianapolis, IN 46202

S100, an astrocytic marker in adult brain, appears predominately in the radial glia during early development of the brain. Immunocyto-chemical staining for S100 shows that it appears first in radial cells vertical to the neuraxis during early gestation stage of neural development. These cells appear in brainstem regions during E13-14 gestation stages of Sprague-Dawley rats, and then propagate from the brainstem center in two directions —rostrally to forebrain and caudally to spinal cord. In contrast, cells positive for glial fibrillary acidic protein (GFAP), a cytoskeletal marker for astrocytes, appear in a much later stage (E16) and do not overlap spatially with S100 immunostaining in adjacent sections. At the E17-19 stages, both GFAP and S100 appeared in the forebrain. The distribution of GFAP-positive cells is different from that of S100. This evidence reopens the hypothesis on ontogenesis of glial cells. In the brainstem and forebrain S100-positive cells at the early gestation stage represent a subpopulation of glial cells other than astrocytes, or radial glia-like cells develop into astrocytes in late gestation and postnatal stage. Further study of radial glia confirmation is in progress. (supported by NIH grant NS23027 to FCZ)

471.10

The Isolation of a novel oligodendrocyte specific c-DNA

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Myelin contains a variety of unique proteins (e.g. MAG, MBP, PLP, CNPase). Only a few proteins seem to be selectively expressed by central nervous system (CNS) oligodendrocytes as compared to peripheral nerve Schwann cells. Myelin disorders that are specific for the CNS exist, however, and oligodendrocytes play important additional roles in CNS development and regeneration by their expression of neurite growth inhibitory proteins.

Here we demonstrate the isolation of a c-DNA encoding a novel oligodendrocyte specific protein. The c-DNA clone (p16.111) corresponds to a m-RNA of 4.9 kb. The presently available c-DNA (3.4 kb), including the poly-A-tail, does not show homology to any known oligodendrocyte or CNS c-DNA. The expression is restricted to the postnatal rat CNS (not detected in sciatic nerve (PNS), liver, lung, thymus, heart, kidney, spleen, skeletal muscle, testis) and is higher at P16-20 than in the adult spinal cord. *In situ* hybridisation shows a selective, strong expression in oligodendrocytes in myelinating regions of the brainstem, cerebellar white matter and corpus callosum. In cultured optic nerve oligodendrocytes p16.111 expression was localized in galC-positive oligodendrocytes.

471.11

THE GLIOARCHITECTONICS OF THE HUMAN NEOCORTEX.
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It is well known that the normal cortex shows few fibrillary astrocytes except in the molecular layer where these cells are associated with external glial limiting lamina. We observed in old individuals and in Alzheimer's disease that astrocytes of the molecular layer also show long, straight, radial fibers which penetrate into the depth of the cerebral cortex down to the II and III layers.

We also describe the subcortical glial zone (of the white matter) astroglia. Also, these cells display straight fibers which penetrate deep into the cerebral cortex and could be traced up to the depth of the IV layer.

It was impossible to prove if these fibers penetrate through the whole thickness of the cortex, but in many aged individuals or individuals with Alzheimer's disease, perpendicularly running astroglial fibers were also seen in the mid-cortical layers.

We believe that these fibers represent a perpendicular glial fiber system that most likely is a remnant of the embryonal radial glia. We hypothesize that these fibers under normal circumstances are of the protoplasmic astroglia type and this is why they do not stain with GFAP in younger individuals. Another consideration is that these fibers represent a special type of isomorphic gliosis.

It was also observed that both the glia of molecular layer and subcortical zone seem to respond (proliferate) synchronically in aging and Alzheimer's disease.

471.12

MULLER CELL PHENOTYPE PRECEDES SENESCENCE OF VIRALLY TRANSFORMED CHICK NEURORETINAL CELLS.
G.M. Seigel*, E.L. Imperato, J.T. Hansen, and M.F.D. Notter. Department of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642.

The phenomenon of senescence has been an obstacle in obtaining permanent Rous sarcoma virus-transformed avian cell lines. Our previous studies analyzed this senescence phenomenon in Rous sarcoma virus-transformed chick neuroretinal cells (LA29NR) and demonstrated a decreased mitotic index accompanied by loss of transformed phenotype (Seigel and Notter, submitted, JVI 364-92). After four to five months in culture, immediately preceding cell death, unusual process formation is observed in LA29NR cell populations. We have now characterized these morphologically differentiated cells as immunoreactive for glial fibrillary acidic protein, S-100 antigen, and vimentin, while predominantly negative for neuron-specific enolase, as detected immunocytochemically. Electron microscopic observations revealed large, vacuolated cells packed with longitudinally-oriented intermediate filaments, extensive smooth and rough endoplasmic reticulum, as well as occasional myelin-like figures, characteristic of normal retinal Muller cells. However, unlike normal Muller cells, retroviral particles also were evident, both intracellularly and extracellularly. From these data, we conclude that despite continued retroviral expression, senescent LA29NR cells exhibit a Muller cell phenotype as a state of terminal differentiation at the end of their mitotic lifespan in culture.

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471.13

UNIQUE GLIAL CELLS DEMARCATE NEUROMERES IN THE CENTRAL NERVOUS SYSTEM DURING METAMORPHOSIS IN *DROSOPHILA MELANOGASTER*.
D. Davis , R. Fehon , S. Artavanis-Tsakonas , and W. J. Costello*
Yale Univ., New Haven, CT and Ohio Univ., Athens, OH.

During metamorphosis in Drosophila, the central nervous system (CNS) is extensively remodelled. Adult nerves arise de novo or from modified larval nerves; inherent in the nerve formation is extensive pathfinding. Some glial cells may serve as landmarks to define pathways for developing neurites. At the boundary between neuropil and cortex, unique glial cells have an affinity for Notch antibodies (Ab) in third instar larvae (Fehon et al, J. Cell Biol. 113:657). In early pupae, Notch Ab affinity is most prevalent in the glial cells at the neuromeres where leg nerves are forming. By 48h, the affinity decreases and is barely evident at 72h; this period is coincedent with final leg nerve formation. Related mitotic activity, typical for many other Notch-positive cells, is absent in these glia. These neuromere glial cells are distinguished by intense synthetic activity in parallel with the peak Notch affinity (<48h pupae), as indicated by extensive rough endoplasmic reticulum.

471.14

IN VITRO CULTURE AND INITIAL CHARACTERIZATION OF PUTATIVE GLIAL CELLS FROM THE MOLLUSCS LYMNAEA AND HELISOMA.
R.L. Ridgway*, N.I. Syed, G.C. Hauser, and A.G.M. Bulloch. Dept. of Med. Physiol., Univ. of Calgary, Alta., Canada T2N 4N1 and Biol. Dept., Seattle Pacific Univ., WA, USA 98119.

In this study we have modified techniques for culturing neurons of pulmonate molluscs to obtain populations of cells having glial-like characteristics. The cells were isolated from the cortical regions of the central ganglia of the snails Lymnaea stagnalis and Helisoma trivolvis using proteolysis (see Ridgway et al., J. Neurobiol., 22: 377-390, 1991). The isolated cells were then cultured under sterile conditions in 50% Leibovitz (L-15) medium on various substrates. The putative glial cells displayed an elongate morphology (75-100 µm in length and 10-15 µm in width), the nucleus being situated midway along the cell axis. Lamellipodial processes extending from perimeter of the cells correlated with a high degree of motility in the first 6-12 h after plating. This motility was substrate dependent: on adhesive substrates (e.g., poly-L-lysine) cells decreased in motility after 12 h, whereas those plated on less adhesive substrates (e.g., bovine serum albumen) were motile for 48-72 h. Electrophysiological recordings made from less motile cells revealed resting membrane potentials ranging from -40 mV to -50 mV. The cells were responsive to both dopamine (0.1-1.0 µM) and FMRFamide (0.01-0.1 µM). Cells could be maintained for 4-5 days whereupon they became pycnotic and lost their membrane potentials; reasons for this cell death are under study.

471.15

CALCIUM OSCILLATIONS AND CONTRACTION WAVES IN INSECT GLIAL CELLS.
H. Jindrova#, J. Dallman+, J.W. Truman+ * and M.S. Cooper+, Institute of Parasitology, Branisovska 31, 370-05 Ceske Budejovice, Czechoslovakia# and Department of Zoology, University of Washington, Seattle, WA 98195+.

Agonist-induced Ca-oscillations, Ca-waves, and/or Ca-transients have been observed in glial cells from vertebrate cortex, hippocampus, optic nerve, and neuromuscular junction. To determine whether such intracellular signaling is found in the nervous system of invertebrates, we examined cytoplasmic Ca-dynamics in glial cells isolated from the brains of the insect, Manduca sexta. Primary explants of 5th instar larval brains were cultured on polylysine-coated coverslips, loaded with the Ca-indicator Fluo-3AM, and observed with time-lapse fluorescence video and confocal microscopy. Single Ca-transients, lasting approximately 10-200 seconds, occurred spontaneously in untreated cultures. When aluminum tetrafluoride (AlF_4^-), a general activator of G-proteins, was externally applied to cultures composed predominantly of glia, the following sequence of Ca-transients ensued: (1) an initial Ca-spike, lasting approximately 10 seconds; (2) a subsequent envelope of elevated Ca^{2+} lasting 400-500 seconds, which was often composed of Ca-oscillations whose period ranged from 20 to 170 seconds. Cytoplasmic contraction waves were also seen to propagate in Manduca glial cells, both spontaneously, and in response to externally applied agonists. These results suggest that an active transmembrane signaling system, involving G-proteins and cytoplasmic Ca-transients, exists in these invertebrate glial cells. (Supported by a Sloan Foundation Research Fellowship and NSF PYI Award DCB-9157132 to M.S.C.).

472.1

POSTBUBERTAL EMERGENCE OF MESOLIMBIC DOPAMINERGIC SUPERSENSITIVITY AFTER EXCITOTOXIC LESIONS OF VENTRAL HIPPOCAMPUS IN THE RAT. Barbara K. Lipska*, Ph.D., George E. Jaskiw, M.D., Ingrid Phillips M.A., Daniel R. Weinberger, M.D., Clinical Brain Disorders Branch, IRP, NIMH, Neuroscience Center at St. Elizabeths, Washington, DC 20032.

While early animal models of schizophrenia addressed primary changes in striato-limbic dopamine activity, they did not account for other phenomena associated with the illness (e.g. congenital temporal-limbic abnormalities, cortical deficits, vulnerability to stress, postpubertal onset). We have reported that ibotenic acid ventral hippocampal (VH) lesions in adult rats affected DA-related behaviors in a manner consistent with increased mesolimbic DA activity. These changes were paralleled by biochemical indices suggesting enhanced DA transmission in the nucleus accumbens, a reduction in DA turnover in the medial prefrontal cortex, and an inverse correlation between these (Brain Res., 1992). The behavioral response to pharmacological (FG-7142) or environmental (swim stress) stressors was not affected. In contrast, locomotor activity and response to amphetamine of rats lesioned as neonates (PD7) did not differ from controls 4 weeks after the lesion (PD35). However, 3 weeks later (PD56), the same rats became hyperactive in these conditions. Moreover, neonatally lesioned animals were hyperresponsive to stress. In summary, in rats with neonatal lesions of VH, augmented activity develops only after puberty. Neonatal but not adult-induced lesions also produce an exaggerated response to stressful stimuli. Homologous mechanisms could be involved in schizophrenia.

472.2

DEVELOPMENTAL EXPRESSION OF PARVALBUMIN mRNA IN THE CEREBRAL CORTEX AND HIPPOCAMPUS OF RAT. Lecea, Luis de[1,2], Eduardo Soriano[1], Jose A. del Rio[1], Sonja Forss-Petter[2*,1] Unit of Cell Biology. Faculty of Biology. University of Barcelona. Spain.[2] Dept. of Molecular Biology, MB10. The Scripps Research Institute. La Jolla. CA 92037

Parvalbumin is a calcium binding protein that is thought to play a major role in calcium buffering in metabolically active fast-firing cells. In adult cortex, parvalbumin is expressed in a subset of characterized GABAergic interneurons. The pattern of parvalbumin mRNA expression during the postnatal development of rat cerebral cortex and hippocampus was examined by means of in situ hybridization with an oligonucleotide probe. In animals aged P0-P6, no signals above background were observed in the cortex, although silver grains always occurred in the thalamic reticular nucleus. At P8-P10 a few cortical cells contained low numbers of silver grains in motor and somatosensory cortices and in the hippocampus. By P12, parvalbumin expressing cells were detected within all cortical regions. At P14 an overall increase both in the number of positive cells and in their intensity of labeling was observed. A further maturation pattern was seen at P16-P21 stages, which lead to the appearance of an adult like distribution in cortex and hippocampus. The appearance of parvalbumin mRNA expressing cells does not follow the usual inside out sequence of cortical maturation. Instead, hybridization is first observed in the middle cortical layers to thereafter expand to the immediate adjacent layers. Count of silver grains revealed that hybridization signals, increased progressively during postnatal development. In adult neocortex and hippocampus the distribution of parvalbumin mRNA containing cells is consistent with our previous immunocytochemical findings in rodent cortex. These data suggest that the developmental pattern of expression of parvalbumin follows the needs of calcium buffering and may reflect the functional maturation of cortical interneurons.

472.3

EFFECTS FROM EXPERIMENTALLY INDUCED HYDROCEPHALUS ON HIPPOCAMPAL EPENDYMAL LINING: AN ULTRASTRUCTURAL STUDY. R.M. Kriebel* and J.P. McAllister. Depts. Anatomy, Phila Col Osteopath Med and Temple Univ Sch Med, Phila PA 19131.

The neurological deficits found in infantile hydrocephalus have most often been explained by pathological changes in cerebral cortex. It has been the primary goal of our studies to provide a cellular basis for the residual neurological deficits observed even though surgical intervention may have relieved the effects of ventriculomegaly on the cerebral cortex. Previous studies have shown significant structural changes in cerebral cortex, basal forebrain region, especially in septal nuclei, and hippocampus. A primary difference in the structural alterations within these regions was the minimal increase in extracellular space in the hippocampus. In the present study we compared the ependymal surface adjacent to the cerebral cortex, basal forebrain region, and hippocampus. Kaolin injection induced hydrocephalus; aldehyde fixed brains were sectioned coronally with subsequent staining for cells and fibers. The ependyma underlying the cerebral cortex was thinned but appeared to remain intact. In contrast, the ependyma adjacent to the hippocampus was not altered in comparison to control tissues. The cuboidal shape as well as surface projections appeared normal. The potential relation of these structural differences to extracellular space in the neuropil is discussed. Supported by AOA901031,RMK;NIHHD21527,JPM

472.4

CYTOARCHITECTURAL, NEURONAL MORPHOLIGIC AND MOLECULAR ASPECTS OF HUMAN HIPPOCAMPAL DEVELOPMENT

Steven E. Arnold* and John Q. Trojanowski, Departments of Psychiatry and Pathology, University of Pennsylvania School of Medicine

Cytoarchitecture, neuronal morphology and the expression of various neuronal cytoskeletal proteins were monitored in subfields of the developing human hippocampus. Fixed sections from the hippocampal formations of 18 cases from 10 weeks gestational age through 2 years were stained with cresyl violet for cytoarchitectural and morphologic analysis and processesed for immunohistochemistry. Monoclonal antibodies (mAbs) used were directed at components of the neuronal cytoskeleton that are believed to be important determinants of neuronal polarity. These included microtubule-associated proteins MAP2, MAP5 and tau, alpha and beta tubulins, and poorly, moderately and highly phosphorylated neurofilament (NF) isoforms. Differential patterns of cytoarchitecture and neuronal morphology were noted across time and both between and within anatomic regions. The expression of MAP2, MAP5, alpha and beta tubulins, NFM-P$^-$, and NFH-P$^+$ was evident at the earliest time point studied and persisted throughout development. Immunoreactivity to tau and NFH-P^{+++} appeared at subsequent points, with tau diminishing in intensity later on in development. MAP2, MAP5 and NFM-P$^-$ immuno- reactivity allowed recognition of nascent dendrites and pyramidal shaped neurons prior to their definition with conventional cresyl violet staining. At all times, there were differences in intensity of immunoreactivity between the different ammonic, subicular and entorhinal subfields. (Supported by NIMH grant 1 K20 MH00978-01)

472.5

CALBINDIN-D28K DISTRIBUTION IN HIPPOCAMPAL FORMATION OF LATE GESTATION FETAL SHEEP IS INDEPENDENT OF PLASMA GLUCOCORTICOID CONCENTRATION. T.J. McDonald* & R.H. Wasserman Department of Physiology, Cornell University, Veterinary Research Tower, Ithaca, NY 14850.

Glucocorticoids regulate the appearance of Calbindin-D28K in the granule cells of the dentate gyrus of adult rats with adrenalectomy causing complete loss of immunoreactivity in the granule cell layer after 4 weeks (Iacopino and Christakos, 1990. J.Biol.Chem. 265:10177-10180). In late gestation, fetal sheep undergo a logarithmic rise in peripheral plasma cortisol concentration that starts at approximately 125 days of gestational age (dGA) with baseline concentrations of 5-10 ng.ml^{-1} to peak at term with concentrations over 100 ng.ml^{-1} (Magyar et al. 1981. J.Ster.Biol. 14:1091-1099). This cortisol rise is driven by the pituitary and hypothalamic paraventricular nuclei and is indispensable for parturition to occur at normal term (Liggins et al., 1973. Rec.Prog.Horm.Res. 29:111-159; McDonald and Nathanielsz, 1991. Am.J.Obstet.Gynecol. 165:764-770). In this study the brains of fetal sheep at 105, 125, 135, 147 (in labor) dGA and newborn lambs were examined immunocytochemically. While Calbindin-D28K was found in diverse areas of the brain, eg. brainstem, cerebellum, neocortex, basal ganglia and hypothalamus, no Calbindin-D28K immunoreactivity was detected in the dentate gyrus or Ammons horn at the ages examined. However, many Calbindin-D28K immunopositive cells were found in the subiculum at all ages, but intensity of staining appeared to be independent of gestational age. It is concluded that unlike in adult rats, endogenous plasma glucocorticoids have no effect on the appearance immunocytochemical distribution of Calbindin-D28K in the hippocampal formation of the late gestation fetal sheep.

472.6

NEUROGENESIS OF THE VASOPRESSIN NEURONS IN THE BED NUCLEUS OF THE STRIA TERMINALIS AND MEDIAL AMYGDALOID NUCLEUS OF THE RAT. H.A. Al-Shamma* and G.J. De Vries. Program in Neuroscience and Behavior, Univ. of Massachusetts, Amherst, MA 01003.

The vasopressin-immunoreactive (AVP-ir) neurons of the bed nucleus of the stria terminalis (BST) and medial amygdaloid nucleus (MA) share many neurochemical and neuroanatomical characteristics, e.g., in their neurotransmitter content, cell morphology, and steroid sensitivity. It is unclear, however, how BST and MA neurons develop these similar characteristics. To get more information about the development of these cells, we determined the day of birth of these AVP-ir subgroups with the cell birth marker bromo-2-deoxy-5-uridine (BrdU).

Pregnant Long-Evans rats received intraperitoneal injections of BrdU on one of gestational days 14-20, day 1 being the day that a copulatory plug is found. At three months of age, the male offspring of these treated females were sacrificed and their brains were processed for both BrdU and AVP immunostaining. Approximately 20% of the AVP-ir cells in the BST and MA were also immunoreactive for BrdU, the majority of which were found in the BST and MA of animals exposed to the cell birth marker on embryonic day 16 and 14, respectively. These preliminary findings are in agreement with earlier studies using [^{3}H]thymidine autoradiography which suggest that most of the cells in the divisions of the BST and MA that contain the AVP-ir neurons are born on embryonic days 15-16 and 14-15, respectively.

472.7

PRE- AND POSTNATAL CHANGES IN THE EXPRESSION OF PROTO-ONCOGENES DURING THE DEVELOPMENT OF ROSTRAL CEREBRAL CORTEX. P.E. Kuhn* and M.W. Miller. Dept. Biology, Rutgers Univ., Piscataway NJ 08854, Res. Serv., V.A.M.C., Iowa City IA 52242, and Dept. Psychiatry & Pharmacology, Univ. Iowa Coll. Med., Iowa City IA 52242.

Zones containing post-mitotic cells and neurons forming neurites express proto-oncogenes. The change in the expression of c-*fos*, c-*neu*, c-*src*, and c-*ras*, was examined using immunoblots. Tissue from the frontal pole of rat cortex was harvested from fetuses and pups (between gestational day (G) 16 and postnatal day (P) 21) and in 3- to 5-month-old rats. Blocks of tissue were disrupted in buffer containing detergent and proteinase inhibitors. Standard gel electrophoresis and transfer techniques were used in producing immunoblots. Anti-c-*fos* identified 2 proteins, one with a molecular weight of 64 kD and the other 80 kD. c-*fos* expression (of both proteins) appeared on G16 and waned by P6 and peaked on G19. Anti-c-*neu* also labels 2 proteins, 65 kD and about 185 kD. The temporal expression of the 65 kD protein was similar to that of c-*fos*. On the other hand, the 185 kD protein appeared on the day of birth (P0) and virtually disappeared by P9; peak expression occurring on P3-P6. Anti-v-*src* identifies a doublet with molecular weights of 53 and 62 kD. The expression of both proteins begins as early as G16 and persists beyond P9. This expression was rather stable throughout this period. Trace amounts of each of these proto-oncogenes were detectable in the adult rat. The pattern of c-*ras* expression is quite different for it builds during the postnatal period to adult levels by P21. Based upon timing, the data are consistent with the concept of a cascade of events, c-*src* being related to cell proliferation or neuronal migration, c-*neu* and c-*fos* being related to neuronal differentiation and or death, and c-*ras* being related to neurite outgrowth, synaptogenesis, and the maintenance of neuronal integrity. Funded by DE 07734, AA 06916, and AA 07568.

472.8

MIGRATION OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IMMUNOREACTIVE NEURONS TO RAT CORTEX. M.W. Miller*. Res. Serv., Iowa City V.A.M.C., and Dept. Psychiatry & Pharmacology, Univ. Iowa Coll. Med., Iowa City IA 52242

Most neurons migrate to cortex by an orderly inside-to-outside sequence. Accordingly, neurons in deep cortex are generated before those in superficial cortex. In visual cortex, this migrational pattern is followed by cortico-cortical projection neurons and by GABAergic local circuit neurons. A combined autoradiographic-immunohistochemical method was used to determine the time of origin of VIP immunoreactive neurons. VIP is expressed by a subpopulation of local circuit neurons, mostly small bipolar neurons, which do not co-localize GABA. The birth of neurons in each layer of cingulate cortex (areas 24 and 29), somatosensory cortex (area 3), and visual cortex (area 17) was examined by administering [^{3}H]thymidine on gestational day 13, 15, 17, 19, *or* 21 and sacrificing the pups on postnatal day 30. Although the timing differed slightly in each cytoarchitectonic area, the basic pattern of neuronogenesis was similar in all areas. VIP-positive neurons with a particular time of origin were not distributed in a tangential band of cortex; rather such neurons were distributed through the full depth of cortex. Such a pattern was evident regardless of the time of origin. Thus, VIP immunoreactive neurons migrate into cortex via a mode other than the standard inside-to-outside sequence. Funded by DE 07734, AA 06916, and AA 07568.

472.9

POSTNATAL DEVELOPMENT OF PROTEIN KINASE C GAMMA EXPRESSION IN RAT BRAIN. B. Buwalda, C. Nyakas, E.A. van der Zee, S. Cazaubon, P.G.M. Luiten*. Dept. of Animal Physiology, University of Groningen, P.O.Box 14, 9750 AA Haren, The Netherlands.

Protein kinase C (PkC) is a key enzyme in intercellular signal transduction, and can be activated through receptor-mediated hydrolysis of phosphoinositol. PkC activation has also been found to play a crucial role in cellular processes related to development. Here we examined the developmental changes in hippocampal and cortical expression of the gamma isoform of PkC. The PkC immunoreactivity was studied in rats on postnatal day 7 (PD7), 10, 14, 20, 30 and 47. PkC-gamma immunoreactive neurons on PD7 are visible in neocortical layer 5 and in the hippocampal subicular and hilar region. On PD10 PkC in the neocortex is increased and expression is also seen in layer 6 and in allocortical areas. Furthermore pyramidal cells throughout all parts of the cornu ammonis (CA) become immunoreactive. On PD14 PkC gamma expression is visible in cortical layers 2,3,5 and 6. Whereas on PD7 and PD10 the PkC immunostaining shows a relative uniform neuronal cytoplasmic distribution, the PkC immunoreactivity within hippocampal CA1 pyramidal cells and entorhinal cortical neurons on PD14 appears to become translocated to the cellular membrane. This translocation of PkC gamma is extended to cortical layers 2,3 and 6 on PD20. After PD20 PkC levels increase in dendritic fields and fiber systems. The translocation of PkC gamma to cellular membranes after PD14 may be related to the receptor linked activation of PkC and in this way reflect maturational processes of central nervous systems.

472.10

Corpus Callosum: EM Measures of Sex and Infantile Handling Effects. G. W. Boehm*, C. M. Mack, A. S. Berrebi and V. H. Denenberg. Biobehavioral Sciences Graduate Program, University of Connecticut, Storrs, CT 06269.

Findings of sexual dimorphism in the rat corpus callosum, using gross size measures, prompted the use of electron microscopic techniques to view ultrastructural parameters. Since neonatal handling has been shown to enhance certain differences between gross measures of female and male rat callosa, both handled and nonhandled rats were used to study this phenomenon. All rats were transcardially perfused at 110 days of age, and midsagittal sections were obtained. Based on previous findings, sampling was restricted to the first 20% of the genu, although the posterior portions of the callosa have been retained and embedded for possible analysis. Preliminary findings indicate a greater quantity of unmyelinated axons than myelinated axons in the areas sampled, and a distinct Sex x Handling interaction in total number of unmyelinated axons.

472.11

CHARACTERIZATION OF A NOVEL **ANTENNAPEDIA**-CLASS HOMEOBOX GENE ISOLATED FROM HUMAN 11 WK FETAL BRAIN LIBRARY. J.F. Leckman*, X. Lin, A. Swaroop, F.M. Vaccarino, M.T. Murtha, F.H. Ruddle. Child Study Ctr., Depart. of Biol., Psychiatry, and Pediatrics, Yale University, New Haven, CT 06510 and W.K. Kellogg Eye Ctr., Depart. Ophthalmol. and Human Genetics, Univ. Michigan, Ann Arbor, MI 48105.

A novel **Antp.**-class homeobox gene has been isolated from a human 11 wk. fetal brain cDNA library. PCR with two sets of oligonucleotide primers (specific for highly conserved regions of the **Antp.**-class homeobox) was used to amplify portions of homeobox genes present in the fetal brain library. Sequencing 100 clones identified 2 novel **Antp.**-class genes. Screening the fetal brain library with a probe specific for one of the novel genes led to the identification of a 1.7 kb cDNA containing the novel homeobox sequence. This clone encodes a protein of approximately 327 amino-acid residues. By Northern analysis, this cDNA detects multiple transcripts in the developing human CNS as well as other tissues. These observations suggest that in early mammalian development this homeobox gene may exert a spectrum of control functions in a variety of organ systems including the CNS.

472.12

ANALYSIS OF THE CONEL HUMAN DEVELOPMENTAL CORTICAL DATA USING OPTIMAL INTERPOLATION W.R. SHANKLE*, J.P. DeFIGUEREDO, B.H. LANDING, UCI 1617 MEDICAL PLAZA, IRVINE, CA 92717

We introduce a developmental neuroanatomical database from J.L. Conel as a potentially useful framework for computational models and analysis of the developing human brain to 6 years and its relation to developing behaviors. We have investigated the relation between functions related to gyri and anatomical measures of cortical thickness, cell density, cell body length, cell body width, # of axoms/ unit area across all layers and over 40 gyri. Using the analytical method, Optimal Multi layer Neural Network Interpolation, inputs consisting of the 6 variables per gyrus per layer were used to find the best separation of the gyral functions used the fewest variables possible. We wished to see which variables, layers and gyri mapped to gyral functions found anatomic correlations relevant to the given classes of gyral functions. Results will be presented. Also presented are data on total cortical thickness. Figure 1 graphs the total cortical thickness in mm., for the 37 loci for all eight age-points in his data. The curves for the majority of loci are not smooth, with many loci showing episodes of reduction in cortical thickness despite the general tendency to increased thickness with age. Figure 2 graphs the slopes (rates of change in cortical thickness per mm. per month) of the values for total cotical thickness, for the seven age-blocks the graph showing that all loci show appreciable change in cortical thickness, either positive (increasing in thickness) or negative (decreasing in thickness) over the age-block 0-1 month, with the rate of change in total cortical thickness per month generally decreasing with age.

472.13

CORRELATION OF CORTICAL GROWTH AND METABOLISM IN THE DEVELOPING RAT. D.R. Riddle*, K. Alexander, A. Richards, F. Zsuppan and D. Purves. Dept. of Neurobiology, Duke University Medical Center, Durham, NC 27710.

We have used enzyme histochemistry and digital image analysis to examine the relationship between regional metabolic activity and postnatal growth in the cerebral cortex. Juvenile (one week-old) and adult (10-12 weeks-old) rats were anesthetized and perfused with saline followed by 10% glycerol. The cortical mantle was then removed, flattened and rapidly frozen; serial tangential cryostat sections were stained for succinic dehydrogenase (SDH) or cytochrome oxidase (CO). Each section was digitized and the images corrected for shading errors. The density of the reaction product within defined regions of the primary somatic sensory cortex (SI) was determined using a computer-based image analysis system. A robust and consistent gradient of enzymatic reactivity was evident in juvenile but not adult rats. The density of the enzymatic reaction product (SDH or CO) was greatest in the representation of the anterior snout and least in the representations of the paws, particularly the hindpaw. This gradient of metabolic activity corresponds to the pattern of differential postnatal growth (Riddle et al., 1992; J. Neuroscience, in press). The covariance of growth and metabolism in juvenile rats must in part reflect the metabolic demands of increased biosynthesis; this gradient may also indicate higher average levels of electrical activity in those regions of the developing cortex that grow the most.

472.14

REGIONAL CEREBRAL BLOOD FLOW DURING VISUAL STIMULI PROCESSING IN TWO-MONTH-OLD ALERT INFANTS. N. Tzourio*, S. De Schonen, B. Mazoyer, A. Boré, U. Pietrzyk, B. Bruck, Y. Aujard, C. Deruelle. S.H.F.J, C.E.A, Orsay, 91406, and Lab. Cogn. Neurosci., C.N.R.S., Marseille, France, Max Planck Instit., Cologne, Germany.

Evidence for regional cerebral metabolic changes accompanying human brain maturation was first demonstrated in sleeping children using PET) and fluoro-deoxyglucose (FDG). In the present study, we have adapted the regional cerebral blood flow (rCBF) PET-[15O]-water activation method to investigate the brain regions involved in visual stimuli processing in awake infants.

Six neonates were recruited during their first week after birth from the R. Debré Hospital neonatalogy intensive care unit. All were suffering from acute fetal stress, birth asphyxia or neonatal convulsions. At the age of two months, rCBF was measured twice in the same session while infants were involved in the following tasks: 1- visual fixation of a circle of flashing diodes, 2- visual fixation of slides of female faces presented at .25 Hz (in two cases, only condition 2 was recorded). Tasks were started one minute before i.v. injection of [15O]-water (0.7 mCi/kg body weight) and a single 80 second scan was acquired on a high resolution PET camera (ECAT 953B/31). Individual magnetic resonance images were also acquired.

Overall, the rCBF cortical pattern at this age appeared similar to that observed with the FDG method. In addition, a right hemisphere dominance was observed during both visual tasks (right-to-left ratio = 1.017, p = 0.014, N = 10, t-test) with no overall significant difference between the two (p = 0.51). Besides demonstrating the feasibility of PET activation studies in alert babies, these preliminary results argue for (i) either differential sensitivity of the two hemispheres to perinatal stress, (ii) a time lag between the maturation rate of the two hemispheres, or, (iii) a right hemisphere advantage in visual attention. (supported by a grant MRT/MEN Sciences de la Cognition)

TRANSPLANTATION: CORTEX

473.1

CHOLINE ACETYLTRANSFERASE IN THE CEREBRAL CORTEX OF NBM-LESIONED RATS RECEIVING CHROMAFFIN CELL GRAFTS. S. A. Welner* and Z.C. Koty. Douglas Hospital Research Centre, Department of Psychiatry, McGill University, Montreal, Quebec, Canada H4H 1R3.

Lesions of the nucleus basalis magnocellularis (NBM) in rats produce spatial memory deficits as well as changes to cholinergic markers in the cerebral cortex, the target area of projections from the NBM. We have previously reported that adrenal chromaffin cells transplanted to the cerebral cortex of rats with NBM lesions are able to ameliorate lesion-induced spatial memory deficits and increase acetylcholinesterase (AChE) staining in the host cortex (Welner et al., *Brain Research*, **527**: 163, 1990). In the present experiments, we test whether grafting chromaffin cells to the cerebral cortex of NBM-lesioned rats will have an effect on a different cholinergic marker in the cerebral cortex, choline acetyltransferase (ChAT), at various time points post-grafting. Sprague-Dawley rats received bilateral partial lesions in the NBM and, two weeks post-lesion, were either left unoperated or had suspensions of adrenal chromaffin cells grafted to six sites in their frontal and parietal cortices. A group of unoperated rats served as controls. ChAT activity was measured at 2, 6, 12 and 16 weeks post-graft. In the lesioned-alone group it was found that ChAT progressively decreased over time, whereas in the lesion plus graft group, ChAT levels were significantly decreased at 6 weeks and appeared to be increasing at the 12 and 16 week time points. These results support previous findings that the cholinergicity of cerebral cortex following grafting of chromaffin cells to the cortex of NBM-lesioned rats is increased. (Medical Research Council, Canada & Fonds de la Recherche en Santé, Québec.)

473.2

BASIC FIBROBLAST GROWTH FACTOR IN CHROMAFFIN CELL GRAFTS TO THE CEREBRAL CORTEX OF NBM-LESIONED RATS. E.S. Yuzda*, Z.C. Koty and S. A. Welner. Douglas Hospital Research Centre, Department of Psychiatry, McGill University, Montreal, Quebec, Canada H4H 1R3.

Chromaffin cell grafts to the frontal and parietal cortices of nucleus basalis magnocellularis (NBM)-lesioned rats can ameliorate the spatial memory deficits which result from the lesions; this is accompanied by an increase in staining for a cholinergic marker in the host cerebral cortex (Welner et al., *Brain Research*, **527**: 163, 1990). Since chromaffin cells of the adrenal medulla are known to contain basic fibroblast growth factor (bFGF), a known neuroprotectant, it is of interest to determine whether bFGF may be involved in the graft effects. As a first step, it is necessary to test whether bFGF is present in these grafts at various time points post-grafting. T-maze trained rats were lesioned and then administered either no graft, kidney cell grafts as a control or chromaffin cell grafts, two weeks post-lesioning. Rats were retested on the T-maze at various time points post-graft; subsequently, the density and distribution of bFGF in grafts and the area surrounding the grafts was measured using immunocytochemical techniques. Whereas the host cortex is virtually devoid of staining, bFGF was clearly visible in chromaffin cell grafts. As expected, control kidney cell grafts contained no bFGF. Further, the presence of bFGF in the grafts correlated with groups that showed behavioral recovery of spatial memory, as measured in the T-maze. These results indicate that bFGF may be involved in producing the effects of chromaffin cell grafts to cerebral cortex. (Supported by the Alzheimer Society of Canada and the Medical Research Council of Canada.)

473.3

HYPERTROPHY OF CALBINDIN-D_{28K}-POSITIVE AND PARVALBUMIN-POSITIVE CELLS IN INTRASTRIATAL CORTICAL GRAFTS. E.-C. Liu*[1], S.B. Dunnett[2] and A.M. Graybiel[1]. [1]Dept. Brain and Cognitive Sciences, MIT, Cambridge, MA 02139, USA and [2]Dept. Experimental Psychology, Univ. of Cambridge, Cambridge, CB2 3EB, UK.

Calbindin-D_{28K}- and parvalbumin-positive neurons in the frontal and temporal cortex are severely degenerated in Alzheimer's disease (AD) (Arai et al., 1987; Ichimiya et al., 1988). Degeneration of these cortical neurons is evidenced by the reduction of their numbers and perikaryal sizes. In the present study we report a finding of hypertrophy of calbindin-D_{28K}-positive (CB) and parvalbumin-positive (PV) cells in intrastriatal grafts derived from embryonic cortical primordia. Ibotenate lesions were made unilaterally in the host caudoputamen 7 days prior to grafting. At grafting, cell-suspensions were prepared primarily from ventrolateral or dorsolateral cortical primordia and then were injected into the damaged caudoputamen. Survival times for the grafted rats were 4 to 8 months. CB and PV cells were present in all cortical grafts. Substantial hypertrophy of CB and PV cells was found in the cortical grafts derived from the ventrolateral cortical primordia. They had well-stained processes and were larger than the CB and PV neurons in the host neocortex (ca. 25-56 μm vs. ca. 11-19 μm in diameter). By contrast, very few hypertrophied cells were found in the grafts derived from dorsolateral cortical primordia. The earliest and most affected regions in AD are in the ventrolateral cortical areas including medial temporal cortex in and around the olfactory areas, whereas the motor, somatosensory and primarily visual areas of the dorsolateral cortex are less affected. Our results suggest that the neurodegenerative striatum induced by ibotenate lesions may provide a trophic environment for CB and PV positive neurons in such ventrolateral cortical regions. Further study of the mechanisms underlying this hypertrophy thus has considerable interest for understanding the aetiology of AD. Supported by NSF BNS-8702475.

473.4

FUNCTIONAL AND MORPHOLOGICAL CHARACTERIZATION OF NGF-PRODUCING GRAFTS IMPLANTED INTO LESIONED RAT FIMBRIA-FORNIX. K.L. Eagle *, L.J. Fisher, G.R. Chalmers, and F.H. Gage. Dept. of Neurosciences, UCSD, La Jolla, Ca. 92093

Nerve growth factor (NGF) has been shown to sustain axotomized cholinergic neurons. Previous work from our group investigated the grafting within the rat CNS of primary fibroblasts genetically modified to produce NGF. When suspended in a collagen matrix and placed into the lesion cavity following bilateral fimbria-fornix (FF) ablation, such grafts promoted the survival of medial septal neurons, and induced sprouting of new processes into the graft and the hippocampal formation. We are investigating functional and morphological correlates of these graft effects. In the present study, unilateral FF lesions were used rather than bilateral lesions because the smaller lesion cavity causes less collateral damage to tissue, as well as allowing use of the contralateral side as a control. However, behavioral deficits have been difficult to elicit in unilaterally lesioned rats. We have used a paradigm which allows for behavioral analysis of these rats in the Morris water maze. Fischer rats were given unilateral aspirative lesions of the FF; they then received grafts of either NGF-producing or control ß-Galactosidase (ßGal)-producing fibroblasts, or no graft (lesion-only). After a 4 month recovery period, the rats were trained to find a hidden platform in a fixed location in the maze using spatial cues, given 4 trials a day for 10 days. After all rats had successfully learned the task, the platform location was reversed 180° from its previous location. Performance in learning the new location was measured by distance travelled in each trial before finding the platform. Over the course of 5 days, the mean distance travelled by ßGal-grafted and lesion-only rats was significantly higher than that of normal control rats, while NGF-grafted rats showed no difference from controls. These results suggest that the reversal paradigm exposes behavioral deficits in unilaterally lesioned rats, and that rats grafted with NGF-producing fibroblasts show attenuation of those deficits. Morphological analysis is under way.

473.5

FETAL CORTICAL GRAFTS: EVALUATION OF CONNECTIVITY BETWEEN GRAFTS AND HOST STRIATUM. A. Herranz*, H.E. Cannon-Spoor and W.J.Freed. NIMH Neuroscience Center at St. Elizabeths, Washington, DC 20032.

The major input to the basal ganglia is the excitatory input from the cerebral cortex to the medium spiny striatal neurons. The purpose of this investigation has been to reconstruct the cortical pathway by using fetal cortical tissue transplants. Frontal cortical lesions were performed by aspiration in adult rats. After 15 days animals received grafts of 16-day gestational cortical tissue into the lesion site. Connections from the graft to the host striatum were studied after 6 weeks using immunostaining for neurofilaments and anterograde tracing with DiI. SMI-31 and SMI-35 inmunoreactive neurites were found crossing the interface with corpus callosum and striatum with a relatively high frequency (13.7 and 29.8 per mm of interface respectively), but only rarely entering the adjacent cortex. DiI tracing demonstrated a large number of graft-derived neurites projecting from 0.5 to 0.8 mm into the host caudate putamen. It is concluded that fetal cortical grafts in adult animals send limited projections into the host caudate-putamen.

473.6

FETAL NEOCORTICAL TISSUE SURVIVES TRANSPLANTATION INTO A RAT MODEL OF NEONATAL HYPOXIC-ISCHEMIC BRAIN DAMAGE. M.H. Elsayed, T. F.Myers, C.L .Anderson and A.I. Castro*. Depts. of Pediatrics and Cell Biology, Neurobiology and Anatomy, Loyola Univ. School of Medicine, Maywood, IL 60153.

Several studies demonstrate that fetal neocortical tissue will survive, grow and form axonal connections with the host brain after transplantation into newborn rats with cortical aspiration lesions. In the present study fetal neocortical grafts were transplanted into Long-Evans, black-hooded rats that sustained hypoxic-ischemic brain damage at 7-8 days of age by permanent right common carotid artery occlusion under methoxyfluorane anaesthesia followed by hypoxic exposure (8% oxygen) for 2-2.5 hr. Twenty-one out of 25 animals survived this procedure. One week later, 1-2 mm^3 block neocortical grafts obtained from E13 fetuses were transplanted into the right hemisphere of these animals just caudal to the coronal suture at 1-2 mm from the midline. All animals survived the transplantation procedures and were sacrificed at 2 (n=8), 4 (n=6) and 6 (n=7) weeks after transplantation. Brains were cut frozen at 30 µm and processed for acetylcholinesterase (AchE) immunocytochemistry and Nissl stain. Generalized atrophy and shrinkage of the right cerebral hemisphere was seen in 20/21 animals. Various degrees of ischemic brain lesions with neuronal loss were observed in several structures including the cerebral cortex, thalamus, hippocampus, and striatum. Well developed transplants were found adjacent to the ischemic areas. AchE positive fibers were seen crossing the transplant-host interface providing evidence that the grafts became integrated into the host brain circuitry.

(Supported by NIH Grant 13230 and the Potts Foundation)

473.7

MORPHOLOGIC ASSESSMENT OF MONOAMINERGIC NEURAL TRANSPLANTS TO THE RAT FRONTAL CORTEX. C.E. Sortwell*, J.Sagen and G.D. Pappas. Dept. of Anat. and Cell Biol., Univ. of IL at Chicago, Chicago, IL 60612.

Monoaminergic neural transplants to the rat frontal neocortex have been demonstrated in our laboratory to alleviate depression in animal models. These findings suggest that neural transplants may provide a long term source of monoamines to correct the central imbalance of serotonin (5-HT) and norepinephrine (NE) functioning implicated as the cause of depression. In order to support this suggestion, grafted monoaminergic tissue must survive well and continue to produce high levels of monoamines. Using electron microscopy the ultrastructural characteristics of 5-HT-containing pineal gland tissue, NE-containing adrenal medulla tissue, cografted pineal and adrenal medulla tissue and control sciatic nerve were examined at least six weeks following transplantation. The transplanted pinealocytes maintained their characteristic *in situ* features of numerous and dense mitochondria, a highly convoluted nucleus and the presence of lipid droplets. Similarly, transplanted adrenal chromaffin cells retained their *in situ* cuboidal morphology and displayed numerous granules. Neither type of graft was highly vascularized. The cografted transplants contained collagen matrices separating the pinealocytes from the chromaffin cells. Immunohistochemical studies indicated that the surviving chromaffin cells continued to produce tyrosine hydroxylase and dopamine-ß-hydroxylase and the pinealocytes continued to produce 5-HT. Sixth month old pineal implants also appeared to sprout densely into the surrounding host parenchyma. These results demonstrate that the monoaminergic tissues retain many features of their *in situ* morphology when transplanted to the frontal neocortex of rats.

473.8

ANALYSIS OF ADRENERGIC RECEPTORS IN THE CORTEX SURROUNDING MONOAMINERGIC TRANSPLANTS IN RATS. D. Dougherty*, J.R. Unnerstall, C.E. Sortwell, and J. Sagen. Dept. Anat. and Cell Biol., Univ. IL at Chicago, Chicago, IL 60612.

Previous findings in our laboratory have indicated that the transplantation of monoaminergic tissue into the frontal neocortex of rats can reduce behavioral deficits in rodent depression models. If the reduction in behavioral deficits by the transplants is due to the release of monoamines, it should be blocked by specific monoaminergic antagonists. We have shown that both alpha and beta adrenergic antagonists not only reversed the effects of the transplants on the behavior but in fact exacerbated the deficit, suggesting changes in receptor sensitivity. This study investigates this effect at the receptor level. Monoaminergic tissue (adrenal medulla, pineal gland, or a combination of both) was transplanted into the frontal cortices of separate groups of rats. Sciatic nerve and muscle were transplanted as controls in other groups. Another group was treated with imipramine (15 mg/ml/kg/day; i.p.) for six weeks. Six weeks later animals were tested for behavioral deficits using the forced swimming test. Following testing, a small section (5 x 5 x 7 mm) of cortex was removed from around the transplant of each animal and the transplant was discarded. The remaining tissue underwent receptor binding studies. ^{3}H-prazosin was used as a ligand to investigate alpha-1 binding sites while ^{125}I-iodocyanopindolol was used for beta binding sites. In these initial studies, K_d concentrations of ligand were used to screen for any changes in binding site sensitivity or concentration. No significant differences in binding were seen between transplant and control groups for either ligand (prazosin: 3.6-4.1 fmoles/mg tissue wet wt; pindolol: 4.6-5.4 fmoles/mg tissue wet wt). However, binding was significantly reduced in imipramine treated animals. These results suggest that the previously noted behavioral and pharmacologic changes in the transplanted animals are not associated with a corresponding change in the number of monoaminergic receptor sites.

473.9

Morphological assessment of grafted cortical neurons, J. Lübke*, M. Wood and D. J. Clarke, Dept. of Human Anatomy, University of Oxford, South Parks Road, Oxford OX1 3QX, U.K.

The morphology of cortical neurons grafted near /or into the rat striatum was studied by means of intracellular Lucifer yellow injections in fixed slices. Rat donor cortical tissue (from postnatal day 1 old rats; AO-strain) as well as mouse donor cortical tissue (prenatal E 19; C3H/HE strain) was grafted as solid pieces (a volume of 4 µl of donor tissue was inserted via a Hamilton syringe) in 8-12 week old rats (AO strain). Animals with xenografts were immunosuppressed with an antibody against the interleukin-2 receptor. 40-60 days postoperatively animals were perfused with buffered 2% paraformaldehyde and 1% glutaraldehyde. 120 µm vibratome sections were taken at the site of the transplant. The graft could be identified by a surrounding rim of astrocytes after incubation of the slice in the DNA-stain DAPI for 10 min. Cortical neurons (over 50 neurons in each transplant) were intracellularly filled with Lucifer yellow, DAB-photoconverted, embedded in resin, photographed and drawn with the aid of a camera lucida. In addition, tissue was prepared for electron microscopy to study the ultrastructural morphology and synaptic inputs of the injected neurons. In general, no cortical lamination could be observed in the grafted cortical tissue, but neurons were loosely packed throughout the graft. Two major cell types could be identified. The majority were spiny neurons (95%), of which some could be described as pyramidal-like with somata, ranging in size between 10-20 µm in diameter. The remaining 5% resembled non-spiny neurons with a basket-like morphology. Dendrites of both cell types were never seen to cross the astrocytic border, but some main axons and axonal collaterals were found to leave the graft. On the basis of light microscopical observations no difference was found between mouse and rat grafted cortical neurons. In conclusion, we have shown that grafted neurons retain some characteristic features of cortical neurons, although there appears to be a greater preponderance of spiny neurons. This may reflect an immaturity of the graft tissue or a response to the striatal environment.

474.1

BEHAVIORAL IMPROVEMENTS PRODUCED BY INTRAOCULAR GRAFTS OF LIVING RETINAL CELLS ARE NOT DUPLICATED BY GRAFTS OF OTHER NEURAL CELLS OR CELL HOMOGENATES. M. del Cerro*, G. Bowen, J. Ison, D. Grover, E. Lazar, and C. del Cerro. Depts. of Neurobiology, Psychology, & Ophthalmology, University of Rochester, Rochester, N. Y., 14642.

In normal animals light flashes suppress acoustic startle reflexes. In light blinded animals inhibition is lost and replaced at certain intervals by an anomalous peak of excitation, which is not seen in enucleated rats. Fischer 344 rats (N=18) were blinded by exposure to continuous fluorescent light and half of the animals were grafted with dissociated fetal rat retinal cell into 1 eye. Compared to non-grafted controls, grafted rats showed both statistically significant reductions of anomalous facilitation and increases in inhibition (Exp. 1: del Cerro et al., NeuroReps, 2:529,1991). To test for the specificity of this effect, blinded rats were grafted with fetal retinal cell homogenates (Exp. 2, N=20), or dissociated perinatal cerebellar cells (Exp. 3, N = 19), with control rats left untouched in each experiment. In contrast to the grafted rats of Exp. 1, the grafted animals in Exps. 2 and 3 were not different from their controls. Histologically, clusters of photoreceptor cells were consistently found in the retinae of Exp. 1 animals, but not on those of Exps. 2 or 3. We conclude that only retinal grafts of living fetal retinal cells partially repair the blindness caused by the action of continuous light exposure.

(Supported by EY 05262, the Rochester Eye Bank, and private gifts).

474.2

A SMALL ANIMAL MODEL OF HIV-1 INFECTION OF NEURAL TISSUE: INTRA-OCULAR XENOGRAFTS OF HUMAN FETAL RETINA AND BRAIN. E. Lazar*, T. Cvetkovich, H. Gendelman[1], B. Blumberg, K. Dzenko, M. del Cerro, C. del Cerro, and L. Epstein. University of Rochester Medical School, Rochester, NY, 14642. and Walter Reed Army Institute of Research[1], Washington, D.C. 20307-5100.

Human immunodeficiency virus type 1 (HIV-1) infection is highly specific for its human host. To study HIV-1 infection of the human nervous system, outside of the human body, we have established a small animal model of the disease. Second trimester human fetal brain or neural retina is transplanted into the anterior chamber of the eye of adult rats immunosuppressed with Cyclosporine A. Tissue procurement was in strict accordance with established guidelines. The human xenografts vascularize, form a blood-brain-barrier, and differentiate forming neurons and glia. The xenografts can be infected with cell free HIV-1 or with HIV-1 infected human monocytes. Analysis by polymerase chain reaction (PCR) revealed HIV-1 sequences in DNA extracted from xenograft tissue exposed to HIV-1 virions, and in-situ hybridization demonstrates HIV-1 mRNA localized in macrophages and multinucleated giant cells. Pathological damage was observed only in neural xenografts containing HIV-1 infected human monocytes supporting hypothesis that these cells are neurotoxic. Interestingly, the host retinal neurons were unaffected. This animal model allows the study of the direct and indirect effects of HIV-1 infection on developing human fetal neural tissues, and may be useful in the evaluation of antiviral therapies, which must target HIV-1 brain infection.

Supported by AI 32305-01, EY 09217-01, and generous private gifts.

474.3

HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISTICS OF Y79 RETINOBLASTOMAS IN VIVO. D. DiLoreto Jr.*, G. M. Seigel, D.H. Brooks, E. Lazar, D.A. Grover, C. del Cerro, M. del Cerro, and G. Chader[1]. Department of Neurobiology, University of Rochester Medical School, Rochester, NY, 14642 and the National Institutes of Health[1] Bethesda, MD.

Retinoblastoma is the most common intraocular neoplasia of childhood. Continuous human retinoblastoma cell lines, such as Y79, have been used primarily for analysis of tumoral cell growth and phenotype in vitro. Recently, we developed a useful in vivo model of retinoblastoma which involves subretinal transplantation of Y79 cells into immunosuppressed Fischer 344 rats (ARVO 92). Thirty to sixty days following transplantation, we examined the histochemical properties of Y79 tumor-bearing retinal tissue. The tumors are formed by fast-dividing, pleomorphic cells which invade the host retina and vitreal cavity. These cells were immunoreactive for the neuronal markers PGP 9.5 and neuron-specific enolase, but remained negative for S-antigen. These tumors showed no sign of a glial cell phenotype in vivo, as the only GFAP immunoreactivity present was comprised of host-derived Muller cell fibers which traversed layers of tumoral tissue. Our results show that intraocularly grafted Y79 cells survive, actively grow, and exhibit a primitive neuronal phenotype in vivo. Furthermore, these cells, even as xenografts, fully retain the features of poorly differentiated, highly invasive, neuro-tumoral cells.

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474.4

ENHANCEMENT OF SURVIVAL OF INTRAOCULAR NEURAL RETINAL GRAFTS BY ANTIGEN-SPECIFIC IMMUNE DEVIATION. Luke Qi Jiang* and J. Wayne Streilein, Department of Microbiology and Immunology, University of Miami, Miami, FL, USA.

The ultimate goal of our studies is to implant functional retinal grafts into the blind eye to restore vision. In order to explore the possibility of using immunological manipulation to enhance survival of intraocular retinal grafts, we implanted neural retinal grafts from newborn BALB/c mice into one eye of C57BL/6 mice, which induces immune deviation (ACAID). In parallel experiments, conventional immunity was induced by implanting similar grafts into the subconjunctival space. Control C57BL/6 mice received a sham operation. Two weeks later, a second graft (identical to the first) was implanted into the contralateral eye of all recipients. The results revealed that all control mice rejected the test graft by 36 pid, at which time the graft size was reduced to 24% of original size. Mice which received prior SCon grafts rejected BALB/c retinal test grafts sooner. In contrast, mice which developed ACAID maintained their retinal test graft for an extended time. In this ACAID group, the graft in the first eye retained 60% of its original size by 50 pid and the second graft in the contralateral eye maintained 63% of its original size by 36 pid. Microscopic examination showed the graft in the ACAID group to have well-differentiated cell layers which resembled the structure of the normal retina. In contrast, AC retinal grafts displayed obvious histologic regression in both control mice and in mice which developed a conventional immunity. These results suggest that 1) antigen-specific suppression (ACAID) can be used to enhance the survival of intraocular neural retinal allografts and 2) immunological manipulation may provide a novel way to prevent rejection of intraocular neural retina grafts.

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474.5

SYSTEMATIC EXPRESSION OF CELL TYPE-SPECIFIC NEURAL GENES IN DEVELOPING AND MATURE TRANSPLANTED RETINAE. J.D. Radel*, F. Hoover†, D. Goldman† and M. Hankin§. *Univ. Pittsburgh, Pittsburgh, PA, †Univ. Michigan, Ann Arbor, MI and §Med. College of Ohio, Toledo, OH.

Retinae transplanted to intracranial locations develop many properties characteristic of normal retina and form anatomically and functionally appropriate connections in the host brain. While resembling normal retinae, the degree to which basic cell populations are represented in transplants (TPs) has not been addressed fully. Embryonic rat retinae (E12-15) were transplanted into newborn rats. After various post-transplantation (PT) periods (4-180 days), animals were perfused and the brains sectioned. Recipients with mature transplants were tested for pupillary responses to assess functional connections or stained with silver to show TP-derived projections to the host superior colliculus. Gene expression was assayed in the transplants using in situ hybridization. Two classes of genes were studied: (1) those encoding proteins involved in neural communication represented by the nicotinic acetylcholine receptor (nAChR) α-3 and β-3 subunits, glutamate receptor (GluR3) subunit, and opsin; and (2) a gene encoding a protein involved in axonal outgrowth (GAP-43). At 4dPT, GAP-43 and β-3 nAChR subunit gene expression was seen at the vitreal margin of the transplant, suggesting the presence of differentiated retinal ganglion cells. At 180dPT in situ hybridizations demonstrated that all genes examined were expressed in a normal spatial fashion. We detected RNA levels of GAP-43, nAChR α-3 and β-3 subunit genes in a heterogeneous population of soma in the ganglion cell layer (GCL) and inner part of the inner nuclear layer (INL). GluR3 RNA was expressed by >50% of cells in the GCL, suggesting that both ganglion and displaced amacrine cells express this gene. In addition, it appeared that the majority of INL soma expressed their GluR3 gene. Unlike normal adult retinae, however, β-3 and GluR3 gene expression was detected in cells located ectopically in the inner plexiform layer, perhaps the result of abnormal cell migration. Finally, opsin mRNA was expressed solely by rod photoreceptors in the outer nuclear layer. In summary we have assayed for the presence of cell-type specific genes in transplanted retinal neurons and report that these genes are expressed in a spatial and temporal pattern similar to the normal retina.

474.6

IMMUNOLOCALIZATION OF OLFACTORY MARKER PROTEIN (OMP), GROWTH ASSOCIATED PROTEIN (GAP-43), AND PLATELET DERIVED GROWTH FACTOR B-CHAIN (PDGF-B) IN THE TRANSPLANTED RAT OLFACTORY BULB. [1]I.N. Kott*, [3]E.W. Raines, [3]R. Ross, [3]M. Sasahara and [1,2]L.E. Westrum. Depts. of [1]Neurol. Surg., [2]Biol. Struct, and [3]Pathol., Univ. of Wash., Seattle, WA 98195.

We are examining the development of transplanted rat olfactory bulbs (OBs) using antibodies against OMP, GAP-43 and PDGF-B. OMP identifies mature primary olfactory neurons (ONs), GAP-43 is a marker for growing axons and PDGF-B is heavily localized in the specialized glia that ensheath the ON. Donor OBs are taken from fetuses of embryonic days 14-15 and transplanted directly into the cavity produced by removal of an OB in one day postnatal rats of the same strain.

Adjacent 8 μm paraffin sections are examined using the three antibodies. Both OMP and GAP-43 are common to many large fiber bundles within the transplanted OB. Fiber bundles in sections identified with GAP-43 antibody (AB) have sharply defined borders and are slightly smaller than homologous bundles in adjacent sections reactive with OMP AB. The borders of OMP reactive fiber bundles are diffuse and may represent collateral branches around the bundle. In adjacent sections reactive for PDGF-B, homologous bundles are often (but not always) surrounded or ensheathed by reactive cell bodies and fibers. Scattered PDGF-B reactivity is also seen within some fiber bundles. The relationships between fiber bundles identified with OMP, GAP-43 and PDGF-B AB remain similar at survival times ranging from 2 weeks to several months and generally recapitulate the patterns, but not the overall architecture, seen in the normal OB. Antibodies kindly provided by Dr. Frank Margolis (OMP) and Mochida Pharmaceutical Co. (PDGF-B).

(Supported by NIH Grants NS09678 and HL18645. L.E.W. is an affiliate of the CDMRC).

474.7

OLFACTORY NERVE REINNERVATION PATTERNS CORRELATE WITH BEHAVIORAL RECOVERY IN OLFACTORY BULB TRANSPLANT AND OLFACTORY BULB LESION RATS. [1,2]K.R. Hendricks*, [2]J.N. Kott, [3]M. Gooden, [3]S. Harman, [3]M.E. Hanson, and [2,3]L.E. Westrum. Depts. of [1]Psychol., [2]Neurol. Surg., and [3]Biol. Struct., University of Washington, Seattle, WA 98195.

We are examining the anatomical correlates involved with potential recovery of function in the rat under the following conditions: neonatal olfactory bulb (OB) transplants (nOBT), neonatal olfactory bulb lesions (nOBL), and adult OBLs (aOBL). The nOBT and nOBL animals can eventually find hidden cookies as well as normal rats but the aOBL rat does not show recovery. Since the aOBL rat does not display general learning disabilities in passive avoidance tests, the loss of cookie-finding ability appears to be specific to olfactory ability. Histological analysis, blind of the behavioral results, was used to place the animals into groups. Techniques included: olfactory marker protein (OMP)* immunocytochemisty for olfactory nerve (ON) penetration, cell and fiber staining, and tritiated thymidine autoradiography for donor-labeled transplant verification. All animals with incomplete OBLs were excluded from the study. Innervation of brain tissues by ON was achieved only in the nOBL and nOBT but not the aOBL and correlated perfectly with recovery of olfactory ability. Analysis of ON fiber reinnervation patterns in the nOBL rat revealed that ON targets included olfactory peduncle (OP) and olfactory cortex (OC). Analysis of nOBT rats revealed that ON innervation of OBT was usually accompanied by innervation of OP and OC. These results suggest that in the absence of secondary OB neurons, ON connects directly with "tertiary" neurons in the pathway and that this novel connection in the nOBL rat may be responsible for recovery of olfactory ability. It is not possible at present to say whether it is ON innervation of OBT or OP/OC or both that is responsible for recovery of olfactory ability in the nOBT rat. (Supported by NIH Grant NS 09678. L.E.W. is an affiliate of the CDMRC). *OMP was kindly provided by Dr. F.L. Margolis.

474.8

RECONNECTIVITY OF TRANSPLANTED OLFACTORY BULBS IN RATS. [1]M.E. Hanson, [1]J.N. Kott, [1,3]K.R. Hendricks, [4,5]D.F. Farrell* and [1,2,5]L.E. Westrum. Depts. of [1]Neurol. Surg., [2]Biol. Struct., [3]Psychol., [4]Medicine (Neurol.), and [5]Child Devel. & Ment. Retard. Ctr., University of Washington, Seattle, WA 98195.

Using lesion-degeneration methods it has previously been shown that successfully transplanted (TX) olfactory bulbs (OB) send their axons to appropriate target areas in the adult host brain (Westrum et al., 1990 Neurosci. Lett.). We are using WGA-HRP transport to study reconnectivity in the developing TX OBs in Sprague-Dawley rats. Time-mated dams received subcutaneous injections of tritiated thymidine on embryonic days (E) 12-14. OBs from fetal rat donors of E 14-15 were immediately grafted into neonatal rats in the site from which the host OB had been removed. Following survival times of 2 weeks and longer, 0.1 µl of a 2 per cent WGA-HRP solution is injected into the TX OB and subjects are perfused after 24 hours. Alternate frozen sagittal sections are processed using TMB as the chromagen or olfactory marker protein (OMP)* immunolocalization. Autoradiography will be included to verify that the injections remained within the TX OB. WGA-HRP transport is seen in fibers from the TX OB into layer I of the host anterior olfactory nucleus (AON) and pyriform cortex (PC) and in cell bodies in layers II and III of the AON and PC. OMP material shows that primary fibers are seen within the TX OB and in the peduncle. These preliminary findings reaffirm that the axons from a TX OB make connections with some appropriate areas of the host brain, and also suggest that axons from cells in the target areas of the host brain innervate the TX OB. Supported by NIH Grants NS09678 and HD02274. LEW is a research affiliate of the CDMRC. *Anti-OMP kindly provided by Dr. Frank Margolis.

474.9

SYNAPTIC CONNECTIVITY OF SEROTONIN RAPHE TRANSPLANTS IN THE SUPRACHIASMATIC AND SUPRAOPTIC NUCLEI IN ADULT RATS. O. Bosler[1]*, S. Boulaïch[1], M. Geffard[2] and A. Daszuta[3]. [1]Lab. de Neurobiologie and [3]Lab. de Neurobiologie Cellulaire et Fonctionnelle, CNRS, Marseille, and [2] Lab. d'Immunologie et Pathologie, Univ. Bordeaux II, France.

We have previously reported that cell suspensions of fetal mesencephalic raphe, transplanted at mid-distance between the suprachiasmatic nucleus (SCN) and the supraoptic nucleus (SON) in adult rats after intraventricular administration of 5,7-dihydroxytryptamine, induced partial 5-HT reinnervation of the SCN vs hyperinnervation of the SON. We have further investigated the ultrastructural relationships of reinnervating vs normal 5-HT axon terminals in both nuclei following immunodetection and systematic sampling on ribbons of 4-12 consecutive sections. About 48% of the 5-HT positive terminals photographed in the ventral part of the normal SCN (n=77, from 4 animals), where they normally predominate, showed synaptic membrane specializations. Graft-derived 5-HT terminals in the same portion of the SCN (n=88, from 4 animals) showed a comparable synaptic incidence (around 46%). The frequency with which 5-HT varicosities made synaptic contacts was also found to be equally high in the SON from normal and grafted rats (more than 40%). These results indicate that the extent of reinnervation after grafting are not solely dependent on the mode of termination of normal 5-HT axons in the denervated territory. Target-specific trophic influences, i.e. target-derived stimulatory or inhibitory signals, should also be determinant in promoting graft-induced reinnervation. Such influences, together with innate programing of transplanted neurons committed to supply 5-HT fibers to the SCN and/or the SON, could also account for the fact that, irrespective of the extent of reinnervation (hypo- or hyperinnervation), the new 5-HT fibers in both nuclei re-established the same relational features as in control tissue.

474.10

THREE-DIMENSIONAL EXTRACELLULAR MATRIX AS PRIMARY CULTURE SYSTEM FOR FETAL HAMSTER HYPOTHALAMIC SCN CELLS. K.Z. Doll, P.W. Coates†, and M.N. Lehman*. Dept. Anat. & Cell Biol., Univ. Cincinnati Coll. Med., Cincinnati, OH 45267; †Dept. Cell Biol. & Anat., Texas Tech Univ. HSC Sch. Med., Lubbock, TX, 79430.

Transplants of the fetal hamster hypothalamic suprachiasmatic nucleus (SCN), the site of a circadian pacemaker, restore rhythmic behavior to SCN-lesioned hamsters (Lehman et al., J. Neurosci. 7:1626). We explored the use of a three-dimensional (3-D) extracellular matrix consisting of hydrated native rat tail collagen (type 1) as an alternative method of culturing SCN cells for use in grafting studies. Fetal (E13) anterior hypothalamic cells were dissociated by a combination of gentle trituration and enzymatic treatment. Cells were rinsed and plated at 5 x10^5 cells/ml/16mm well on either the 3-D matrix or control poly-L-lysine coated coverslips. At various intervals following seeding (1, 4, 7 and 14 days) cultures were rinsed with serum-free media and fixed with 4% paraformaldehyde. The 3-D matrix was processed for immunocytochemistry (Dudley et al., Peptides 10:1205) to detect a variety of neuronal and glial markers. 3-D matrices contained far greater numbers of isolated neurons and few glia in contrast to the clusters of neurons and glia seen in poly-L-lysine cultures. A 4 days positive staining for neuron-specific tubulin (class III, type II) was abundant; at 14 days staining was decreased. Neurophysin immunostaining suggested that SCN neurons survived in the 3-D matrix. The matrix may be useful in providing a scaffolding for neuronal cultures to be used for grafting studies, including those in which isolated SCN neurons are tested for their ability to restore rhythmicity. [Supported by NIH R01 NS28175 to M.N.L.]

474.11

TRANSPLANTATION OF FETAL SUPRACHIASMATIC NUCLEI (SCN) INTO MIDDLE-AGED RATS INFLUENCES LIGHT-INDUCED C-FOS EXPRESSION IN THE HOST SCN A.Cai[1], J.M. Lloyd[1], M.N.Lehman[2], and P.M. Wise[1]*. Dept. Physiology, U. Maryland Sch. Med., Baltimore, MD 21201[1] and Dept. Anatomy & Cell Biology, U. Cincinnati Coll. Med., Cincinnati, OH 45267[2].

Aging disrupts multiple diurnal rhythms that are regulated by the SCN. c-Fos expression in the SCN is induced by light, and may be part of the mechanism for photic entrainment of the circadian pacemaker in the SCN. The purpose of this study was to determine whether transplantation of fetal SCN into middle-aged rats can influence light-induced c-Fos expression in the host SCN. We transplanted fetal SCN tissue into the third ventricle of middle-aged female constant estrous rats (9-12 mo). Sham operated middle-aged and young (3-4 mo) rats served as controls. After one month, rats were ovariectomized (day 0) and treated with estradiol (day 7) to maintain a constant and similar hormone environment in both age groups. On day 11, rats were transcardially perfused within 90 min before and after lights on. Brains were prepared for immunocytochemical localization of c-Fos (Cambridge Research, OA-11-823). Young rats exhibited virtually no c-Fos expression in the SCN prior to lights on, and a dramatic increase in c-Fos by 90 min. In contrast, c-Fos-containing cells are evident in some middle-aged rats prior to lights on. Transplantation of the fetal SCN into middle-aged rats appears to reinstate the pattern of c-Fos expression observed in young rats; that is, no c-Fos expression was detectable before lights on. The results suggest a possible trophic influence of the donor SCN on the host SCN function. Supported by NIH AGO2224.

475.1

NANOASSAY FOR NEUROTOXIN–TARGET RECEPTOR BINDING BY FLUORESCENT LIGAND EXCLUSION ANALYSIS (FLEA). D. Yoshikami*. Dept. of Biology, Univ. of Utah, Salt Lake Cty, UT 84112

A simple and efficient approach has been developed to assess the binding of ligand with receptor in solution. The approach is called fluorescent ligand exclusion analysis (FLEA), and requires: 1) a fluorescently labeled ligand (**fL**); 2) a microscopic speck of a steric exclusion matrix, such as a polyacrylamide or dextran bead normally used for gel filtration chromatography, which is permeable to **fL** but not to receptor (**R**); and 3) a fluorescence microscope fitted with a video camera or photodetector to measure fluorescence within the semipermeable matrix. Whereas **fL** can permeate the matrix, **fL** bound to **R** cannot; hence, fluorescence within the matrix provides a direct measure of the free **fL** concentration which can be used to determine the equilibrium binding constant.

By use of a flow cell (internal volume, ≤ 150 nanoliters) containing an immobilized steric exclusion bead (diameter, ~ 50 microns), different solutions can be readily FLEA-assayed in quick succession (≤ 1 minute/sample). In addition, since **fL** can diffuse into (and out of) the bead relatively rapidly (time constant of seconds), the kinetics of binding of **fL** to **R** may also be examined. Thus in-line, as well as near-real time, binding assays can be performed by FLEA. Only nanomolar concentrations of reagents in nanoliter volumes are required, so FLEA is referred to as a "nanoassay."

The practicality of FLEA has been verified with experiments involving fluorescently labeled α-Bungarotoxin used in conjunction with acetylcholine receptors from *Torpedo*, and we are exploring the feasibility of examining other neurotoxin–target receptor interactions with FLEA. It should be noted that FLEA may also be used to examine the interactions of other biological molecules, including those of antibody with antigen, enzyme with substrate, and protein with each other as well as with nucleic acid.

475.2

IMAGING CELL VOLUME CHANGES IN RAT HIPPOCAMPAL SLICES. R.D. Andrew* and B.A. MacVicar. Anatomy Department, Queen's University, Kingston, Ontario and Department of Medical Physiology, University of Calgary, Calgary, Alberta.

Brain cell swelling is a consequence of seizure, ischemia or excessive rehydration therapy yet the process is difficult to study unless cells are isolated. By imaging light transmittance in the hippocampal slice, we studied how the signal is affected by osmotic manipulation of cell swelling where neuron-glia relationships are intact. Brief exposure to 20 mM K^+ (1-2 min, n=6) or hypo-osmotic saline (4-6 min, n=16) consistently and reversibly increased light transmittance. The increase was greatest in CA1 dendritic regions. Synaptic blockade using zero-Ca^{2+} saline did not alter the transmittance increases. In hypo-osmotic saline (-40 mOsm), an increase in the CA1 population spike (PS) evoked from Schaffer collaterals developed concomittantly with the transmittance increase. Both findings are consistent with a swelling-induced reduction of extracellular space. Hyperosmotic saline (+40 mOsm) using impermeant mannitol (n=9), but not permeant glycerol (n=5), consistently reduced light transmittance and PS amplitude. Recovery from mannitol (+40 mOsm) was markedly slower than from -40 mOsm ACSF although recovery could be accelerated by brief application of hypo-osmotic saline.

We conclude that increased light transmittance results primarily from reduced light scattering caused by cell swelling, particularly by astrocytes. This imaging technique may prove useful in examining brain cell swelling in epileptiform and excitotoxic states.

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475.3

GLIAL SWELLING IN CA1 STRATUM RADIATUM IN RESPONSE TO OSMOTIC AND ELECTRICAL STIMULATION. P. Osehobo*[1], B.A. MacVicar[2] and R.D. Andrew[1]. Anatomy Department[1], Queen's University, Kingston, Ontario and Department of Medical Physiology[2], University of Calgary, Calgary, Alberta.

Light transmittance in CA1 stratum radiatum (st. rad.) of rat hippocampal slices increases dramatically upon stimulation of Schaffer (Sch) collaterals (MacVicar and Hochman, 1991) or when saline osmolality is lowered (Andrew and MacVicar, this meeting). In both cases, we suspected that neuronal and/or glial swelling reduced light scattering, thus increasing transmittance. Previously we've shown that neither pyramidal cell intrinsic properties nor synaptic input were altered by lowering osmolality. Glial resting potentials in st. rad. in this study were unaffected by -40 mOsm ACSF (n=10) or -80 mOsm (n=4). A stimulus train to Sch. collaterals (10-100 Hz, 0.5 - 2.0 s) consistently increased light transmittance and depolarized glia in st. rad. The amplitude of the depolarization was increased in -40 mOsm ACSF (n=15) probably due to increased K^+ accumulation in the reduced extracellular space. Antidromic stimulation from alveus in ϕ Ca^{2+} saline caused only minor increases in light transmittance in CA1 stratum pyramidale where glia are sparce (n=4). Apparently rapid firing by neuronal somata without dendritic activation caused comparatively small increases in cell volume.

We conclude that glial swelling in regions of dendritic depolarization underlies the large change in light transmittance observed during orthodromic stimulation. However glial swelling, in itself, does not alter glial resting potential in hippocampal slices.

475.4

AFFERENT CONNECTIONS OF NUCLEUS RAPHE PALLIDUS. A STUDY WITH FLUORESCENT TRACERS IN RATS. M.I. Nogueira, L.E. Ribeiro do Valle*,Dep. de Fisiologia e Biofísica, and J.C.Bittencourt, Dep. Anatomia, Instituto de Ciências Biomédicas, USP. SP. 05508 - Brasil.

Nucleus raphe pallidus (NRP) has been implicated in the modulation of visceral and somatic activities. It is an important source of serotonergic innervation to the brain stem and the spinal cord. We are investigating in detail its afferent connections.

Fluorogold was injected iontophoretically in the NRP of male rats, after a survival time of 30 days the animals were sacrified and their brain processed. Marked cell bodies and dendrites were found mainly in the hypothalamus (preoptic, anterior and lateral areas), in the colliculus superior , in eye related movment nuclei, pretectal areas, in the central gray, in vestibular and coclear nuclei and in the spinal cord layer 10 and 7. These results should be confirmed by using an anterograde tracer technique.

The present findings contribute to a better understanding of the functional role of the NRP.

Grants from CNPq and FAPESP.

475.5

"Miniruby": A Fluorescent Biocytin Compound for Intracellular Labeling Of Neurons In Fixed Slices. W. -L. Liu, M. M. Behbehani[1] and M. El-Etri*, M. T. Shipley. Depts. of Anatomy & Cell Biology and [1]Physiol & Biophysics, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

Intracellular labeling can be done not only in living, but also in fixed slices. Biocytin is useful for intracellular injection because it is highly soluble, has high affinity for avidin and high electrophoretic mobility. In fixed slices, membrane potential cannot be used to signify that a cell is impaled. Thus, it is necessary to inject cells with a fluorescent compound so that impalement and filling can be visualized. As biocytin does not fluoresce it cannot be used in fixed slices. Here, we report that a biocytin compound, "miniruby" (MR), dextran(MW~10K)-tetramethylrhodamine-biocytin, is a useful intracellular marker for injecting neurons in fixed slices.

Fixed adult slices (300-400 μm)of olfactory bulb, piriform cortex and periaqueductal gray were used. Slices were stained by 0.001% ethidium bromide so that cell bodies could be visualized under fluorescent illumination. A cell was impaled with a pipette containing 3-5% MR; positive pulsed constant current (2-5 nA ; 300-400 msec on- 600-700 msec off; ~10 min) was applied until the fine dendrites were brightly fluorescent. Slices were post-fixed for 6-12 hours, then reacted by ABC-DAB.

MR has several advantages: (1) It is easy to visualize the electrode in relation to the cell bodies. (2) The histochemical staining is very sensitive and is light stable. (3) Injected neurons, dendrites and axons are well visualized by brightfield microscopy. In addition, it should be possible to analyze MR filled cells at the EM level.

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475.6

IMMUNOPEROXIDASE LABELING OF FLUORO-RUBY H. T. Chang* Department of Anatomy & Neurobiology, The University of Tennessee, Memphis, College of Medicine, 875 Monroe Ave., Memphis, TN 38163

Fluoro-Ruby (tetramethylrhodamine - dextran amine conjugate, Molecular Probe # D-1817) has been shown recently to be an effective anterograde tracer readily visible in brain sections using conventional fluorescence microscopy. However, several inherent disadvantages have limited the use of Fluoro-Ruby by many neuroanatomists. For example, since the fine fluorescent signals are difficult to resolve at low magnifications, the analysis of the macroscopic distribution of Fluoro-Ruby labeled fibers within tissue sections has been difficult. This is in contrast to the ease of using low magnification dark-field microscopy to analyze the distribution of fibers labeled by either the autoradiographic or the immunoperoxidase methods. On the other hand, high magnification analyses of Fluoro-Ruby labeled fibers also have been problematic due to the labile nature of the fluorescent signal and the lack of a convenient method to render the labeled fibers electron dense for electron microscopic analysis. In this study, I will present results which show that most of these inherent limitations of Fluoro-Ruby can be overcome by conventional immunohistochemical procedures using primary antisera raised against tetramethylrhodamine, the fluorescent moiety of the Fluoro-Ruby. Problems of false negative results associated with anterograde tracing methods revealed during the course of this study will also be discussed during the Annual Meeting.

(Supported by NIH Grant AG05944)

475.7

BEHAVIOR OF LIPOPHILIC DYES IN THE EMBRYONIC MAMMALIAN VISUAL SYSTEM. J. Kelly Johnson[1] & Vivien A. Casagrande.[1,2] Depts. of Cell Biol.[1] & Psychol.[2], Vanderbilt Univ., Nashville, TN 37232-2175.

Lipophilic dyes have been used in studies of neuronal pathways since Godement et al (Development, 1987, 101:697-713). However, continued reports of cell:cell (transcellular) movement of the dyes have been made by a number of investigators since this time. The embryonic rat visual system was used to examine what conditions affect cell:cell movement of these dyes and which cell types and cellular interactions are involved.

Midgestation (E15.5) rat embryos were immersion fixed in buffered paraformaldehyde. DiI, DiA, or 4di10ASP (Molecular Probes; Eugene, OR) were placed in one eyecup, covering the optic disk. Brains were dark incubated 2-12 weeks at room temperature, sectioned biweekly, and mounted and photographed the same day.

All dyes label similar cell types, but at different rates (DiI/DiA at ~0.18 mm/day, 4di10ASP at ~0.21 mm/day). After incubating 2 wk , 1) DiI and DiA label a few optic axons from the opposite eye, 2) DiI labels a few, and DiA many, midline glia at the optic chiasm, 3) neither DiI, nor DiA, label glia along the optic tracts, 4) 4di10ASP labels a large number of optic axons, ganglion and Müller cells in the opposite eye, most midline glia at the optic chiasm and glia along both optic tracts. After 8 weeks, 4di10ASP labels optic radiations into cortex, and a glial compartment dividing dorsal and ventral thalamus. All dyes label more of the same cell types with longer incubations.

These results suggest that, 1) the dyes move cell:cell via specialized contacts between axons, and axons and glia, within the optic chiasm, diencephalon, and retina, and 2) the amount of transcellular labeling correlates with the rate of diffusion and length of incubation.

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475.8

RETROGRADE LABELING OF EMBRYONIC RAT SEPTAL NEURONS FOLLOWING IN VITRO HIPPOCAMPAL INJECTIONS OF FLUORESCENT TRACERS. B. Webb*, M.B. Heaton, M.A. King, and D.W. Walker. University of Florida College of Medicine and V.A. Medical Center, Gainesville, FL, 32610.

A technique was developed in which tracer substances were injected into isolated brains and retrogradely labeled cells were subsequently analyzed. Specifically, septal neurons that project to the hippocampus were retrogradely labeled using FITC, fluorescent microspheres, and Di-I in embryonic rat brains on days E18, E21, and P1. The embryonic rat brains were removed and immediately immersed in oxygenated Tyrode's Balanced Salt Solution. Dye was injected bilaterally throughout the rostral-caudal extent of each hippocampus. Each injection (total of 4/hippocampus) consisted of 0.1 ul of fluorescent dye delivered through a micropippette. Following 17 h of transport time in oxygenated Tyrode's, each medial septum was removed. The neurons were dissociated (DNAase and trypsin) and cultured (L-15 media, with fetal bovine serum (FBS), heat inactivated horse and FBS, glutamine, penicillin-streptomycin, and fungizone) in 35 mm dishes at medium density (1 septum/2 dishes). A hole had been drilled in the bottom of the dishes and covered by a coverslip with a grid. The neurons were followed daily with a low light video system (intensified CCD cameras) and images were digiitized daily for analysis. The number of labeled septal-hippocampal neurons increased progressively from E18 to P1 which is consistent with developmental studies of septo-hippocampal projections. The neurons showed considerable process outgrowth and differentiation during culture. The cultures were fixed (4%paraformaldehyde) and stored in phosphate buffer for immunocytochemical analysis. This technique will allow the identification and experimental manipulation of a subpopulation of medial septal neurons, in particular those septal neurons forming the septohippocampal pathway. Supported by the Veterans Administration, NIAAA AA00200, and A.B.M.R.F.

475.9

FLUORO-GOLD REVISITED: TOXICOLOGICAL AND PHARMACOLOGICAL PROPERTIES. L.C. Schmued[1]*, J.F. Bowyer[1], C. Beltramino[2], H.W. Broening[1] and W. Slikker Jr.[1] [1]Division of Neurotoxicology, NCTR/FDA Jefferson AR 75209-9502 and [2]Dept. of ENT, Univ. of Virginia, Charlottesville, VA 22908.

Since its introduction in 1985, Fluoro-Gold (FG, hydroxystilbamidine) has been widely used as a retrogradely transported fluorescent tracer to reveal neuronal connectivity. However, little is known regarding its neurotoxic or pharmacological properties. To address its neurotoxic potential, FG was injected into the rat striatum at different concentrations (2.5-10%), volumes (100-250 ηl), and vehicles (saline and .1M cacodylate). Survival intervals ranged from one week to one day. The tissue was processed according to a modified de Olmos' cupric-silver stain for degeneration. At the injection site degenerating neurons and neuropil could be observed at higher dye concentrations and volumes. Necrotic debris without any cellular profiles is seen at survival times exceeding 2 days. Examination of the substantia nigra indicated degenerating axon terminals in some cases, however no neurons retrogradely labeled with FG also stained for degeneration. The second phase of this study involved looking at the effects of FG on the dopamine (DA) release from striatal slices *in vitro*. Slices were first incubated in a medium containing [^{3}H]DA, and then exposed to 10^{-6} to 10^{-4}M [FG]. FG completely blocked the basal, 15 mM K^+-, and 1 mM glutamate- evoked [^{3}H]DA release in a concentration dependent manner. Basal and glutamate-evoked [^{3}H]DA release were inhibited by lower [FG] than K^+-evoked release. These studies indicate that high [FG] can be neurotoxic. However, since FG can also inhibit neurotransmitter release, it might be neuroprotective in some instances.

475.10

LABELING OF SPECIFIC CELL TYPES IN THE RAT CNS BY INTRAVENTRICULAR INJECTIONS OF BIOCYTIN. A.J. McDonald* and F. Mascagni. Dept. of Cell Biology and Neuroscience, Univ. of South Carolina Sch. of Med. Columbia, S.C. 29208.

Recent experiments performed in this laboratory have demonstrated that large injections of biocytin into the lateral ventricle (or brain) produce labeling of particular neuronal subpopulations in the rat CNS. Rats were perfused 24 hr following injection and brain sections were processed with the ABC technique. In many cases the staining of neurons was very complete and resembled that obtained with the Golgi technique. Regions containing labeled cells included the olfactory bulb, cerebral cortex, hippocampus, amygdala, striatum, hypothalamus, tectum, cerebellar cortex, and dorsal horn of the spinal cord. Only particular cell types were labeled in each of these regions (e.g., only nonpyramidal neurons were strongly labeled in the cerebral cortex; all neurons but Purkinje cells were labeled in the cerebellar cortex). Labeled cells in most brain regions appeared to be small local circuit neurons. Cases with short survival times (1-6 hr) exhibited strong labeling of glial cells and light labeling of a large percentage of neurons in many brain regions. Cases with 48 hr survival times exhibited no labeled cells. Injections of d-biotin did not produce neuronal labeling. Injections of biotinamide (neurobiotin, Vector Labs) produced a pattern of labeling that was much different from that produced by biocytin. The results of this study suggest that there is differential uptake and retention of biocytin by subpopulations of CNS neurons. This uptake system may be associated with biotin-dependent metabolic pathways in these cells. Double-labeling studies are in progress to further characterize neurons that exhibit biocytin uptake. Supported by NIH Grant NS19733.

475.11

ULTRASTRUCTURE OF PHYSIOLOGICALLY CHARACTERIZED CORTICAL NEURONS AND THEIR RELATION TO GABAERGIC AND CATECHOLAMINERGIC TERMINALS IN RAT BRAIN. R.L. Cowan*, S.R. Sesack, J. Chan, E.J. Van Bockstaele and V. M. Pickel. Div. of Neurobiology, Dept. of Neurology and Neuroscience, Cornell University Med. Center, New York, N.Y. 10021.

In vivo intracellular recording and immunocytochemical labeling were used to determine the ultrastructure and synaptic associations of physiologically characterized pyramidal neurons in the superficial layers of the rat frontal cortex. After each recording, individual neurons were filled with biocytin, and the animals were transcardially perfused with acrolein. Sagittal serially collected sections were processed for visualization of biocytin using avidin biotin peroxidase. Sections containing the filled neuron were processed using immunogold silver for detection of GABA or tyrosine hydroxylase (TH), a marker of catecholaminergic afferents. Filled neurons had branched and spiny dendrites as seen by light microscopy. At the ultrastructural level, filled perikarya and dendrites had irregular (ruffled) contours. Although these were densely filled with peroxidase, synaptic contacts were usually identifiable. In dually labeled sections, (i) GABA immunoreactivity was seen in perikarya, dendrites and terminals, some of which formed synapses on proximal filled dendrites, and (2) TH-labeling was seen in terminals in neuropil near the filled dendrites in the superficial layer. The combined use of *in vivo* intracellular labeling with ultrastructural identification of transmitters provides an important method for determining the synaptic basis for modulation of cortical activity. (Supported by grants MH00078, DA04600, HL18974).

475.12

COMBINED ANALYSIS OF CALCIUM BINDING PROTEINS, GAMMA AMINOBUTYRIC ACID, ANTEROGRADELY TRANSPORTED WHEATGERM AGGLUTININ HORSERADISH PEROXIDASE AND ANTEROGRADE DEGENERATION IN THE CNS OF THE PRIMATE. Antonia M. Milroy,*Diane Daly Ralston and Henry J. Ralston, III. Department of Anatomy and the W.M.. Keck Foundation Center for Integrative Neurosciences, University of California, San Francisco, California, 94143.

Our laboratory has explored the use of several neuroanatomical techniques simultaneously to analyze projections in the primate central nervous system to neuronal populations that have been characterized for the presence of neurotransmitters and calcium binding proteins. As an example, one M. facicularis, as part of a series of studies in which our laboratory is currently involved, was injected with wheatgerm agglutinin-horseradish peroxidase (WGA-HRP) in somatosensory/motor cortices for anterograde transport in cortico-spinal/thalamic/rubral pathways and underwent ablation of the area of hand representation in the prefrontal cortex, area 4, for cortico/rubral/spinal afferents. According to protocol the animal was anaesthetized and perfused intracardially with 2% paraformaldehyde and 2% glutaraldehyde, pH7.4 at room temperature. The brain was kept in cold fixative for 4-5 hours , then transferred to cold phosphate buffered saline. Vibratome sections of 100µm, were 1) reacted with tetramethyl benzidine (TMB) for electron microscopy 2) incubated with the appropriate antibody for calbindin or for parvalbumin using the Avidin-Biotin method, reacted with either diaminobenzidine (DAB) or TMB. (Triton-X was not used in the Avidin-Biotin method). Areas for study were blocked for EM and placed in 1% OsO_4 overnight and then embedded according to standard EM procedures. Post embedding GABA immuno-gold was then applied to the tissue containing either transported HRP, anterograde degeneration, parvalbumin or calbindin. (Triton-X was used in low concentration in all solutions of the post-embedding GABA immuno-gold labeling instead of etching). We have found that these techniques can be employed in a variety of combinations to maximize the information while preserving the fine structural integrity of the tissue. Supported by NS-21445 and NS-23347.

475.13

LIGHT AND ELECTRON MICROSCOPIC STUDIES OF BOUTONS ON ISOLATED RAT NEURONS. W.Liu* and M.DeSantis. Dept. of Biological Sciences and WAMI Medical Program, University of Idaho, Moscow, ID 83843, USA

The size of axosomatic boutons was measured on isolated neuronal somas using scanning electron microscopy (SEM). Neuronal cell bodies were isolated from the trigeminal motor nucleus by ultrasonic disruption after perfusion of the anesthetized rat with aldehyde fixative and postfixation in osmium tetroxide. The isolated neuronal somas were first identified by light microscopy and then examined by SEM. They ranged in size from 23.0 to 41.6 um average diameter (n=23). Preparation for SEM resulted in an average shrinkage of the soma by 20% of its diameter. There was a direct relationship between the size of cell body and the amount of shrinkage. SEM revealed bulbous structures from 0.6 to 3.6 um in size at the surface of neuronal somas. Our interpretation that they were boutons was confirmed when transmission electron microscopy (TEM) was used to study the same somas after SEM. The surface of the isolated somas was generally free of adhering elements except for the boutons. (Supported by Idaho State Board of Education grant 92-010).

475.14

ULTRASTRUCTURAL LOCALIZATION OF GLIAL HYALURONIC ACID BINDING PROTEIN (GHAP). M.A. Hosley.* Spinal Cord Injury Research, V.A. Med. Ctr., West Roxbury, MA

GHAP (glial hyaluronic acid binding protein) is a 60 kDa glycoprotein which binds to hyaluronic acid in the CNS. Functionally, GHAP is thought to inhibit neurite adhesion and outgrowth. In this study, the ultrastructural localization of GHAP was performed in rat spinal cord and cerebellum, the two portions of the CNS with the highest GHAP staining. The localization was performed via an indirect immunoperoxidase staining with the monoclonal antibody 12C5 as the primary antibody. Animals were anesthetized and then perfused via a gravity flow system through the left ventricle, first with 50cc of PBS (phosphate buffered saline)at pH 7.3-7.4, containing 2% procaine-HCl and 3mg% heparin sulfate (169 units/ml). This was followed by fixation with 100-300cc of PBS at pH 7.3-7.4 containing 2.0% paraformaldehyde, 2.5% glutaraldehyde and 1% CPC (cetylpyridinium chloride); the CPC serves to stabilize the water soluble hyaluronic acid in the extracellular matrix to which the GHAP binds. After perfusion, the brain and spinal cord were removed and stored overnight (12-16 hrs.) in the same fixative. Vibratome sections were subsequently collected and stained by indirect immunoperoxidase using the DAB protocol of Streitt and Reubi (1977).

Electron micrographs will be presented to illustrate the labelling of GHAP in both the spinal cord and the cerebellum.

This research supported in part by NIH Grant NS 13034 and V.A. Gen. Res. Service Merit 0002.

475.15

A PRE-EMBEDDING TRIPLE-LABEL EM IMMUNOHISTOCHEMICAL METHOD AS APPLIED TO THE STUDY OF MULTIPLE INPUTS TO DEFINED NIGRAL NEURONS. A. Reiner*, K.D. Anderson and E.J. Karle. Dept. of Anat. & Neurobiol., Univ. of Tennessee, Memphis, TN, 38163.

Both dopaminergic and nondopaminergic neurons are present in the substantia nigra, and the substantia nigra receives input from both enkephalinergic (ENK+) striatal neurons and substance P-containing (SP+) striatal neurons. To determine the types of nigral neurons receiving these inputs and to determine whether such individual neurons receive input from both types of terminals, we have developed an approach for carrying out pre-embedding triple-label EM immunohistochemistry, using three distinct markers.

Following fixation with a paraformaldehyde-glutaraldehyde-acrolein fixative, vibratome sections were sequentially labeled for the localization of: 1) SP+ terminals using a PAP/DAB procedure with rat anti-SP; 2) ENK+ terminals using a 1nm gold-conjugated secondary with mouse anti-ENK; and 3) tyrosine hydroxylase-containing (TH+) neurons using a PAP/BDHC procedure with rabbit anti-TH. The sections were then osmicated and the immunogold labeling was silver intensified. Thin sections examined with EM showed that all three labels could be distinguished at the EM level. Although individual SP+ and ENK+ terminals contacted both TH+ and non-TH+ perikarya and dendrites, profiles receiving simultaneous contact from both SP+ and ENK+ terminals were typically non-TH+. These results show that pre-embedding triple-label EM immunohistochemistry is feasible, and that SP+ and ENK+ striatal terminals may be segregated on nigral dopaminergic neurons (i.e. on different neurons or different parts of the same neurons). Supported by NS-19620 & NS-28721 (A.R.)

475.16

PREDOMINANCE OF Na/Ca EXCHANGER (NCX) IMMUNOREACTIVITY IN SELECTED SYNAPTIC FIELDS OF RAT HIPPOCAMPUS. R.K. Yip*[1], A. Ambesi[2], G.E. Lindenmayer[2] and M.P. Blaustein[1]. [1]Dept. of Physiology, U. of MD Med. Sch., Baltimore, MD 21201 & [2]Dept. of Pharmacol., Med. U. of SC, Charleston, SC 29425.

The Na/Ca exchanger (NCX) is prevalent in neurons and appears to be concentrated at nerve terminals; the exchanger is also present in glia (*Ann. N.Y. Acad. Sci.* 639:254-274, 1991). Polyclonal antisera specific to canine cardiac NCX were used to localize the NCX in adult rat hippocampus. We compared the distribution of the NCX immunoreactivity to the distribution of synapsin I and synaptophysin (synaptic vesicle markers), and glial fibrillary acidic protein (GFAP; a glial marker). Immunostaining of 25 μm cryosections of hippocampus was performed by an indirect immunoperoxidase procedure. The anti-NCX antibodies labeled the strata oriens and radiatum most intensely, followed by stratum lacunosum-moleculare and the inner third of the dentate molecular layer where axons from both the ipsilateral and contralateral hilus terminate. The regions of the hippocampus where the neuronal cell bodies are located (the stratum pyramidale and the dentate granular layer) were poorly labeled by the anti-NCX antiserum. No specific labeling in any region of hippocampus was observed with preimmune serum. Immunostaining patterns for synapsin I and synaptophysin were similar to the NCX pattern although antibodies to synapsin I and synaptophysin, but not NCX, strongly stained the entire molecular layer of the dentate gyrus. The GFAP immunostaining pattern differed markedly from the NCX pattern. These results suggest that much of the NCX immunoreactivity is associated with synapses since synapsin I and synaptophysin are present in all nerve terminals. We concluded that the NCX is present in relatively high concentration in the hippocampus, and that it is especially prevalent in some synaptic fields where it may play an important role in Ca homeostasis.

475.17

CYTOCHROME OXIDASE SUBUNIT IV PRECURSOR POLYPETIDE: IMMUNOHISTOCHEMICAL LOCALIZATION IN THE RAT BRAIN S. Liu*and M. Wong-Riley. Dept. Cellular Biology & Anatomy, Med. Coll. Wis., Milwaukee, WI 53226

Cytochrome oxidase (C.O.) contains 13 subunits which are individually encoded by mitochondrial or nuclear genes. Nuclear-derived mitochondrial proteins are cytoplasmically synthesized as precursors carrying cleavable polypeptides (presequence) at the N-terminus, which functions as the targeting signal for directing precursors into the mitochondria. In order to determine if nuclear-derived precursors are localized mainly in neuronal cell bodies or in dendrites, we generated polyclonal antibodies against the presequence of the rat C.O. IV precursor. Immunoreactivity could be detected in all brain regions examined (cerebellum, hippocampus, brain stem, somatosensory barrel cortex, and olfactory bulb). There is a distinct heterogeneous immunohistochemical pattern among different brain regions, laminae, and cell types. The pattern of immunoreactivity for the precursor matched that for the mature enzyme and the pattern shown by C.O. histochemistry. Antisera were localized in both somata and dendrites in the hippocampus and the cerebellum, where somatic and dendritic layers are spatially segregated. This is the first evidence that precursors of the rat C.O. subunit IV are detectable immunohistochemically in the mammalian brain. (Supported by NIH grant NS 18122 to MWR)

475.18

QUANTITATIVE DIFFERENCES IN CALCITONIN GENE-RELATED PEPTIDE (CGRP)-IMMUNOREACTIVITY IN RAT SPINAL CORD MOTONEURONS INNERVATING FAST AND SLOW MUSCLES. J. Calderó, A. Sorribas, E. Hengesbach, V. Yuste, J.X. Comella* and J.E. Esquerda. Unitat de Recerca Neuromuscular, Departament de Ciències Mèdiques Bàsiques, Facultat de Medicina de Lleida, Universitat de Barcelona, E-25006, Lleida, Spain.

When CGRP is detected by immunocitochemistry a substantial variation on the intensity of the reaction is found among the spinal cord motoneuron population. The significance of such heterogeneity is unknown. We have explored whether or not motoneurons innervating fast or slow muscles shown correlative differential content in CGRP. Motoneurons innervating the slow soleus or the fast extensor digitorum longus (EDL) muscles from adult Sprague-Dawley rats were labelled by intramuscular injection of fluoresceinated-wheat germ agglutinin or cholera toxin (B subunit). CGRP-like immunoreactivity (CGRPLI) was evidenced by immunofluorescence using rhodamine as a label. The intensity of CGRP reaction was measured in traced neurons with a microspectrofluorimeter. Recorded data from each set of samples were analyzed by means of an automatized classification procedure for individual intensities in each neuronal population. The classification algorithm is based on a pattern recognition method with no initial membership defined for each individual cell. In female rats, an inverse frequency distribution of the intensities of CGRPLI was consistently observed when histograms from soleus and EDL-innervating motoneurons were compared: the strong CGRPLI was more frequent in the body of soleus-innervating motoneurons. When the same evaluations were done in male rats, the above mentioned clear-cut differences were not consistently observed. It is concluded that the type of motor units and/or metabolic state of the muscle fibers may influence the neuronal CGRP.

475.19

A SILVER-REDUCING AXONAL PROTEIN IN THE CENTRAL NERVOUS SYSTEM OF ADULT RATS. C.J.Tandler, A.Pellegrino de Iraldi* Instituto de Biología Celular, Facultad de Medicina, UBA. Paraguay 2155, (1121) Buenos Aires, República Argentina.

A selective "argentaffin" staining of nerve fibers after mercuric acetate postfixation was reported (20th Ann Meet Soc Neurosc, Abstract 28.9,1990). The technique (Hg-Ag) also stained specifically proteins within lateral components of triads and diads in striated muscle cells (Histochemistry 92:15,23, 1989). The axonal argentaffinity is dependent on both glutaraldehyde and mercuric ions. It is not suppressed after extraction of lipids, heating in 1N perchloric acid or incubation with alkaline phosphatase. Methylation of glutaraldehyde fixed tissues abolished the Hg-Ag reactivity indicating that carboxyl groups in protein are involved in the formation of the organic mercurial responsible for silver staining. The electron microscope shows that the silver-reducing protein localizes inside the axon. The procedure stained white and gray matter fibers in cerebrum, cerebellum and spinal cord but not the neuronal perikarya or their dendrites and proximal axons. In the cerebellum the basket cell axons were strongly reactive but not the parallel fibers. The possibility of the method to distinguish between functional stages of proteins in relation to cytotypic variations in cytoskeleton structures is suggested.

Work supported by grants from the CONICET and UBA, República Argentina.

475.20

ANATOMICAL ORGANIZATION OF INTRINSIC NEURONS OF THE RABBIT TRACHEA

R.Hendriks, S.R.Knoper*, and D.L.Kreulen. Department of Pharmacology, College of Medicine, University of Arizona, Tucson, Arizona. 85724.

The mammalian trachea is likely to be under the efferent influence of both intrinsic and extrinsic neurons. Using a variety of neuronal markers, we have investigated the anatomy of intrinsic tracheal neurons of the rabbit. Individual trachea were variously labeled with *Neutral Red* (Sigma, USA) and antibodies to *Neuron Specific Enolase* (NSE) or *S-100* protein and were prepared for viewing either as wholemounts or tissue sections. All three markers clearly labeled intrinsic neurons of the rabbit trachea. The nerve cells were found to be organized into small ganglia containing less than ten cell bodies each. The ganglia were primarily between the cartilaginous rings and at the esophageal border. Most were found to lie at the surface of the tissue although occasionally ganglia could be found lying deep within the trachea. No clear plexus arrangements for these neurons were discernible. Individual fibers of some neurons could occasionally be followed to the mucosa. In conclusion, the intrinsic neurons of the trachea form a diffuse neural network that, accordingly, is likely to have a unique role in the functioning of this tissue. Support HL-46471.

475.21

CEREBRAL ATROPHY AND HYDROCEPHALUS IN CONTROL AND GANGLIONECTOMIZED SPONTANEOUSLY HYPERTENSIVE RATS. D. Livingstone, F. Hans, L. Wei, P. Brink*, J. DeMaro, W. Finnegan, and J. Fenstermacher. Dept. Neurological Surgery, SUNY, Stony Brook, NY 11794-8122.

The spontaneously hypertensive rat (SHR) is hypertensive, hyperactive, and hydrocephalic relative to Wistar-Kyoto rats (WKY). In young adults, glucose metabolism is lower in SHR than WKY and is elevated in SHR when one superior cervical ganglion is removed at 4 weeks of age. In view of these cerebral oddities, tissue volumes and neuronal density were measured in control, ganglionectomized (uGX), and sham-operated (sham) SHR and WKY. In control SHR the volume of the whole brain and of some areas was 5-10% less than in control WKY; ventricular (CSF) volume was nearly 2 times larger in SHR than WKY. Neuronal density was similar in control SHR and WKY. Relative to controls, the volumes of brain and all brain areas were 15-25% less in uGX and sham SHR as well as uGX and sham WKY. In both SHR and WKY, neuronal density in some areas tended to be less in uGX and sham rats. The uGX procedure at 4 weeks of age, but not ganglionectomy *per se*, seems to affect brain structure and function.

475.22

PHOSPHOTYROSINE-CONTAINING PROTEINS IN THE RAT BRAINSTEM AND SPINAL CORD. J.W. Unger*, W. Lange and J.N. Livingston[1]. Department of Anatomy, University of Munich, FRG, and [1]Department of Endocrinology University of Rochester, Rochester, NY 14642, USA.

The regulation of cell activity by a number of growth factor receptors and proto-oncogene products involves tyrosine kinases (TKs). The presence of phosphotyrosine-proteins (PY) is an index of the activity of Tks. Light- and electronmicroscopy demonstrated PY-immunoreactivity in numerous nuclei of the brainstem and spinal cord of the adult rat, including afferent and efferent nuclei, i.e. nucleus of the solitary tract, hypoglossal, lateral reticular nucleus, area postrema. Ultrastructural analysis revealed PY in the cytoplasm, in the perinuclear Golgi complex and at pre- and post-synaptic sites. Several receptor and cytosolic TKs may contribute to the PY-substrates, i.e. a high overlap was evident with the presence of insulin receptors. Our results suggest that protein tyrosine phosphorylation may play an important role for the regulation of neuronal function in the adult central nervous system. (Supported by the Walter-Schulz-Stiftung, NIH, and NATO Collaborative Research Grant).

BLOOD-BRAIN BARRIER I

476.1

PERMEABILITY COEFFICIENT-SURFACE AREA PRODUCTS OF THE BLOOD NERVE AND BLOOD BRAIN BARRIERS FOR NGF AND ALBUMIN. J.F. Poduslo,* G.L. Curran, and C.T. Berg. Molecular Neurobiology Laboratory, Departments of Neurology and Biochemistry/Molecular Biology, Mayo Clinic and Mayo Foundation, Rochester, MN 55905 USA.

Neurotrophic molecules will likely prove to be of importance in the treatment of neurodegenerative diseases of the peripheral and central nervous system. Although their administration to patients with Alzheimer's and other neurodegenerative diseases may represent a new era of treatment of these degenerative diseases, the development of a reliable, non-invasive, delivery system is clearly needed prior to the initiation of clinical trials. We have utilized the i.v. bolus injection technique in the catheterized brachial vein and artery of normal rats to quantify the permeability coefficient-surface area product (PS) for NGF across the blood nerve and blood brain barriers. The PS obtained for 2.5S NGF across the blood nerve barrier was $1.726 \pm 0.394 \times 10^{-4}$ ml/gm/second ($\bar{x} \pm$ S.D.). When compared to the PS for normal albumin (0.101 ± 0.088), a 17-fold increase was observed for NGF. Although a 5-fold M, difference exists between NGF and albumin, the PS for NGF was significantly higher suggesting a possible different mechanism of uptake into the nerve. Values obtained for different brain regions ranged from 12.7-32.5 fold higher for NGF compared to albumin. No changes were observed in the residual plasma volume for either the endoneurium or the different brain regions. Data will also be presented which suggest that selective modifications of NGF can enhance its permeability into the nervous system while still preserving biological activity of the protein. Such approaches might be useful in formulating a reliable delivery system of this and other neurotrophic factors for the potential treatment of Alzheimer's and other neurodegenerative diseases.

476.2

SATURABLE TRANSPORT OF TNFα ACROSS THE NEONATAL BBB. L.M. MANESS*, A.J. KASTIN, W.A. BANKS, and E.G. GUTIERREZ, Dept of Med and Neurosci Training Prog, Tulane Univ Sch of Med and VA Med Ctr, New Orleans, LA 70146.

The effects of tumor necrosis factor-α (TNFα) on the development of the CNS raise the possibility that circulating TNFα might enter the brain. We examined the blood-to-brain transport of recombinant murine TNFα across the blood-brain barrier (BBB) in 3 day old rat pups. ^{125}I-TNFα and ^{99m}Tc-albumin were injected IV, and the unidirectional influx constant (Ki) into the brain was calculated from the levels of radioactivity in the brain and blood. No entry of albumin was detected for the 30 min period tested, while the Ki for ^{125}I-TNFα was $8.12(10^{-4})$ ml/g-min. A 0.5 μg excess of unlabeled TNFα decreased the Ki to $0.79(10^{-4})$ ml/g-min, which represented an inhibition of over 90% of ^{125}I-TNFα entry [$F(2,7)=10.9$, $p<0.01$]. The excess TNFα, however, did not alter the brain/blood ratio of ^{99m}Tc-albumin (11.7±2.9 μl/g brain for controls vs 11.5±3.9 μl/g for excess TNFα). These results show that TNFα does not disrupt the neonatal BBB and support the existence of a blood-to-brain saturable transport system for TNFα in the neonatal rat.

476.3

THE IN VIVO TRANSPORT OF DOPAMINE AT THE LUMINAL SIDE OF THE BLOOD-BRAIN BARRIER IN GUINEA PIGS. C.L. Martel*, J.B. Mackic, M.H. Weiss, J.G. McComb and B.V. Zlokovic. Depts. Neurol. Surg. and Divn. Neurosurg. CHLA, USC Sch. Med., Los Angeles, CA 90033

Studies in animal models with high monoamine oxidase B (MAO-B) activity in cerebral microvessels have suggested that circulating dopamine (DA) is prevented from entering brain parenchyma by the enzymatic blood-brain barrier (BBB). In the present study, blood-brain DA transport is examined in an animal model with low BBB MAO-B activity - the guinea pig, Cavia porcellus, which in this respect is similar to humans. A vascular brain perfusion method [J.Neurochem. (1986) 46: 1444-1451] and a capillary depletion technique [J.Neurochem. (1990) 54: 1882-1888] were used to study BBB transport and/or binding of plasma derived DA. The initial rapid uptake (V_i), and rate of entry (K_{in}) of [^{3}H]-DA into the brain tissue were both found to be almost ten times higher than that of simultaneously infused [^{14}C]-sucrose, a metabolically inert cerebrovascular space marker. A substantial in situ DA binding to cerebral microvessels was also demonstrated. Brain uptake of [^{3}H]-DA (21 nM) was significantly inhibited in the presence of 500 nM unlabeled DA (by 49%); concentrations of 1-250 μM produced further inhibition (83%-92%). The D_2-receptor antagonist spiperone (25 μM) virtually abolished [^{3}H]-DA uptake, as evidenced by both brain homogenate and capillary-depleted tissue. In situ DA binding to cerebral microvessels was completely displaced by spiperone. The BBB DA transport and capillary sequestration were not affected by the MAO inhibitor pargyline (50 μM). These results suggest that DA is indeed transported across the BBB in guinea pigs by a specific MAO-independent mechanism. Brain uptake of circulating monoamine may be mediated by a capillary membrane D_2-receptor. (Supported by Childrens Hospital of Los Angeles grant 1102/3846).

476.4

OPIATES GIVEN DURING THE NEONATAL PERIOD ALTER THE BLOOD-BRAIN BARRIER TRANSPORT OF TYR-MIF-1. L.M. HARRISON*, A.J. KASTIN, and W.A. BANKS Dept of Med and Neurosci Training Prog, Tulane Univ Sch of Med and VA Med Ctr, New Orleans, LA 70146.

Tyr-MIF-1, a peptide with opiate-modulating properties, and Met-enkephalin are removed from the brain by a peptide transport system (PTS-1). Since morphine given during the neonatal period is known to alter opiate function, we examined whether PTS-1 was also affected. Neonatal rats were injected subcutaneously (SC) during the first week of life with either morphine sulfate (MS), MS+naltrexone (MS+Nal), MS+methyl naltrexone (MS+MNal), or vehicle at a dose for each drug of 50 μg/rat. On postnatal day 22, the brain-to-blood transport rate of ^{125}I-Tyr-MIF-1 was measured as previously described (Methods in Enzymology 168:652-660, 1989). Pups treated with MS had significantly smaller brain weights than controls ($p<.05$) or MS+MNal treated pups ($p<.001$). After correction for brain weight, transport of ^{125}I-TMIF-1 by PTS-1 in MS treated pups was over 21% faster than in control rats ($p<.01$) and 16% faster than in MS+MNal treated pups ($p<.05$). These results indicate that treatment with opiates during the neonatal period can affect the development of PTS-1.

476.5

BLOOD-BRAIN GLUTATHIONE TRANSFER IN GUINEA-PIGS OF DIFFERENT AGE. R.K. Kannan*, J.B. Mackic, N. Kaplowitz and B.V. Zlokovic. Dept. Neurol. Surg., Divns. Liver and GI Dis. and Neurosurg., CHLA USC Sch. Med., Los Angeles, CA 90033

Glutathione (GSH) is the major endogenous anti-oxidative agent in brain cells. Recently, it has been demonstrated that blood-brain transport of GSH may be of primary importance to preserve high levels of this cerebroprotective peptide in the central nervous system [J. Clin. Invest. (1990) 85: 2009-13]. In the present study, a vascular brain perfusion technique [J. Neurochem. (1986) 46: 1444-51] was used to examine blood-brain barrier (BBB) uptake of plasma-derived GSH in weanling (10 days) and 9 months old guinea-pigs. [^{35}S]-GSH (4 nM) was infused simultaneously with [^{14}C]-sucrose for 10 min, and blood-brain unidirectional transport rates (K_{in}) were estimated from uptake vs. time plots by single and/or multiple-time point graphic analysis. In weanling guinea-pigs, regional cerebrovascular permeability to GSH was 5 to 7 times higher than for sucrose. In older animals, however, there was no statistical difference between GSH and sucrose rates of entry into the brain. In both groups, K_{in} values for [^{14}C]-sucrose (cerebrovascular space marker) were comparable. Based on this preliminary evidence, it is suggested that blood-brain transport of GSH is guinea-pigs is lessened by aging possibly due to inefficient specific transport mechanism (Supported by VA funds).

476.6

GLUTATHIONE TRANSPORT ACROSS THE BLOOD-BRAIN BARRIER IN GUINEA-PIGS. J.B. Mackic*, M.N. Lipovac, R. Kannan, D. Tang, J.G. McComb, N. Kaplowitz and B.V. Zlokovic. Dept. Neurol. Surg., Divns. Liver and GI Dis. and Neurosurg., CHLA USC Sch. Med., Los Angeles, CA 90033

Glutathione (GSH) cerebroprotective function(s) involve detoxification of xenobiotics, reduction of hydrogen peroxide and lipid peroxides, and maintenance of thiol-disulfide status of brain cells. Although brain is rich in GSH, GSH level becomes significantly reduced under certain pathologic conditions (e.g., cerebral ischemia, focal seizures). Thus, strategies for GSH central delivery are of obvious therapeutic potential. The presence of GSH-specific blood-brain barrier (BBB) transporter that is independent of gamma-glutamyl transpeptidase (GGT), has recently been suggested in rats [J. Clin. Invest. (1990) 85: 2009-13]. In this study, we used the guinea-pig model with high BBB GGT activity to re-examine transport of circulating [^{35}S]-GSH across the BBB. Brain uptake index (BUI) technique confirmed original data in rats by demonstrating regional BUI values of 19.9% to 28.1% relative to [^{3}H]-water standard. GSH BUIs were not affected by 5 mM serine-borate (GGT inhibitor). Vascular brain perfusion technique [J. Neurochem. (1986) 46: 1444-51] revealed that regional rates of GSH blood-brain entry were 4-6 times higher relative to simultaneously infused sucrose (cerebrovascular space marker). There was no significant capillary sequestration of circulating GSH, but the uptake of [^{35}S]-GSH (4 nM) was abolished by 1.5 mM unlabeled tripeptide. HPLC analysis demonstrated that more than 95% of plasma-derived GSH in the brain was in its original form after a 10 min perfusion. It is concluded that intact GSH is transported across the BBB in guinea-pigs, by a mechanism that may be similar to that previously described in rats. (Supported by VA funds).

476.7

EXPRESSION OF THE mRNA FOR THE BASIC AMINO ACID TRANSPORTER (SYSTEM Y+) AT THE BLOOD-BRAIN BARRIER. I. Stoll*, K. C. Wadhwani and O. R. Smith
Laboratory of Neuroscience, National Institute on Aging, Bethesda, MD 20892.

Basic amino acids (Lys, Arg and Orn) are transported into brain from blood by a Na-independent, saturable carrier at the blood-brain barrier (BBB). The carrier is stereospecific and exhibits a Km for L-lysine of approx. 70μM. Recently, the cell-surface receptor for ecotropic murine retroviruses was shown to be a basic amino acid transporter with similar selectivity and affinity attributes (Wang et al., Nature (1991) 352, 729). To determine if the same protein is expressed at the BBB, transporter mRNA expression was assessed in purified rat brain capillaries and compared to expression in whole rat brain, brain parenchyma (brain minus capillaries) and desheathed rat sciatic nerve. We designed oligonucleotide primers based on the published sequence of the ecotropic retroviral receptor and used them to generate a cDNA from mouse brain total RNA by polymerase chain reaction. Partial sequence analysis of the amplified cDNA showed identity with the published sequence. Expression of the transporter mRNA in rat brain and peripheral nerve was observed by both Northern blot analysis or by RNAse protection, with the highest level observed in capillaries (greater than 10-fold higher than whole brain). These results suggest that the ecotropic murine retrovirus receptor may be the basic amino acid carrier of the BBB.

476.8

STRUCURE-ACTIVITY STUDY OF HIGH-AFFINITY NEUTRAL AMINO ACID TRANSPORT ACROSS THE BLOOD-BRAIN BARRIER. O. R. Smith*, Y. Takada, and N. Greig. Lab. of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

Large neutral amino acids are transported from blood into brain by a saturable carrier at the blood-brain barrier (BBB). This carrier exhibits many of the properties of the amino acid "L System" including sodium-independent, BCH-inhibitable transport. Previous studies from our laboratory have identified several high affinity drugs that are also taken up into brain by this mechanism. In the present study we examined structure-activity relations for two series of drugs in order to better understand the molecular factors that influence binding and transport. Brain uptake was measured in pentobarbital-anesthetized rats using the in situ brain perfusion technique (Takasato et al., 1984). As previously reported for the natural, endogenous amino acids (Smith et al., 1987), transport affinity ($1/K_m$) for amino acid drugs was critically influenced by side chain hydrophobicity. However, other factors contributed as well, including the nature and location of side groups. For example, placement of a nitrogen mustard at the 7-position of 2-amino-1,2,3,4-tetrahydro-2-naphthoic acid enhanced affinity by >30 fold (K_m ~0.2 μM). In contrast, transfer of the same group to the 5-position or movement of the amino acid group to the 1-position reduced affinity by > 90%. Comparable differences were seen for structural analogues of phenylalanine, including melphalan and meta-L-sarcolysine. V_{max} varied among compounds and was quite low for some analogues. The results demonstrate that amino acid transport capacity and affinity are critically influenced by solute structure and that careful positioning of hydrophobic side groups can markedly enhance delivery to brain.

476.9

AMINO ACID TRANSPORT INTO MEMBRANE VESICLES ISOLATED FROM THE BLOOD-BRAIN BARRIER. M.M. Sánchez del Pino, R.A. Hawkins* and D.R. Peterson, Dept. Physiology and Biophysics. Univ. Health Sci./Chicago Med. Sch., North Chicago, IL 60064.

Luminal and abluminal membranes from bovine brain endothelial cells were separated on Ficoll gradients. The membranes formed sealed vesicles that were adequate for transport studies. Transport of N-(methylamino)-isobutyrate (MeAIB), a specific substrate of the A-system, and L-phenylalanine were studied in both membrane populations. Luminal vesicles showed a high affinity transport system for L-phenylalanine that was independent of Na^+ and H^+ gradients and inhibited by other large neutral amino acids, including D-phenylalanine; it was not inhibited by MeAIB. In contrast, abluminal vesicles contained a Na^+-dependent phenylalanine transport system that was inhibited by MeAIB. Similarly, uptake of radiolabeled MeAIB was stimulated by a Na^+ gradient in abluminal vesicles, whereas no significant effect of a Na^+ gradient was observed in luminal vesicles. The results provide direct evidence for the presence of the Na^+ dependent A-system exclusively in the abluminal membranes of the BBB. The data are consistent with the location of an L-type system in both membranes.

476.10

RECEPTOR-MEDIATED TRANSCYTOSIS THROUGH THE BLOOD-BRAIN BARRIER (BBB): FERRO-TRANSFERRIN AND ITS RECEPTOR. R. Broadwell, B. Baker, P. Friden, M. Tangoren and A. Wolf*. Div. of Neurosurgy, Univ. Maryland Sch. Med., Baltimore, MD 21201 and Alkermes, Inc., Cambridge, MA 02139

The potential for receptor-mediated transcytosis of ferro-transferrin (fTRF) and antibody against its receptor through BBB endothelia was investigated in Sprague Dawley rats. TRF, a blood-borne peptide, conveys iron to tissues and cells. BBB endothelia exhibit the receptor for fTRF on the luminal surface. Rats were injected into the carotid artery with fTRF conjugated to HRP (6mg/0.5ml; 1-3hrs) or intravenously with native HRP (50mg/0.5ml; 1hr), HRP-conjugated or non-conjugated antibody to the fTRF receptor (0X26; 200-700ug/ 0.5ml; 5mins-6hrs) and to Major Histocompatibility Complex Class I (0X27; 210ug/0.5ml; 6hrs) against the PVG rat strain; the latter antibody and native HRP were employed as controls. Non-conjugated antibodies were identified immunohistochemically. fTRF-HRP, each of the antibodies, and native HRP labeled BBB endothelia, perivascular phagocytes and pial surface macrophages. fTRF-HRP and OX26-HRP were seen ultrastructurally within perivascular clefts; fTRF-HRP only was observed in cells of the neuropil; OX26-HRP only was localized in saccules of the Golgi complex in BBB endothelia. Native HRP and OX27 labeling of perivascular and pial surface phagocytes occurs through extracellular routes circumventing the BBB. Potential transcytosis of blood-borne fTRF-HRP and OX26 through the BBB very likely occurs, but the exact intracellular pathways are elusive. Supported by NIH grant #NS18030.

476.11

IN VIVO LABELING OF BRAIN MICROVASCULAR TRANSFERRIN RECEPTORS USING COLLOIDAL GOLD-ANTITRANSFERRIN RECEPTOR ANTIBODY-COMPLEXES. J. Yang, U. Bickel, T. Yoshikawa, H. Weiner*, and W.M. Pardridge, Departments of Medicine and Psychiatry and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The transferrin receptor (TfR) is highly expressed at the plasma membrane of brain microvascular endothelial cells, which form the blood-brain barrier (BBB) in vivo. A monoclonal antibody to the rat TfR, OX26, has recently been evaluated as a model of brain transport vectors for drugs unable to pass through the BBB. Transcytosis of OX26 and OX26-drug conjugates could be demonstrated in pharmacokinetic studies, and OX26 was successfully used for brain delivery of a vasoactive intestinal peptide analog, resulting in a pharmacological response (increased cerebral blood flow).

To obtain ultrastructural information about the in vivo transcellular trafficking of OX26 at the BBB, a direct receptor labeling study with 5 nm-colloidal gold/OX26 complexes was performed. The complex was infused for 10 min into anesthetized Sprague-Dawley rats via the internal carotid artery (about 200 μg OX26/rat). Immediately after the end of the infusion, the animals were perfusion fixed via the ascending aorta with 2% glutaraldehyde. Vibratome and semithin sections of the brain were silver enhanced and examined at the light microscopic level. A widespread staining of brain capillaries was found. Selected regions were subsequently processed for transmission electron microscopy. A pattern of gold particles at the lumenal membrane of endothelial cells and intracellular clusters of gold particles in defined cytoplasmic compartments resembling endosomes was seen. In conclusion, this study presents a novel approach to in vivo receptor labeling and ultrastructural analysis.

PRESYNAPTIC MECHANISMS VI

477.1

A GABA-T BLOCKER SELECTIVELY ENHANCES PRESYNAPTIC INHIBITION. H. Golan and Y. Grossman*. Unit of Physiology, Faculty of Health sciences, Ben-Gurion University of the Negev, Beer-Sheva, 84105, Israel.

Blockers of GABA transaminase (GABA-T) elevate GABA concentration in the nervous system and increase convulsive threshold. In order to determine how administration of a GABA-T blocker influences synaptic transmission, we studied the effect of ethanolamine-O-sulfate (EOS), an irreversible blocker, on spontaneous and evoked PSPs in the opener muscle of the crayfish walking leg. EPSPs and IPSPs were recorded intracellularly; corresponding single axons were stimulated separately. EOS (1 mM) was applied to the cut end of the nerve in a suction electrode. Picrotoxin (0.05 mM) caused an increase in R_m by 24.7% (n=7) indicating tonic GABA release. EOS had no effect on R_m (n=3) and did not alter reversal potential or conductance of IPSPs. Inhibition was tested by delivering a short train of stimuli to the inhibitory axon 0-70 ms before evoking EPSPs. In 7 of 12 cells EOS enhanced inhibition by 15-60% without changing the time course of EPSP decay. In cells not sensitive to EOS, control inhibition was also weak. The data suggest that the predominant effect of the GABA-T blocker is to selectively enhance presynaptic inhibition.

477.2

GAP JUNCTIONS MEDIATE SELF AMPLIFICATION OF AFFERENT SIGNALS IN CRAYFISH. D. Cattaert, A. El Manira, F. Clarac*. CNRS NBM, 31 Chemin Joseph Aiguier BP 71, 13402 Marseille CEDEX 9 FRANCE.

Sensory information plays an important role in the control of motor behaviour. Nevertheless the strength of sensory-motor loops fluctuates during motor tasks. The modulation of reflexes not only involves neurones that are postsynaptic to the afferents, but sensory cells themselves, either at the periphery by neuromodulators that modify their sensitivity, or centrally by presynaptic inhibition. We present here evidences for a new type of presynaptic control of sensory afferents that involves electrical coupling between sensory terminals. This study has been achieved in the crayfish walking system in *in vitro* experiments. Sensory terminals from the coxo-basipodite chordotonal organ, were intracellularly recorded in the central neural network in the proximity of their synaptic endings. Paired recordings demonstrate electrical coupling between sensory terminals. The analysis of dye coupling (Lucifer Yellow) between those terminals by confocal microscope reveals large close apposition sites. The functionnal role of sensory coupling is discussed taking into account the enhancement of synaptic transmission from sensory terminals by subthreshold depolarizations, such as those produced by spikes in coupled terminals. By this mechanism only meaningfull sensory messages would be effectively transmitted.

477.3

ANALYSIS OF PRIMARY AFFERENT INPUT.TO RAT DORSAL HORN
S. Jeftinija*, Lj. Kojic, T.-H. Chen and L. Urban[1]. Dept. Vet. Anatomy and Neuroscience Program, Iowa State University, Ames, IA 50011, USA; Laszlo Urban, MD, Ph.D., [1]Present address, Sandoz Institute for Medical Research, Gower Place, London WC1E 6BN, UK.

Horizontal spinal cord slice preparation with attached dorsal root (DR) and dorsal root ganglion (DRG) was used to study the functional interaction of different afferent fibers at DH neurons. Brief perfusion of 50mM potassium to the DRG depolarized large sensory neurons (29±2.2 mV, mean ±SEM, n=32) and blocked firing of AP. Simultaneous intracellular recording from superficial DH neurons revealed that in cells receiving input from both small and large fibers, excitatory postsynaptic potentials (EPSP) evoked by electrical stimulation of large myelinated afferents (10-20V, 0.02ms) were selectively blocked during high potassium perfusion. Bath application of TTX (0.2-10μM) to the DRG blocked electrically-evoked APs in large DRG neurons and consequently EPSPs in DH neurons. Depolarizing effect of high potassium on DRG neurons and excitatory effect on DH neurons was not blocked by TTX. During this potassium-induced increased activity in DH neurons, EPSPs evoked by stimulation of small unmyelinated fibers were potentiated. Our data suggest that in large DRG neurons with TTX sensitive Na channels high K evoked depolarization inactivates the fast Na-current and blocks AP generation. As TTX resistant Na channels require higher levels of depolarization C-cells remain active in the presence of high K and maintain synaptic input to the DH. This difference could be responsible for the selective activation of C afferent fibers by high K which produced EPSPs in the spinal cord. This hypothesis is further supported by the finding that when Na ions in the DRG bathing media were totally removed in the presence of TTX, both high K and electrical activation of afferents were without effect on DH neuron. Work was supported by NIH Grant NS27751.

477.4

PURIFICATION OF β-LEPTINOTARSIN-h BY IMMUNOAFFINITY CHROMATOGRAPHY. R.D. Crosland*, T.H. Hsiao¶, and B.C. Lidgerding#. Toxinology Div. and #Virology Div., U.S. Army Med. Res. Inst. Infec. Diseases, Frederick, MD 21702 and ¶Biol. Dept., Utah State Univ., Logan UT 84322.

β-Leptinotarsin-h (β-LPT-h) is a proteinaceous toxin(s) that promotes Ca++ influx and neurotransmitter release at mammalian presynaptic nerve terminals. To date, the most purified preparations of β-LPT-h have been demonstrably heterogeneous. Our goal was to further purify β-LPT-h by immunoaffinity chromatography.

We immunized Balb/C mice with the heretofore most purified preparation of β-LPT-h. Monoclonal antibodies were generated by cell fusions with SP2/0 parent myeloma cells. Antibodies of interest were located by determining the ability of antibody + protein G-Sepharose to remove ^{45}Ca++ uptake activity from solutions containing β-LPT-h. We found four activity-binding antibodies, one of which (2C3-1-1) we chose for further study. 2C3-1-1 bound to only 80% of the activity in the preparation used for immunization, revealing the presence of at least two immunologically distinct Ca++ uptake activities. Column chromatography of β-LPT-h with 2C3-1-1 coupled to Affi-Gel 10 and subsequent SDS-PAGE chromatography revealed two bands of 55 kD and 60 kD.

477.5

THE EFFECT OF CALCIUM AND SODIUM ON THE EXTRACELLULAR CONCENTRATION AND RECOVERY OF DOPAMINE USING QUANTITATIVE MICRODIALYSIS. A.D. Smith* and J.B. Justice, Jr., Department of Chemistry, Emory University, Atlanta, Georgia, 30322.

The point of no net flux method was used to study the effects of varying the sodium (Na^+) and calcium (Ca^{2+}) concentrations (conc.) in artificial cerebrospinal fluid (CSF) on the in vivo probe recovery and extracellular conc. of dopamine (DA) in the nucleus accumbens of the rat. Anesthetized animals were implanted with 20 gauge guide cannula in the nucleus accumbens at coordinates AP +3.4, ML +1.5 from bregma and DV -7.2 from dura. The night prior to the experiment a 2 mm microdialysis probe was inserted and perfused with artificial CSF at 0.2 μl/min. On test day, dialysate samples were collected at 20-minute intervals and injected on a smallbore HPLC system. Samples were analyzed until stable levels of DA were obtained, after which time, depending on the experiment, the perfusate was changed to an artificial CSF solution containing 0, 1.2, 3.0, 5.0 or 7.2 mM Ca^{2+} or 0, 72.5, 145, or 217.5 mM Na^+. Each ionic conc. was run with 0 and 50 nM DA (n = 4 or 5). Linear regression was performed at each ionic conc. to obtain the extracellular DA conc. and probe recovery. The results indicate that DA increases with increasing Ca^{2+} conc. from 0 to 46.1 nM at 6.7 nM DA/mM Ca^{2+} and decreases with increasing Na^+ conc. from 18.3 to 1.8 nM at -0.07 nM DA/mM Na^+. There was no effect of Na^+ on recovery. However, the effect of Ca^{2+} on recovery was complex. Above physiological Ca^{2+}, the recovery tended to decrease, whereas below there was an increase in the recovery. Linearity of the method was confirmed using 0, 1.25, 2.5, 5, 10, 12.5, 25 and 50 nM DA in a separate group of animals (n = 12).

477.6

ELECTRICAL STIMULATION REPROGRAMS THE METABOLIC AND SYNAPTIC PHENOTYPES OF CRAYFISH MOTONEURONS. P.V. Nguyen* and H.L. Atwood. MRC Group in Nerve Cells & Synapses, Department of Physiology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada, M5S 1A8.

Can impulse activity in motoneurons determine metabolic efficacy? Using Rhodamine-123 (Rh) as a supravital, mitochondria-specific fluorochrome, we compared the mitochondrial activities and synaptic physiology of crayfish tonic and phasic abdominal motoneurons. Tonic flexor motoneurons showed greater activity-dependent resistance to synaptic depression than phasic extensor motoneurons. Axonal mitochondria from the former showed significantly higher mean Rh fluorescence intensities than did the phasic axons' mitochondria (as measured using confocal microscopy). Mitochondrial metabolic inhibitors (dinitrophenol, azide, CCCP) induced synaptic depression in tonic axons and reversibly abolished the Rh fluorescence of their mitochondria, suggesting that Rh fluorescence signals metabolic competence. In vivo stimulation of an identified phasic motoneuron significantly increased both neuromuscular synaptic stamina and mean axonal mitochondrial Rh fluorescence. Axotomy prior to conditioning stimulation abolished both changes. Thus, there exists a direct correlation between mitochondrial metabolic capacity and synaptic stamina in single living motor axons, while imposed electrical activity reshapes both metabolic and synaptic performance.

477.7

ELECTROGENIC Na-K PUMP IN SYMPATHETIC PRE-GANGLIONIC FIBRES IN BULLFROG LUMBAR SYMPATHETIC CHAIN. T.Hashiguchi[1], M.Hashiguchi[1], T.Tosaka[1] and A.L.Padjen[2]*. [1]Dept. of Physiology, Tokyo Medical College, Tokyo, Japan and [2]Dept. of Pharmacology and Therapeutics, McGill University, Montreal, Qc, Canada.

Excitability of nerve terminals in vertebrate nervous system is difficult to study in a direct way. We have used the sucrose gap technique applied on the sympathetic chain to measure membrane potential of preganglionic terminals of bullfrog sympathetic ganglia (X segment). A switching amplifier (T.Hashiguchi, Pflueg.Arch.406:540,1986) was used for simultaneous current injection and potential measurement.

Injection of a current pulse (200 - 400 ms) was followed by biphasic hyperpolarizing afterpotential (HAP) only after voltage displacement reached threshold for action potentials generation. HAP was unaffected by d-tubocurarine (10 μM) or atropine (1 μM), and thus not likely result of endogenous acetylcholine release. Removal of extracellular sodium (choline replacement), but not of Ca^{++} or Cl^-, abolished the HAP. Na,K-pump blockers (3-10 μM ouabain, Na-free lithium Ringer) as well as Na-azide blocked the HAP generation; however, 2,4-DNP (0.1 mM) only depressed it. Addition of cesium (.2 - 5 mM) caused a conc dependent increase in HAP amplitude (> 250%; 110% at ED_{60} of .7 mM Cs) and duration and slowing of the rate constant (control: 0.039/sec ± .014, n=12; 2 mM Cs: 0.017/sec ± .0024, n=4), more than could be explained by a depression of inward rectification. HAP was not accompanied by changes in membrane conductance.

These results suggest that HAP in preganglionic terminals results from activation of an electrogenic pump. (This work was supported in part by MRC and DHRC)

477.8

ALTERNATIVE SOLUTIONS OF AXONAL IMPULSE PROPAGATION INTO A LARGER-DIAMETER REGION. M.D. Goldfinger*, Dept. of Physiology & Biophysics, Wright State University, Dayton, OH 45401-0927.

Impulse propagation was simulated for unmyelinated axons whose diameter changed from 1 to 2-10 μm. The 1-dimensional cable equation was solved with a finite-difference discretization (1). The spatial integration step (Δx=3 μm) was constant; hence, the electrotonic step Δx/λ was geometry-dependent (2). The Hodgkin-Huxley equations (3) defined excitability. First-order differential equations were solved numerically with a second-order method which averaged the forward (explicit) and backward (implicit) Euler integrations over very small time steps (e.g., Δt = 0.1 μs) kept constant during each run (4). No predictor/corrector methods were used. The H&H temperature parameter was 10°C. It was assumed that Na, K, and leak maximal conductances and reversal potentials were geometry-independent. The point-to-point conduction velocity [CV(x)] was computed from the time of the action potential peak (T) at any point: $CV(i) = \Delta x/(T_i - T_{i-\Delta x})$ (2,4).

A single impulse propagated from the 1 μm-diameter region into a region with diameter d=2-10 μm. In the 1-μm region and within 200 μm of the diameter expansion, CV(x) changed axially: first increased, then slightly decreased, and finally increased at the expansion to a maintained value θ(d), where: $\theta(d) = \theta_{d=1}\sqrt{d}$. Impulse amplitude and duration were basically unaffected by the diameter expansion (cf 5). The CV(x) pattern was paralleled by axial changes in maximal negative membrane current density.

The CV(x) pattern with diameter expansion was due entirely to the decrease in axial load, since: (1) the pattern was duplicated in uniform-diameter axons with a reduction of internal resistivity instead of a diameter expansion; (2) the pattern reverted to a step change of CV(x) at the diameter expansion if the electrotonic step in the expanded region equalled that of the smaller-diameter region. Correcting the discretization for non-uniform diameter did not affect the result (6). However, the CV(x) pattern was sensitive to Δt and to inappropriate discretization.

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477.9

MODULATION OF LOCAL ANESTHETIC BLOCKADE IN SINGLE FIBERS BY ACTIVATION OF NEIGHBORS IN PERIPHERAL NERVE. S.A. Raymond*, S.C. Steffensen, J.G. Thalhammer and G.R. Strichartz. Anesthesia Research, Brigham & Women's Hospital, Boston, MA 02115.

Single axonal units from isolated frog sciatic nerves recorded via suction electrodes were stimulated with current pulses (1.5-3 times the tonic threshold for the recorded unit) during exposure of the nerve to stable concentrations of the local anesthetic lidocaine, [lido]. At [lido] just below the concentration needed to produce "tonic" conduction failure for above-threshold stimuli at rates $\leq$0.5 Hz, units conducted impulses at a slowed velocity but with 100% fidelity. Increasing the number of neighboring units also firing to each stimulus by increasing the stimulus current by a factor of 10 further slowed the conduction velocity, or CV, in 28 of 28 fibers studied, and led progressively to a full block of conduction. The impulse slowing began after the first impulse at the tenfold intensity and then progressively increased over about 30 s. Slowing or block also persisted for at least 30 s after the stimulus intensity was returned to control. No cumulative increase in latency nor failure was observed without lidocaine. Instead, the tenfold stimulus intensity with [lido] = 0 increased the CV by about 10% (reduced latency by 0.3-0.5 ms).

The recovery time for latency to return to the tonic latency, recorded prior to the tenfold increase in stimulus intensity, depended on the number of stimuli given at the higher intensity. If the intensity was maintained at the high level, activating more neighboring fibers, then the slowing or block was likewise maintained. Repeated activation of conditioning bursts of 8 impulses at tenfold intensity produced conduction failure that persisted for more than 30 min. The cumulative slowing and block was observed only for fibers near blocking concentration since in cases where multiple fibers were recorded, the ones not near block did not slow when the intensity was increased. Because of the 30 s accumulation and recovery times and because of a transient increase we observed in the CV after tetanic conditioning, which is known to result in post-tetanic hyperpolarization, we attribute the modulation of block to a potentiation of lidocaine binding by a depolarization of the affected axon by its activated neighbors, possibly resulting from increased extra-axonal potassium.

477.10

A NOVEL TARGET OF NITRIC OXIDE-STIMULATED ADP-RIBOSYLATION.
L.G. Hammerland*, A.M. Ferro and B.M. Olivera. Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112

Nitric oxide (NO) has been implicated as a retrograde messenger necessary for the induction of long-term potentiation (LTP). Although it is not understood how NO acts in this pathway, the two known physiological effects of NO are the activation of a soluble guanylate cyclase and the stimulation of an ADP-ribosyltransferase that targets a group of 39-42 kDa proteins which are found in many tissues, including brain. Here we report the identification and partial characterization, in a neural preparation, of a novel target of NO-stimulated ADP-ribosylation.

Because NO likely acts via presynaptic mechanisms, the membrane-associated targets of NO-stimulated ADP-ribosylation were identified using a biochemical model of the synapse, a presynaptic membrane fraction isolated from electroplax of *Torpedo californica*. ADP-ribosylation (using ^{32}PNAD as the ADP-ribose donor) of *Torpedo* presynaptic membranes resulted in the labeling of several proteins. However, the ADP-ribosylation of just one protein (apparent Mr = 110 kDa) was enhanced in the presence of the NO-generating compound sodium nitroprusside. This enhancement of ADP-ribosylation by nitroprusside was dose-dependent and was blocked by calcium. Furthermore, the 110 kDa protein was photoaffinity labeled by 8-azido-GTP, suggesting that the target of NO-stimulated ADP-ribosylation also binds guanine nucleotides. These results suggest that the NO-stimulated ADP-ribosyltransferase and its 110 kDa target protein may modulate synaptic transmission.

477.11

INTERLEUKIN-1β (IL-1β) INHIBITS SYNAPTIC TRANSMISSION IN THE BASOLATERAL AMYGDALA (BLA). B-J.Yu, D.G.Rainnie and P.Shinnick-Gallagher*. Dept. of Pharmacology, Univ. of Texas Medical Branch, Galveston, TX 77555

IL-1β, a cytokine, has central actions including anticonvulsant activity. We analyzed IL-1β effects on synaptic transmission using intracellular recording in the rat BLA, a nucleus involved in limbic epilepsy. In the BLA, stimulating the stria terminalis (ST) or lateral amygdala (LA) elicits excitatory postsynaptic potentials (EPSPs) and fast- and slow-inhibitory postsynaptic potentials (f-and s-IPSPs) via both feed-forward and direct pathways. The ST- and LA-evoked EPSPs were reversibly depressed by IL-1β (2 ng/ml). F- and s-IPSPs evoked by stimulating the ST and LA were also reduced or completely blocked suggesting direct and indirect effects on GABAergic transmission. Furthermore, the reversal potential for the IPSP was not altered by IL-1β. Analysis of input-output relationships indicated that the inhibitory effect of IL-1β on synaptic transmission was not overcome by increasing the stimulus intensity. We tested whether IL-1β inhibition was due to a pre- or post-synaptic action by applying agonists exogenously. Muscimol responses were not altered by IL-1β suggesting presynaptic inhibition of the f-IPSP. Responses to AMPA were slightly decreased but the ST- and LA-evoked EPSPs were decreased to a greater extent; IL-1β effects on AMPA responses were not reversible. These data suggest that IL-1β inhibits excitatory and inhibitory transmission at a presynaptic site and provide evidence that IL-1β, a mediator of the immune response, has an inhibitory effect on synaptic transmission in CNS neurons. Furthermore, these results suggest that the BLA nucleus may be a neuronal substrate for interactions between the immune and central nervous systems.(supported by NS 29265).

477.12

ENDOGENOUS ADENOSINE MEDIATES NEUROMUSCULAR DEPRESSION WITHOUT AFFECTING CALCIUM CURRENTS IN THE FROG. R.S. Redman* and E.M. Silinsky. Dept. Pharm., Northwestern Univ. Med. School., Chicago Illinois, 60611.

Studies at normal levels of acetylcholine output have suggested that at selected nerve endings, a proportion of presynaptic neuromuscular depression in frog is mediated by adenosine released by active neuromuscular junctions (Meriney & Grinnell, J. Physiol 443:441-455). Under such conditions, presynaptic facilitation occurs concomitantly with depression, thus partly obscuring the action of adenosine. In addition, Ca currents cannot be measured, precluding an investigation as to mechanism of depression. We have found that simultaneous measurements of Ca currents and quantal ACh release may be made in normal Ca solutions with the addition of 3,4, diaminopyridine (DAP, 100 μM) to block a proportion of K currents in the motor nerve. In this study we examined the contribution of endogenous adenosine to evoked ACh release and Ca currents in the frog cutaneous pectoris muscle in Ringer containing DAP, using fast flow methods for the rapid application of adenosine receptor reagents to the neuromuscular junction. Nerve stimulation at 0.1 Hz causes a gradual decrease in ACh release that is consistently and rapidly reversed by the selective adenosine receptor blocker 8-cyclopentyltheophylline (CPT, 1 μM) or by adenosine deaminase (5 i.u.). The normal inhibitory effect of adenosine or 2-chloroadenosine is occluded during synaptic depression. All of these effects occur without changes in perineural Ca currents. The results suggest that endogenous adenosine mediates neuromuscular depression by a mechanism unrelated to changes in Ca entry. This work was supported by NIH grants T32 NSO7140 and R01 NS12782.

477.13

EXTRACELLULAR MESSENGERS PRODUCED BY ECTO-PROTEIN KINASE IN DEVELOPING NEURONS. H. Yang, M.V. Hogan and Y.H. Ehrlich*. CSI/IBR Ctr. Dev. Neurosci. CUNY at Staten Island, NY 10301 and the Biology Doctoral Program of CUNY Graduate School.

Ecto-Protein kinase (ePK) utilizes extracellular ATP to phosphorylate proteins localized at the external surface of the neuronal plasma membrane. To determine the role of ePK in neuronal development, we investigate primary neurons cultured from the telencephon of 7-day chick embryos. Phosphorylation reactions are carried out with cells attached to 48-well plates. For ePK assays we add [γ-^{32}P]ATP (0.1μM; 15μCi per well) and incubate for 10 min. To label intracellular proteins an equivalent dose (15μCi per well) of inorganic ^{32}Pi is added. We found that two proteins with apparent MW of 11.7K and 13K became rapidly phosphorylated upon addition of [γ-^{32}P]ATP to the medium, but were not labeled at all even after 1-2 hrs incubation with ^{32}Pi. These proteins have been identified as exclusive substrates of ePK. The extracellular phosphorylation of these proteins was found to peak at the onset of rapid neuritogenesis. In the present study, attached cells were rinsed three times with Krebs-Ringer buffer just prior to the ePK reaction, to remove all soluble components. Immediately after 10 min incubation with [γ-^{32}P]ATP, the medium was separated from the attached cells and spun at 1000 x g/5 min. A soluble fraction was prepared from this supernatant by high speed centrifugation (150,000 xg/90 min). The 11.7K and 13K phosphorylated substrates of ePK were found in the soluble fraction. Thus, during the extracellular phosphorylation reaction these phosphoproteins *detach* from the cell surface. In developing neurons this process may provide a unique, novel means of communication: a signal sent from growing neurites to target neurons, and/or for interaction with components of the extracellular matrix.

478.1

AMINO ACIDS REQUIRED FOR FAST Na^+ CHANNEL INACTIVATION.

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Previous work with site-directed and cut mutants has shown that the intracellular peptide loop between homologous domains III and IV (LSP 19) of the rat brain Na^+ channel α subunit may function as the inactivation gate. To further resolve the role of LSP 19 in inactivation, we constructed Na^+ channel cDNAs encoding extensive changes in the amino acid sequence of LSP 19 and analysed their effects by expression in oocytes and in mammalian cells. The results of the experiments presented here show that charged amino acids in LSP 19 are not essential for inactivation of the channel. Mutations of charged residues caused only small increases or decreases in inactivation rate. Rapidly inactivating Na^+ currents are recorded in oocytes expressing mutant Na^+ channels with 10 of the 12 basic amino acids and 2 of the 3 acidic amino acids substituted with glutamine. In contrast, substitution of three contiguous nonpolar amino acids within LSP 19 removes fast inactivation when expressed in oocytes. Furthermore, substitution of a single phenylalanine with glutamine removes most of the fast inactivation. Na^+ currents were also recorded in mammalian cells expressing the phenylalanine mutation. Single channel recordings from cell-attached patches on these cells reveal rapid transitions between open and closed states which continue throughout 100 ms depolarizations indicating nearly complete removal of inactivation. Slow inactivation is retained. Further analysis of the gating properties of these mutant Na^+ channels lacking fast inactivation is in progress.

478.2

BIPHASIC MODULATION OF SODIUM CHANNELS BY PHOSPHORYLATION AT TWO DIFFERENT SITES.

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Phosphorylation of the voltage-dependent rat brain sodium channel by protein kinase C (PKC) has 2 effects: 1) Inactivation of the current is slowed and 2) Peak currents are reduced. We have previously shown that both effects are blocked by mutation of serine to an alanine in a PKC consensus site in the conserved intracellular loop between homologous domains III and IV of the α subunit (Numann et al, Science 254:115, 1991; West et al, Science 254, 866, 1991). Here we report that although phosphorylation at this site is necessary for both effects, a second site is also involved. Modulation by the protein kinase C activator 1-oleoyl-2-acetyl-*sn*-glycerol (OAG) was studied using cell-attached patches from Chinese hamster ovary cells stably expressing the rat brain type IIA sodium channel α subunit (CNaIIA-1 cells; West et al, Neuron 8, 59, 1992). Dose-response experiments showed that the slowing of inactivation saturated near 40 μM OAG whereas the reduction in peak current continued until doses of 70 to 80 μM OAG (n=44). Low concentrations of OAG (<10 μM) often produced slowing of inactivation with no reduction in peak current (n=8). These data suggested the involvement of at least two separate PKC phosphorylation sites. To identify the second site, we screened consensus PKC phosphorylation sites in the cytoplasmic loop connecting homologous domains I and II of the sodium channel α subunit. Mutation of serine to alanine at one site in this loop eliminates sodium current reduction by OAG but slowing of inactivation is unaffected (n=12). Similar mutation of a nearby phosphorylation site had no effect. The PKC system appears to cause biphasic modulation of sodium channels. Phosphorylation of serine 1506 by itself causes slowing of inactivation. Additional phosphorylation at a second site in the intracellular loop between domains I and II causes a reduction in peak sodium current.

478.3

CONVERGENT REGULATION OF BRAIN SODIUM CHANNELS BY CAMP-DEPENDENT PROTEIN KINASE AND PROTEIN KINASE C.

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Direct application of the catalytic subunit of cAMP-dependent protein kinase (cA-PK) and ATP to the cytoplasmic surface of rat brain Na channels in excised inside-out membrane patches reduces channel activity. Protein kinase C (PKC) phosphorylation of Ser1506 in the conserved intracellular loop between domains III and IV of the α subunit slows inactivation and allows reduction of channel activity by phosphorylation at another site in the loop between domains I and II (Numann et al, preceding abstract). Since phosphorylation at Ser1506 is necessary but not sufficient for reduction of Na current by PKC, we examined whether it might also control reduction of channel activity by cA-PK. Na current recorded in 14 macro-patches excised from Chinese hamster ovary cells stably expressing Type IIA sodium channels with the mutation Ser1506Ala were unaffected by application of cA-PK and ATP. Application of cA-PK and ATP to 6 patches containing wild-type channels reduced the current by 40% in the same series of experiments. The reduction in wild-type channel activity by cA-PK cannot be due to phosphorylation at Ser1506 since: 1) this site is not phosphorylated by cA-PK in biochemical experiments and 2) phosphorylation at this site by PKC causes only slowing of inactivation. Evidently, phosphorylation of Ser1506 by PKC is necessary for reduction of Na channel activity by phosphorylation of a second site by cA-PK. The PKC consensus site at ser1506 was converted to a cA-PK consensus site by conversion of Lys1507 and Lys1508 to Gln. In contrast to wild-type, Na currents are slowed and reduced by cA-PK and ATP. The effects of cA-PK are similar to PKC in wild-type and consistent with the conclusion that phosphorylation of Ser1506 by PKC slows Na channel inactivation and regulates the reduction of Na channel activity by both PKC and cA-PK.

478.4

MUTAGENESIS OF THE RAT BRAIN SODIUM CHANNEL PROTEIN KINASE A PHOSPHORYLATION SITES.

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We have previously demonstrated that induction of protein kinase A (PKA) in *Xenopus* oocytes results in an increase in sodium currents expressed from the rat brain type IIa sodium channel clone. We have now used site-directed mutagenesis to determine if the current enhancement by PKA was the result of direct phosphorylation of the sodium channel α subunit. The rat IIa channel has five consensus PKA phosphorylation sites located in the linker region between domains I and II. Phosphorylation at these five sites was either eliminated by replacing the serine residues with alanine, or was mimicked by replacing the serines with aspartates. All five of the serine to alanine substitutions resulted in functional channels in oocytes, with variable levels of current. The voltage-dependent properties and inactivation kinetics of all of the mutants were similar to that of the wild-type channel. All five of the mutant channels also showed some current enhancement following PKA stimulation, but the magnitude of the effect was quite variable and depended on the specific mutation. Four of the five serine to aspartate mutants have thus far been expressed and are similar to the wild-type channel in preliminary experiments. When all five PKA sites were replaced with alanine in a single mutant, however, the level of current was less than 100 nA, at least 1000 fold lower than that seen with the wild type sodium channel. This result implies that phosphorylation at some subset of the PKA sites is necessary for normal sodium channel function in *Xenopus* oocytes. To test whether phosphorylation at any single PKA site is sufficient for normal levels of current, each of the consensus sites in the composite mutant was reverted back to the original wild-type sequence. These mutants are currently being analyzed. Finally, we are examining a composite mutant with all five serines replaced by aspartates to test whether a negative charge at each position is sufficient for normal sodium channel function.

478.5

DEDUCED AMINO ACID SEQUENCE OF A PUTATIVE SODIUM CHANNEL FROM THE JELLYFISH *CYANEA CAPILLATA*.

Robert M. Greenberg*, Molly A. Holman and Peter A.V. Anderson, Whitney Laboratory, Univ. of Florida, St. Augustine, Fl 32086.

The Na^+ channel in *Cyanea* is a Na^+ channel physiologically but a Ca^{++} channel pharmacologically. Using PCR with degenerate primers based on conserved sequences from other Na^+ channels, we isolated a 900bp product with high sequence homology (60% identity) to known Na^+ channels. This fragment was used to probe a Northern blot of *Cyanea* mRNA, resulting in specific labelling of a 8.5kb band. Additional fragments of this putative Na^+ channel have now been obtained and sequenced, yielding a single open reading frame of 4.3kb. Residual portions at the 3' and 5' ends of the cDNA are currently being isolated. The deduced amino acid sequence from this cDNA shows high sequence homology with other Na^+ channels (35-45% identity, 76% conservation) particularly in the transmembrane spanning regions.

Scrutiny of this cDNA in regions corresponding to the verapamil binding site of Ca^{++} channels and the TTX binding site of Na^+ channels reveals important clues as to the amino acids involved in binding these important channel blockers.

Supported by NSF grant BNS-9109155 to PAVA and funds from the Univ. of Florida DSR.

478.6

CLONING AND SEQUENCE OF A PUTATIVE SQUID SODIUM CHANNEL cDNA AND A PROPOSED TERTIARY STRUCTURE MODEL.

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With polymerase chain reaction and recombinant DNA techniques, we have cloned DNA complementary to RNA which seems to encode a sodium channel protein of the optic lobe of squid Loligo bleekeri. The total number of amino acid residues deduced from the cDNA is 1,522, about three-fourths of that of rat brain I, II and III (each being about 2,000) and an even greater proportion in eel (1,820). The estimated molecular weight of the squid sodium channel protein is 174,105 dalton. The squid sodium channel is basically quite similar to those of vertebrates, and consists of four domains I, II, III and IV, each containing five hydrophobic segments (S1, S2, S3, S5 and S6), one characteristic segment with strong positive charge (S4), and short intervening sequences. Both a negative charge and a positive one in S2, and a negative charge in S3 as well, are entirely conserved at identical positions in all the domains; but only 3 positive charges are serially repeated at every third position in II-S4. On the basis of the amino acid sequence data, we propose a tertiary structure model of the squid sodium channel.

478.7

CLONING AND CHARACTERIZATION OF *tip-E* GENE. L. M. Hall*, G. Feng, J. Pursey Lee, P. Deak and E. R. Reynolds. Department of Biochemical Pharmacology, SUNY at Buffalo, Buffalo, NY 14260.

The *tip-E* mutation is an ethylmethane sulfonate-induced recessive mutation which shows a temperature-sensitive paralytic phenotype. Biochemical and electrophysiological studies showed that *tip-E* is associated with reduced binding of saxitoxin to sodium channels and decreased sodium current density. These data suggest that *tip-E* affects the function or regulation of sodium channels. We have induced a chromosome deletion/translocation which gives same phenotype as *tip-E* and have cloned a genomic fragment containing the *tip-E* gene based on the chromosome deletion/translocation breakpoint. Northern analysis showed that this genomic region contains 4 different mRNAs (2.0, 3.2, 4.0, and 5.2 kb). A cDNA clone corresponding to the 4 kb mRNA was sequenced. It encodes a novel protein of 452 amino acids with two putative hydrophobic regions. We believe this is the *tip-E* gene because it is disrupted in the deletion/translocation which gives the *tip-E* phenotype. In addition, the sequence of genomic DNA from *tip-E* mutants showed a single nucleotide change resulting in a stop codon in the region corresponding to the open reading frame of the cDNA. This change would cause premature termination of the 4 kb mRNA encoded protein. Transformation experiments are in progress to determine whether the genomic fragment encoding the 4 kb mRNA can rescue the *tip-E* phenotype.

This work is supported by NIH grant NS16204.

478.8

The SkM2 Na^+-Channel Gene Is Expressed In Rat Dorsal Root Ganglia L.M. Donahue*, K.A. Moffatt and N. Sueoka[1]. Dept. of Cell Biol. and Anat., Texas Tech Univ. H.S.C. Lubbock, TX 79430; [1]Dept. of Mol., Cell. and Dev. Biology, University of Colorado, Boulder, CO 80309.

Previous work by our laboratories has shown that SkM2 is the predominant Na^+-channel gene expressed by the neuronal derivatives of the RT4 cell line family. Since the RT4 family was derived from a rat peripheral neurotumor, our results presented the possibility that the SkM2 Na^+-channel gene might be important *in vivo* in the rat PNS. In addition our findings suggested that the RT4 system may derive from dorsal root ganglia (DRG) since small cell neurons from rat DRG have tetrodotoxin (TTX) resistant Na^+-current and the only known Na^+-channel gene thought to encode a TTX-resistant channel is the SkM2 gene. We are pursuing two lines of investigation: the first to ask whether SkM2 is expressed *in vivo* in rat DRG and the second to ask whether other markers of small cell DRG neurons are expressed by the RT4 neuronal cell types. We have examined the expression of SkM2 from newborn rats by RNase protection assay and detect low levels of SkM2 mRNA. We are currently in the process of examining DRG by *in situ* hybridization histochemistry to determine which DRG cell type(s) express SkM2. We have also determined that the RT4 neuronal derivatives do not express the three neurofilament proteins, as is the case for small cell DRG neurons. Small cell DRG neurons express the intermediate filament protein peripherin and we are presently examining whether the RT4 neuronal derivatives express peripherin.

478.9

ALTERED RELATIVE EXPRESSION OF TWO HUMAN BRAIN SODIUM CHANNEL mRNAS IN PATIENTS WITH EPILEPSY. A. J. Lombardo*, C. M. Lu, R. Kuzniecky and G. B. Brown. Psychiatry and Behavioral Neurobiology, Neurology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Voltage-gated sodium channels are important contributors to the intrinsic excitability properties of CNS neurons. The aim of this study was to determine whether the relative expression of human brain sodium channel (HBSC) subtypes I and II is altered in epileptic brains compared to those of normal controls. Previous studies have shown that the ratio of these two subtype mRNAs varies markedly between different normal brain regions along the neural axis, but for a given region the value is generally consistent between individuals (Lu *et al.*, FEBS Lett., in press).

Epileptic tissue was obtained at surgery from patients undergoing treatment for intractable seizures. Postmortem tissues matched for brain region, age and sex were used as normal controls. Following RNA extraction, the relative amounts of type I and II sodium channel mRNA was compared in these tissues using a modification of the ligase detection reaction as described previously (Lu *et al.*, FEBS Lett., in press). This protocol, combining high sensitivity and high specificity, proves useful for measurements of highly homologous messages such as HBSC I and II, since specificity is provided at the level of single nucleotide differences.

Compared to normal postmortem controls, the data reveal an increased range of the I:II ratio in epileptic tissues, with moderate relative increases in HBSC I-specific transcripts. Additionally, a I:II ratio of 0.43 was determined for normal temporal lobe, a region not previously characterized with respect to sodium channel mRNA expression. These results suggest a potential correlation between epileptic activity and alterations in the expression of subtypes I and II of the human brain sodium channel. Moreover, these results may lend insight into the control of neuronal excitability and its contribution to the state of hyperexcitability in epileptic tissue.

478.10

PORE-FORMING REGIONS ON VOLTAGE-SENSITIVE POTASSIUM AND SODIUM CHANNELS SHARE A COMMON SECONDARY STRUCTURAL MOTIF TARGETED BY STRUCTURALLY SIMILAR SCORPION TOXINS. John A. Schetz*, Department of Neuroscience and The Whitney Laboratory of the University of Florida, Gainesville, Florida 32610.

Secondary structure within the putative S5-S6 pore-forming regions of voltage-sensitive K^+ and Na^+ channels was compared and quantified with a statistical metric (SHC (C) version 6.10). The metric predicts that the α-scorpion toxin receptor region mapped to IS5-IS6 on rat brain II Na^+ channel and the charybdotoxin receptor region mapped to S5-S6 on the *Shaker* A K^+ potassium channel are structurally similar (overall fit = 92%), even though their amino acid sequences are sparingly homologous (18%). The striking secondary structural similarity between K^+ channel and Na^+ channel sequences in comparable S5-S6 regions demonstrates that scorpion peptide toxins target structurally similar channel receptor sites. This result, in combination with the fact that the peptide ligands, charybdotoxin and α-scorpion toxin Aa II, are themselves structurally similar (Bontems *et al* (1991), *Science*, 254, 1521-1523), invites the hypothesis that venomous peptide ligands of comparable structure bind "pore"/receptor regions of comparable structure.

478.11

BIOCHEMICAL EVIDENCE THAT THE TTX-INSENSITIVE SODIUM CHANNEL IN THE JELLYFISH *CYANEA CAPILLATA* IS A HIGH MOLECULAR WEIGHT PROTEIN WHICH BINDS THE CALCIUM CHANNEL BLOCKER AZIDOPINE. Peter A. V. Anderson* and John A. Schetz, Dept. of Neuroscience and the Whitney Laboratory, Univ. of Florida, 9505 Ocean Shore Blvd., St. Augustine, Florida 32086.

Previous electrophysiological studies of neurons from the jellyfish *Cyanea capillata* (Anderson (1987), *J. Physiol.*, 396, 86P) revealed the presence of a fast Na^+ current which is completely insensitive to the classic Na^+ channel blocker, TTX, but it is blocked by the L-type Ca^{++}-channel blockers known as dihydropyridines (DHP). Since *Cyanea* Ca^{++} currents are insensitive to DHPs, this unusual combination of channel pharmacology in *Cyanea* allowed biochemical analysis of the *Cyanea* Na^+ channel. Isolated membranes from nerve-rich tissue in *Cyanea* were digitonin-solubilized, applied to an azidopine affinity column and eluted with drug. Column eluates were concentrated, then size fractionated by SDS-PAGE. Silver staining revealed a distinct protein band with an molecular weight of approximately 200 KDa only in column fractions eluted with a concentrated DHP solution. This size is consistent with that deduced from a putative Na^+ channel cDNA isolated from *Cyanea*.

Special thanks to Dr. H. Glossman for providing Azidopine. Supported by NSF grant BNS 9109155 (PAVA).

478.12

NEUROANATOMICAL DISTRIBUTION AND BINDING CHARACTERISTICS OF SAXITOXIN SITES IN THE RAT AND TURTLE CNS. Y. Xia* and G.G. Haddad. Dept. of Pediatrics, Section of Respiratory Medicine, Yale Univ. Sch. Med., New Haven, CT

Although the literature on saxitoxin (STX) binding in the CNS is abundant, there is little data on the neuroanatomical distribution and STX binding properties in various brain nuclei. In the present study, we examined in detail the saturation profiles of STX binding at several CNS levels using a broad concentration range of ^{3}H-STX (up to 64 nM) and mapped STX sites at low (2 nM) and high (15 nM) concentrations of ^{3}H-STX. Since the density of STX binding sites (Na^+ channels) may be an important factor that determines resistance to anoxia, we also compared rat to turtle CNS. We found that 1) Scatchard plots were linear with Kd values around 5 nM for a number of areas including most cortical areas; 2) in some nuclei, especially in the brainstem, these plots showed an increase followed by a decrease in Bound/Free Versus Bound; 3) STX binding density was heterogeneous at both binding concentrations with a much higher density in rostral areas than in the brainstem; and 4) saturation patterns in the turtle brain were similar to those of the rat, but STX binding density was much lower (several to 10 fold) than in the rat. We conclude that a) STX binds mainly to a high affinity site and b) positive cooperativity occurs with STX binding in some areas. Since previous results from this lab have shown that Na^+ plays an important role during anoxia, we speculate that low binding density in the turtle brain may be partly at the basis of its hypoxic tolerance.

478.13

DEVELOPMENT OF THE SODIUM CHANNEL HIGH AFFINITY SAXITOXIN (STX) RECEPTOR IN RAT BRAIN. R. Villegas, C. Castillo, M. E. Póo, S. Schnell, C. Piernavieja, D. Balbi and G. M. Villegas*. Instituto Internacional de Estudios Avanzados (IDEA), Apartado 17606, Caracas 1015A, Venezuela.

The course of Na channel (NaC) gene transcription in Wistar rat forebrain was followed by measuring the levels of total NaC mRNA and types I, II and III NaC mRNAs, utilizing cDNA probes. Embryos (E) and postnatal (P) rats were studied. Total NaC mRNA increases steadily from E15 up to a maximun level at P14 (100%), declining then smoothly up to P90 (≈65%). Measurements of types I, II and III in E15, P0 and P15 reveal that: II, the most abundant, increases steadily up to P15 (100%); I increases after birth (10% of II at P15), and III increases until birth (21% of II at P15) and then declines. The amount of high affinity STX receptors, measured with ^{3}H-STX, rises to a plateau with a similar course to that observed for the total NaC mRNA, with some delay in the rising phase. Maximum binding (B_{max}) in pmoles/g wet weight and K_D in nM, determined in 200 mM NaCl (pH 7.4), are: 2.6 and 38.3 for E15; 26.3 and 3.1 for P0; 145.1 and 3.3 for P15. The STX affinity of batrachotoxin-modified NaC from E15, P0 and P15, incorporated into lipid bilayers in the same NaCl solution, was studied; the K_D(0 mV) in nM is 31.9 for E15; 9.9 for P0, and 3.9 for P15. Our results provide new information on the development of the high affinity STX/TTX receptor of rat brain Na channels.

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478.14

EFFECTS OF SCORPION VENOM, SEA ANEMONE TOXIN, GRAYANOTOXIN, AND DELTAMETHRIN ON TETRODOTOXIN-SENSITIVE AND TETRODOTOXIN-RESISTANT SODIUM CHANNELS. Mary-Louise Roy* and Toshio Narahashi, Dept. of Pharmacology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Dorsal root ganglion neurons acutely dissociated from 3-12 day old rats express TTX-sensitive (TTX-S, K_D~1 nM) and TTX-resistant (TTX-R, K_D~100 μM) sodium channels, which have been shown to differ in their biophysical and pharmacological properties. Leiurus quinquestriatus scorpion venom and sea anemone toxin ATX-II (1-100 nM) exerted no significant effects on TTX-S and TTX-R currents. Tissue dissociation procedures (collagenase-dispase, papain, trypsin, and mechanical dissociation) were systematically examined, each generating the same results. The binding region(s) of both toxins may thus be absent in these channels. α-Dihydro-grayanotoxin (GTX) and deltamethrin, however, each exerted differential effects on TTX-S and TTX-R channels. GTX (100 nM-10 μM) shifted TTX-S channel activation voltage slightly (-2.5 ± 4.1 mV, n=6), without affecting reversal potential. TTX-R current activation was minimally affected (-3.5 ± 4.3 mV, n=8) whereas the reversal potential was dramatically shifted (-17.9 ± 9.1 mV). Deltamethrin (1-100 nM), a type II pyrethroid, shifted activation and reversal potentials of the TTX-S current, without a significant effect on tail current. TTX-R tail current, however, was greatly prolonged, with τ_{slow} values of greater than 10 seconds. The differential properties of the TTX-R and TTX-S sodium channels continue to be of interest in CNS development and drug interactions. Supported by NIH grants F31 MH09839 and R01 NS14143.

478.15

DIFFERENCES IN THE NEUROEXCITATORY ACTIONS OF SODIUM CHANNEL SPECIFIC NEUROTOXINS IN RAT AND TROUT BRAIN SYNAPTOSOMES. J. T. Eells*, K. A. Pleyte, J. L. Rasmussen and P. A. Holman. Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

The effects of pyrethroid insecticides and other sodium channel specific neurotoxins on synaptosomal membrane potential were investigated in rat and trout brain synaptosomes using a membrane permeant, lipophilic cation [^{3}H]-tetraphenylphosphonium (TPP+). Concentration-dependent and tetrodotoxin-sensitive decreases in TPP+ accumulation indicative of membrane depolarization were produced by aconitine (Acn), veratridine (Vtd), scorpion (*Leiurus quinquestratus*) venom (ScV) and type I and type II pyrethroids in both species. Acn, Vtd and ScV were more potent and efficacious membrane depolarizing agents in rat synaptosomes than in trout synaptosomes. Type II (deltamethrin, cypermethrin and fenvalerate) pyrethroids produced similar depolarizing responses in rat and trout synaptosomes, however, the *1R,cis,αR* isomer of deltamethrin which had no effect on membrane potential in rat synaptosomes depolarized trout synaptosomes. In addition, the type I pyrethroid, permethrin, exhibited significantly greater efficacy in trout synaptosomes producing a maximal estimated membrane depolarization of 14 mV compared to 5 mV in the rat. These results provide evidence of interspecies differences in the membrane depolarizing properties of several sodium channel specific neurotoxins and suggest that some of the neurotoxin binding domains of the voltage-sensitive sodium channel in trout brain differ from those in mammalian brain. The hypersensitivity of fish to the neurotoxic actions of pyrethroid insecticides may be related to these differences. (ES05006 and ES01985)

478.16

THE SODIUM CHANNEL MARKS NEUROELECTROGENESIS IN EPILEPTIC MICE Hatch D.J., Smart M.L., Esplin M.S., Mouritsen C.L., & Litzinger M.J.* Depts. of Pediatrics and Physiology. University of Utah. SLC, Ut. 84132

Neuroelectrogenesis is the initiation of electrical activity in the central nervous system. Our study uses the sodium channel marker saxotoxin (STX) to follow the development of this ion channel in Swiss Webster (non-epileptic) and DBA (audiogenic seizure) mice. In 1972 ,Hafemann and Unsworth used tetrodotoxin, which also lables the sodium channel,to mark a critical period in the sodium spike generating system of Swiss Webster mouse brain. This critical period , postnatal days 10-15 ,corresponds to an increase in cortical width & weight, the maturation of dendritic spines for synapse formation and the onset of electrical activity (Himwhich, 1962). Hafemann and Unsworth concluded that the sodium channel was necessary though not sufficient for action potential propagation. Prior studies from this lab have shown differences in voltage sensitive calcium channel development between Swiss Webster and DBA mice during the critical period (Esplin, et. al., 1991).

Susceptibility of the DBA mice to seizure is greatest at juvenile ages (days 16-21) and dissipates thereafter. Data comparing binding of ^{3}H -STX in Swiss Webster and DBA mice on postnatal days 8 and 16, the critical period , show an increase in both groups. Swiss Webster shows a 33% increase; DBA shows a 20% increase. Hafemann & Unsworth showed a drastic increase (two fold) in tetrodotoxin binding during this time frame. Preliminary data in the DBA audiogenic seizure mice suggest a gradual increase in sodium channel development between days 3-16. This study suggest a difference in soudium channel development which may underlie the basic mechanism of audiogenic seizures. (This work was supported in part by NIH grant # HD00886.)

478.17

EFFECT OF SODIUM CHANNEL MODULATING DRUGS ON LOSS OF EPSPs FROM HYPOXIA/HYPOGLYCEMIA IN RAT HIPPOCAMPAL SLICES IN VITRO. C.P. Taylor* and M.L. Weber. Parke-Davis Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

Previous studies with phenytoin, lidocaine and tetrodotoxin in various models of ischemia have shown neuroprotection, suggesting the involvement of voltage-sensitive sodium channels in ischemic brain damage. We investigated this hypothesis with several modulators of voltage-sensitive sodium channels using rat hippocampal slices under conditions that mimic ischemia. Hippocampal slices were incubated under control conditions and then subjected to a 12-min period of "ischemia" from deprivation of oxygen and reduction of D-glucose from 10 mM to 2 mM. This treatment caused a loss of synaptic potentials over 2-5 min and a sudden negative shift in extracellular voltage corresponding to loss of ion homeostasis after about 8 min. Return of normal medium failed to recover EPSPs in any of 36 control experiments.

However, treatment with phenytoin (20 or 50 μM), lidocaine (50 or 100 μM), carbamazepine (50 μM), or verapamil (10 μM) significantly delayed the onset of negative voltage shifts and increased the number of slices that recovered EPSPs. These neuroprotective effects were seen at drug concentrations that did not alter presynaptic action potential amplitude, indicating that sodium channels were modulated but not blocked. Nimodipine at a concentration (1 μM) that modulates L-type voltage-sensitive calcium channels, had no effect. These results suggest that modulation of voltage-dependent sodium channels by anticonvulsant or local anesthetic drugs may be beneficial for treatment of cerebral ischemia.

478.18

IMPAIRED Na CHANNEL INACTIVATION PRODUCES MYOTONIA IN RAT SKELETAL MUSCLE. S. C. Cannon* and D. P. Corey, Department of Neurology and Howard Hughes Medical Institute, Massachusetts General Hospital, Boston, MA 02114.

The myotonias are a group of heritable muscle disorders that share the common phenotype of delayed relaxation after contraction due to enhanced electrical excitability of the muscle membrane. Several forms of myotonia have recently been postulated to arise from a defect in the inactivation of the Na current. In myotubes cultured from patients with a myotonic form of hyperkalemic periodic paralysis, a small elevation in extracellular [K] causes about 5% of the defective channels to gate in a non-inactivating mode (**Neuron** 6:619, 1991). We used both an *in vitro* assay and a theoretical model to test whether this degree of impaired Na channel inactivation was sufficient to cause myotonia.

Partial impairment of Na channel inactivation was obtained by applying a toxin from *Anemonia sulcata* (ATXII) to excised fast twitch fibers from the rat hindleg. With 10 μM ATX II, unitary Na currents recorded from bleb-attached patches had an average Popen of 0.02 in steady-state. With this degree of inactivation failure, twitch relaxation was slowed ten-fold and trains of repetitive discharges were elicited by 300 msec steps of current injection. Myotonic discharges continued after the end of the current injection. Detubulation by osmotic shock abolished the after-discharges which suggests they are driven by T-tubular K accumulation. Computer simulation predicted myotonic behavior when 1-2% of the Na channels fail to inactivate, and paralysis by depolarization block with ≥4%.

478.19

ANTIMYOTONIC ACTIVITY AND USE-DEPENDENT BLOCK OF SKELETAL MUSCLE Na^+ CHANNELS BY ENANTIOMERS OF TOCAINIDE AND MEXILETINE. A. De Luca*, C. Franchini, V. Tortorella, S.H. Bryant and D. Conte Camerino. Depts. of Pharmacobiology and Pharmacochemistry, Fac. of Pharmacy, Univ. of Bari, Italy and Dept. of Pharmacol. and Cell Biophysics, Univ. of Cincinnati, USA.

The use dependence of class I antiarrhythmic drugs, as tocainide, is related to high affinity receptor interaction during slow inactivation states of Na^+ channels (De Luca et al, Naunyn-Schm., 344: 596, 1991). In mammalian muscle cells this receptor has been described to be stereospecific (Tricarico et al., Pflügers Arch, 418: 500, 1991). To clarify the role of stereospecificity in the use-dependence of these drugs, the ability of enantiomers of tocainide and mexiletine to solve abnormal membrane excitability was evaluated "in vitro", by means of two intracellular microelectrodes technique, on rat extensor digitorum longus (EDL) muscle, made myotonic by previous incubation with 50μM anthracene-9-carboxylic acid (9AC), and on external intercostal muscle fibers from congenitally myotonic goats.On rat EDL, 30μM R(-) tocainide complitely antagonized the 9AC induced hyperexcitability, whereas 50μM S(+) inhibited only by 25% the myotonic repetitive firing. On the other hands, 50μM of R(-) and S(+) mexiletine inhibited 9AC hyperexcitability by 70% and 60%, respectively. On myotonic goats, 10μM R(-) tocainide fully restored normal membrane excitability, whereas 100μM S(+) was almost uneffective. These compounds were also tested on Na^+ currents recorded from frog semitendinosus muscle fibers by means of triple Vaseline gap voltage clamp. External application of 500μM of either R(-), S(+) and RS tocainide blocked Na^+ currents by 25-50%. Similarly, both enantiomers of mexiletine had an IC50 of about 300μM. Either enantiomers or racemates of tocainide and mexiletine were strongly use-dependent, i.e. they further cumulatively reduced peak Na^+ current (up to 50-70%) upon repetitive stimulation at 2Hz frequency. The present data suggest that the presence of a weakly stereospecific receptor (as in frog muscle), does not impair the use-dependence of Na^+ channel blockers. However, the presence of highly stereospecific site, as in mammals, modulates use dependence of drugs with pronounced stereoselectivity, as tocainide and this might have important therapeutical implications. (Granted by CNR 91.00226 and Telethon-Italy, 1991).

478.20

SODIUM CHANNEL BLOCKADE AND ANTICONVULSANT ACTIVITY OF SUBSTITUTED 2-AMINOBENZOTHIAZOLES. Sheryl J. Hays*, Michael J. Rice, Daniel F. Ortwine, Graham Johnson, Roy D. Schwarz, Denise K. Boyd, Laura C. Rodolosi and Peter A. Boxer. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI 48105.

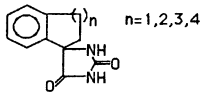
Modulation of sodium channels is a mechanism shared by the clinically useful anticonvulsants, phenytoin and carbamazepine. Riluzole, 2-amino-6-(trifluoromethoxy)benzothiazole was reported to be a potent anticonvulsant agent which initially was believed to function via a glutamatergic mechanism. Recent evidence suggests that riluzole is exerting its activity by a direct blockade of voltage-dependent sodium channels.

To understand the potential of 2-aminobenzothiazoles to function as anticonvulsants, a series of 4-, 5-, and 6-aryl-substituted benzothiazoles have been examined for their ability to inhibit sodium flux. Many of the compounds were found to be potent inhibitors of veratridine-stimulated influx of sodium into rat neocortical slices. A quantitative structure-activity relationship study has been completed on this set of benzothiazoles and suggests a trend toward increased potency with increasing lipophilicity, decreasing substituent size, increasing electron withdrawal capacity, and the absence of a 4- or 5-substituent. These compounds were further examined for anticonvulsant activity in a maximal electroshock (MES) model in mice. Anticonvulsant activity (as well as ataxia at higher doses) was observed in all potent sodium flux inhibitors.

478.21

SODIUM CHANNEL BINDING ACTIVITIES FOR DIPHENYLHYDANTOIN ANALOGS. M.L. Brown, W.J. Brouillette, G.B. Brown*, Departments of Chemistry , Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, AL 35294.

The anticonvulsant 5,5-diphenylhydantoin (Phenytoin or DPH) binds to the neuronal voltage-sensitive sodium channel in a voltage- and frequency dependant manner. Specific binding occurs at physiologically relevant concentrations, suggesting that the sodium channel may be an anticonvulsant "receptor site" for DPH. Previous studies from this laboratory suggested that the DPH binding site may require a specific phenyl ring orientation. We have been interested in determining an optimum phenyl ring orientation and possible stereoselective preference for this site. In order to investigate these possibilities, we have synthesized several spirocyclic hydantoins (general structure I) which restrain the 5-phenyl ring to different angular relationships with the hydantoin ring. Increased binding occurred for compounds able to adopt a phenyl ring orientation relatively "coplanar" to the hydantoin ring. The sodium channel binding activities for some enantiomers showed modest selectivity.



Structure I

(Supported by the NCEA Mentoring Program, a Patricia Robert-Harris Fellowship to M.L.B. and the UAB Department of Chemistry.)

478.22

SPECIES DIFFERENCES IN THE PARANODAL DISTRIBUTION OF SODIUM CHANNELS. M.A. Ferguson, S. Tinkle, J.D. England, A. Koszowski, S.R. Levinson* Departments of Neurology and Physiology, University of Colorado, Denver, Colorado 80262.

A polyclonal antibody was raised against rat skeletal muscle sodium channel (M1 isotype) amino acid residues 1306-1324, a conserved sequence among all known vertebrate sodium channel isotypes. The specificity and high titre of these antibodies permit their reliable use with Western blot, RIA, and immunocytochemistry in a wide range of excitable tissues from fish and mammals. Using these site-directed antibodies for immunocytochemistry, we have demonstrated specific immunoreactivity (IR) at the nodes of Ranvier in both goldfish lateral line nerve and mouse sciatic nerve. In mouse sciatic nerve, but not goldfish nerve, we also observed specific IR on the paranodal myelin. Confocal microscopy demonstrates that this specific IR is patchy but localized to the outer loops of the paranodal myelin. These observations suggest that in mice sodium channels are present in paranodal myelin as well as at the nodes of Ranvier, whereas in fish sodium channels are present only at nodes. Thus, a species difference may exist for the presence of sodium channels within Schwann cells - myelin.

478.23

CHANGES IN THE DISTRIBUTION OF SODIUM CHANNELS DURING ACUTE TOXIC AXONAL INJURY. F. Gamboni, J.D. England, M.Ferguson, S.R. Levinson, Warren Wickelgren* Departments of Neurology and Physiology, University of Colorado, Denver, Colorado 80262.

Potassium tellurite (K_2TeO_3) is a neurotoxin known to cause acute demyelination in the peripheral nervous system of many experimental animals. When 3 ug of this agent is injected intraneurally into the posterior lateral line nerve of goldfish, demyelination is followed by profound axonal degeneration in the segment of nerve distal to the site of injection. The proximal segments of these nerves were analyzed on days 4,7,10,15, and 21 postinjection by both immunocytochemical and RIA methods, using an antibody specific for the sodium channel molecule (NaCh) in fish nerve. These studies showed a transient increase (0.5 to 5 fold compared to normal nerves) in the concentration of NaCh in the proximal nerve segments between days 7 to 10 postinjection. Furthermore, the immunocytochemistry showed that NaChs accumulated in the axolemma immediately proximal to the site of the lesions. These findings support the theory that NaChs in peripheral nerve are synthesized primarily in the neuronal perikaryon, transported down the axon, and then inserted into the axolemma.

478.24

COMPARISION OF THE FUNCTIONAL PROPERTIES OF HUMAN AND RAT NEOCORTICAL SODIUM CURRENTS.

T.R. Cummins[1*], Y. Xia[2] and G.G. Haddad[1,2]. [1]Interdepartmental Neuroscience Program and [2]Dept. of Pediatrics, Yale Univ. Sch. Med., New Haven CT 06510.

Rat and human brain sodium channels have a high degree of homology, but it is not clear how rat and human channels compare in terms of expression and function. We therefore studied the functional properties and STX-binding characteristics of sodium channels in adult human, adult rat and neonatal rat neocortex using whole-cell voltage clamp of acutely dissociated pyramidal neurons and quantitative autoradiography.

Adult human and rat neurons were very similar in terms of Na^+ current properties and current density (~700 and 1000 pA/pF, respectively), while neonatal rat neurons had a significantly lower current density (< 200 pA/pF). The STX binding site density was 10 fold lower in rat neonatal neocortex than in adult human and rat neocortex. In human and rat neocortex, only a single, high-affinity STX binding site was found in both neonatal and adult tissue (Kd ~ 4 nM). Data from our laboratory has shown that the sodium channel density in the adult rat brainstem is 3 fold lower than that found in adult rat neocortex. We conclude that, while in the rat significant regional and maturational differences are found in sodium channel density, human and rat adult neocortical sodium currents are similar in terms of function and level of expression.

478.25

NON-INACTIVATING, TTX-SENSITIVE Na^+ CONDUCTANCE IN RAT OPTIC NERVE AXONS: POSSIBLE PHYSIOLOGICAL AND PATHOLOGICAL ROLES. P.K.Stys*, H.Sontheimer, B.R.Ransom, S.G.Waxman. Dept. Neurology, Yale School of Medicine, New Haven, CT, 06510.

Sodium channels provide the rapid depolarization underlying axonal action potentials. Classical channel kinetics dictate that these channels quickly and completely inactivate at depolarized potentials, contributing to rapid termination of the action potential. We have used the *in vitro* rat optic nerve (RON) as a model to study the ionic mechanisms of anoxic injury in central myelinated axons. Anoxic injury in the RON is critically dependent on Na influx via a TTX-sensitive Na conductance. However, conventional Na channels should be inactivated at the depolarized membrane potentials that occur in anoxic tissue. We therefore hypothesized that a non-inactivating Na conductance must exist, which mediates pathological Na fluxes during anoxia. To test this prediction, we studied RONs with a modified grease gap technique. The recorded DC potential is a reliable fraction of true compound axonal resting potential (Vm), behaving as a linear function of trans-gap impedance. Nerves were studied at rest ([K]o=3mM) and at two levels of depolarization ([K]o=15 and 40 mM; at [K]o=40 mM, resting potential decreased to 44±9 % of control). A TTX-induced hyperpolarization was observed at each value of [K]o, indicating that a finite, non-inactivating Na conductance exists at rest and at depolarized membrane potentials in RON axons. PK:PNa ratios were estimated to be 39±16, 22±14 and 78±59 at [K]o=3, 15 and 40 mM, respectively. Following the TTX-induced hyperpolarization, Vm began to depolarize. We suggest that the depolarization was partly due to inhibition of the Na-K-ATPase secondary to depletion of intracellular Na. We conclude that myelinated axons of the RON possess a TTX-sensitive, non-inactivating Na conductance. This conductance may function as a source of intracellular Na for the Na-K-ATPase under physiological conditions. Under pathological conditions such as anoxia, this conductance plays a central role in mediating irreversible injury by admitting Na, raising [Na]i and driving the Na-Ca exchanger to import damaging quantities of Ca. This non-inactivating Na conductance may have unique pharmacological properties and its blockade may protect CNS white matter from anoxia without abolishing electrogenesis. *(PKS was supported by a Centennial Fellowship from the Medical Research Council of Canada).*

478.26

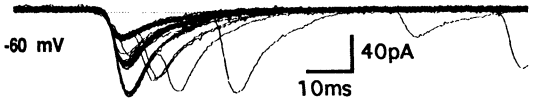
A NOVEL VOLTAGE-DEPENDENT, TTX-INSENSITIVE SLOW SODIUM CURRENT IN ACUTELY ISOLATED STRIATAL NEURONS K. Hoehn,* T.W.J. Watson and B.A. MacVicar. Neuroscience Research Group, University of Calgary, Alberta, Canada, T2N4N1

We have observed a novel voltage-dependent, TTX-insensitive slow Na^+ current which may be important in determining the firing patterns of neostriatal neurons. This current was investigated using whole-cell voltage clamp of neurons which were acutely isolated from rat (21-28 day postnatal) striatum using either trypsin-hyaluronidase or pronase. The pipette solution contained (in mM): trizma phosphate 70, trizma base 28, TEACl 40, EGTA 11, MgATP 2, NaGTP 0.3. The extracellular solution contained (in mM): NaCl 90, TEACl 40, HEPES 10, 4-AP 4, TTX 0.0012, Ca^{2+} 5 and Mn^{2+} 3. In addition to the fast TTX-sensitive Na^+ current, a slow Na^+ was present which was not affected by even a high concentration of TTX (12 μM). Activation threshold was noted around -50 mV and peak currents were observed at around 0 mV. This current decreased in amplitude with decreasing concentrations of extracellular Na^+ and was abolished when Na^+ in the extracellular medium was replaced with either TRIS-Cl, TEACl or sucrose. A small current was observed when 90 mM KCl was substituted for 90 mM NaCl, indicating some permeability to K^+. The slow Na^+ current was not diminished in 0 Ca^{2+} solution or when Ca channels were blocked by either cadmium (25 to 100 μM), cobalt (3 mM) or manganese (3 mM), indicating that it is not a Ca^{2+}-activated current nor a monovalent cation current through Ca channels. Activation of the slow Na^+ current could be fit by a single exponential with time constants ranging from 1400 ms at -40 mV to 200 ms at 0 mV. Deactivation of 'tail currents' could be fit to a single exponential with time constants ranging from 90 ms to 350 ms and revealed a bell-shaped dependence on voltage. A study of 'tail currents' at various times after onset of the slow Na^+ current revealed that the magnitude of the 'tail currents' corresponded closely to the magnitude of the inward current. This slow Na^+ current may underlie the depolarizing afterpotential observed in striatal neurons and may contribute to long-term changes in membrane-potential which have been observed in striatal neurons. (Supported by the M.R.C of Canada and AHFMR).

478.27

SPONTANEOUS TTX-INSENSITIVE Na^+ CURRENTS IN RAT SENSORY NEURONS MAY INVOLVE ALL-OR-NONE Ca^{2+}_i TRANSIENTS. H.-M. H. Saunders, J. Vautrin and J. L. Barker*. Lab. of Neurophysiol., NINDS, NIH, Bethesda, MD

Whole-cell patch clamp recordings in the presence of 1-3 μM tetrodotoxin (TTX) revealed spontaneous transient inward currents (STICs) in cultured (1-3 weeks) dorsal root ganglion cells dissociated from 19-20 day-old rat embryos. The amplitudes of STICs consistently showed preferred levels (see below). Their resting frequency was 2.4 events per minute at -60 mV, and increased as the holding potential was depolarized. Extracellular ATP (5-50 μM) increased the frequency of STICs in a dose dependent manner. Removal of extracellular Ca^{2+} decreased their frequency to 27 % of control, but did not eliminate STICs. Addition of 2.5 mM Mn^{2+} increased their frequency 7-8 fold, but preserved the preferred-amplitude characteristics. Replacement of extracellular Na^+ with N-methyl- D-glucamine reduced the frequency of STICs, shifted the distributions of peak amplitudes to lower levels, prolonged their time- to- peak, and decreased their rate of rise. Extracellular Na^+ -free reversibly eliminated STICs. When a membrane permeable Ca^{2+} chelator, BAPTA-AM, was added to the bath solution, the frequency of STICs decreased progressively to 6% of control. The properties of STICs suggest that these TTX-resistant Na^+ -dependent currents maybe activated by all-or-none Ca^{2+} transients released from intracellular stores.



478.28

REDUCED SODIUM CURRENT IN CULTURED HIPPOCAMPAL NEURONS FROM MOUSE TRISOMY 16, A MODEL OF DOWN SYNDROME. Z. Galdzicki *, E.J. Coan & S.I. Rapoport, LNS, NIA, NIH, Bethesda, MD 20892.

Action potentials in cultured hippocampal neurons from the trisomy 16 (Ts16) mouse have a slower depolarization rate than control neurons. This is likely due to differences in the voltage dependent sodium current. Primary cultures of hippocampal neurons were prepared from control and Ts16 mouse fetuses at day E15-16. Whole cell patch clamp recordings were obtained from 2-4 week old neurons. The extracellular medium included 130 NaCl, the intracellular medium included 5 NaCl. Currents were evoked with a 10 ms pulse in 10 mV steps between -50 mV to 90 mV from a holding potential of -60 mV. The mean membrane resistances were approximately 500 MΩ, the capacitances approximately 30 pF and the reversal potentials approximately +80 mV. None of these values were significantly different between control and Ts16 neurons. The mean maximum inward current was -125 pA/pF for control neurons (n=16) and -62 pA/pF for Ts16 neurons (n=16) (significant at $p < 0.01$), and was abolished by 1 μM TTX, but unaffected by 2 mM $CdCl_2$. This indicates that the current was a voltage-dependent sodium current, and likely explains the reduced depolarization rate of the action potential.

478.29

CORTISOL INDUCES SODIUM CHANNEL EXPRESSION IN CULTURED ASTROCYTES. B.K.Krueger#, D.S. Brougher# and P.J. Yarowsky ▮*. Depts. of Physiology# and Pharmacology & Exp. Therap. ▮, Univ of Maryland Sch. of Med., Baltimore, MD 21201.

We have previously shown (Yarowsky and Krueger, J. Neurosci 9: 1055, 1989) that substitution of serum-containing medium by serum-free G5 medium (A. Michler-Stuke et al., Int. J. Devl. Neuroscience 2:575, 1984) induces a rapid increase in high affinity STX binding in cultured rat astrocytes. We have now investigated whether one of the hormonal constituents of G5 (cortisol) induces high STX affinity Na channels. Serum-free medium was prepared containing DMEM/F12 with transferrin, biotin, sodium selenite, insulin, and fibronectin. Cultures from postnatal day 1 rat cerebral cortex were maintained in serum-containing medium for 4 DIV where a low, basal level of high affinity STX binding was measured. Cultures were then changed to serum-free medium with or without cortisol (10 nM). The astrocytes in cortisol-free medium continued to show the basal level of high STX affinity Na channels. An induction of Na channel expression by cortisol was evident after 24 hr, and was greater than 2.5 times the basal level 4 d after the change. This induction could be specifically blocked by the cortisol antagonist RU-38486 (200 nM and 2 μM) and it was also completely blocked by the transcription inhibitor actinomycin (20ng/ml). Thus, cortisol has a role in the control of Na channel expression during CNS development. Supported by NIH and NSF.

478.30

THE GAMBLER'S RUIN PROBLEM AND TRANSITIONS BETWEEN CLOSED AND OPEN STATES OF SODIUM CHANNELS. C.C. Chancey*[1], and S.A. George[2]. [1]Physics and Chemistry Depts., Purdue University Calumet, Hammond IN 46323, [2]Neuroscience Program, Amherst College, Amherst MA 01002.

Transition times between states of ion channels are generally assumed to be describable using classical statistical mechanics. However, the detailed connection between the distribution of these times and the dynamics of the gating mechanism is unknown. Assuming the models of Catterall and Guy, we have calculated times for sodium channel activation to occur through thermally-activated barrier hopping. We calculated coulomb interactions between the S4 α-helix and negative charges on nearest-neighbor helices, and included longer range interactions by adding a background electric field. Periodic pairing of charges between the S4 and adjacent helices in the model causes the resting and depolarized states of the channel to correspond to local minima in the S4 potential energy curve. Harmonic potentials closely fit the deviations from each local minimum, allowing the vibrational energies of the S4 helix about each minimum to be estimated. The thermally-induced stochastic fluctuations are modelled in terms of the classical Gambler's Ruin problem, producing a distribution of transition times.

478.31

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF CATHa CELLS - A NEW CATECHOLAMINERGIC CNS CELL LINE. M. Lazaroff*, D.M. Chikaraishi, K. Dunlap. Neuroscience Program, Tufts Univ. Sch. of Med., Boston, MA 02111.

A CNS cell line (CATHa) was previously derived from a brain tumor from a transgenic mouse in which SV40 T antigen was under the transcriptional control of rat tyrosine hydroxylase 5' flanking sequences. The cells synthesize catecholamines and have neurofilament and synaptophysin proteins whereas glial fibrillary acidic protein is absent suggesting that the CATHa cells are neuronal in origin.

We have assayed for the presence of voltage-dependent ion channels by whole cell voltage-clamp recording. When CATHa cells are depolarized from a resting potential of -80 mV to between -40 and -20 mV, a fast-activating inward current is observed. Peak current is reached within 2 ms and is followed by a rapid, exponential inactivation (1.6 ms). Current-voltage relations show a maximum current near 0 mV. This current resembles that of the voltage-dependent Na^+ channels in other preparations. This was further confirmed by ion substitution experiments in which external NaCl was replaced with N-methyl-D-glucamine (NMDG); this substitution eliminated the current. In addition, 250 nM tetrodotoxin (TTX) blocked the current in a reversible manner. These findings suggest that CATHa cells express voltage-gated Na^+ channels.

When depolarized to -40 mV, a longer lasting inward current is observed in approximately 40% of the CATHa cells. This current is blocked by 100 uM cadmium and is carried predominantly by calcium ions. In other CATHa cells a long-lasting, more slowly activating outward "K^+- like" current is seen. Therefore, these transformed cells appear to possess voltage-gated sodium channels, calcium channels, and possibly potassium channels.

478.32

NA^+/K^+ ATPase INHIBITORS BLOCK PALYTOXIN INDUCED INACTIVATION OF IONIC CURRENTS IN FROG MUSCLE MEMBRANE. M.B. Rodríguez de Salzberg, S. Ortiz-Miranda and G. Escalona de Motta*. University of Puerto Rico Dept. of Biology and Institute of Neurobiology, San Juan 00901.

Palytoxin (PTX), isolated from zooanthids of the genus Palythoa (Cnidaria) and considered one of the most potent marine toxins, has been proposed to increase Na^+ permeability of cell membranes through a mechanism involving the Na^+/K^+ ATPase. In nM concentrations PTX depolarized frog sartorius muscle fibers in about 20 min. PTX (0.1-10 nM) completely inactivated ionic currents elicited by depolarizing voltage steps using the "loose" patch clamp technique. Holding membrane potentials at more negative values abolished this inactivation. Pretreatment of muscles with various cardiac glycosides produced a dose-related inhibition of PTX action. However, the inhibitory action of these glycosides was dependent on their chemical structure, monoglycosides being the most active inhibitors. These results confirm a relationship between the depolarizing effect of this toxin and the activity of the membrane Na^+/K^+ ATPase. (Work was supported by NIH grants GM08102, NS07464 and NOAA, USDC Sea Grant College Program at UPR).

CALCIUM CHANNEL MOLECULAR BIOLOGY

479.1

CLONING OF THE α1 SUBUNIT OF A VOLTAGE-DEPENDENT CALCIUM CHANNEL EXPRESSED IN THE ELECTRIC ORGANS FROM *NARCINE BRASILIENSIS*. M. Philip, J. Lin, C. Hashimoto, L. Shilo, S. Azhar, J. Fox*, J. Ramachandran and J. Bell. Neurex Corporation, 3760 Haven Avenue, Menlo Park CA 94025.

The electric organ of the fish *Narcine brasiliensis* is a rich source of molecules such as calcium channels involved in neuronal communication. Using two oligonucleotides from motif IV and the carboxy tail of the rabbit skeletal muscle calcium channel as primers, reverse transcribed cDNA from electromotor nuclei as template, and PCR, we cloned a 570 bp fragment and subsequently used it as a probe to screen a cDNA library made from electromotor nuclei. From the library, we isolated several overlapping clones covering almost the full length of the message. Upon sequencing, these clones showed high similarity to Class D channels (Snutch, T.P.*et. al.*, (1990) *Proc. Natl. Acad. Sci.* Vol 87, 3391; Williams, M.E. *et. al.*, (1992) Neuron, Vol 8, 71). Human and fish genes are highly conserved with 79% identity overall at the amino acid level. There is almost complete conservation of transmembrane domains and significant divergence in the intracellular regions between motifs I and II and between motifs II and III. In preliminary results, the C-terminal region of our fish clone is relatively short and diverges completely from the human clone 177 residues from the last transmembrane domain, perhapes suggesting alternate splicing in this region. It appears that the subtypes of calcium channels arose early in vertebrate evolution and are highly conserved among different species. We also obtained a presumptive splice variant, unique from other published sodium or calcium channels, which has two in-frame deletions of 129 bases and 66 bases corresponding to the IVS3 and IVS5 transmembrane domains.

479.2

MULTIPLE CALCIUM CHANNEL CLONES ISOLATED FROM A MARINE RAY. W.A. Horne*, P.T. Ellinor, I. Inman, M. Zhou, R.W. Tsien & T.L. Schwarz. Dept. Mol. Cell. Physiol., Stanford Univ., Stanford CA, Dept. Pharmacol., Cornell Univ., Ithaca NY.

In many neurons, transmitter release from presynaptic terminals is triggered by Ca^{2+} entry via DHP-insensitive Ca^{2+} channels (e.g. ω-CgTx-sensitive N-type channels). In an effort to understand the molecular properties of such channels, we have looked for Ca^{2+} channels in *Discopyge ommata*, a marine ray whose electric organ is the richest known source of binding sites for ω-CgTx. PCR was used to amplify putative Ca^{2+} channel sequences from the electric lobe which gives rise to nerve endings in the electric organ. PCR products were used to rescreen lobe libraries, yielding several clones with strong homology to known Ca^{2+} channels. We find one cDNA (doe-2) homologous to L-type, and another (doe-3) homologous to the BI channel of Mori et al. We also find additional clones (doe-1 and doe-4) with less similarity to L-type or BI channels. doe-4, which encodes a protein of 2326 amino acids, is more similar than the other doe clones to class B channels (Snutch). In all cases, the marine ray clones show higher homology to their mammalian counterparts in transmembrane domains than in external or cytoplasmic linkers. Northern analysis shows that doe-1 is more abundant in brain; doe-3 and doe-4 are more plentiful in the electromotor nucleus, and thus might play some role in motor nerve terminals. Evidently, the familial pattern of Ca^{2+} channel genes that has emerged for mammals also appears in this lower vertebrate.

479.3

CLONING AND CHARACTERIZATION OF A NOVEL α_1 SUBUNIT OF *DROSOPHILA* Ca^{2+} CHANNEL. W. Zheng, G-P. Feng, D.F. Eberl, D. J. Triggle* and L.M. Hall. Dept. of Biochemical Pharmacology, School of Pharmacy, SUNY/Buffalo, NY 14260.

The most abundant Ca^{2+} channels in *Drosophila* head membranes have different pharmacological properties than those of vertebrate L-type Ca^{2+} channels. These *Drosophila* Ca^{2+} channels have high binding affinity for phenylalkylamines, but are insensitive to dihydropyridines and to benzothiazepines whereas the vertebrate L-type channels are sensitive to all three classes of the Ca^{2+} channel antagonists. We report here a complete sequence of the Ca^{2+} channel α_1 subunit cloned from a *Drosophila* head cDNA library. This cDNA encodes a deduced protein estimated to contain 1964 amino acids. It exhibits 79.5, 72.2 and 71.2% sequence similarity to the α_1 subunits expressed in rat brain (D), rabbit skeletal muscle and heart respectively. A comparison of the Ca^{2+} channel sequences from *Drosophila* and vertebrates will be presented which provides important insight concerning the nature of phenylalkylamine and dihydropyridine binding sites. Northern analysis shows that this α_1 subunit mRNA is expressed as a single size class in leg (9.5kb) and body (9.6kb) but as three size classes (9.5, 10.2 and 12.5) in head.. This gene has also been localized on the left arm of chromosome 2 by *in situ* hybridization. Mutant analysis will define the physiological/developmental role of this channel in the whole organism. (Supported by NIH grants HL16003, HL39369 and GM42850-02).

479.4

PARTIAL SEQUENCE OF α_{1x}, A PUTATIVE NOVEL α_1 SUBUNIT OF A VOLTAGE-SENSITIVE CALCIUM CHANNEL L.M. Marubio*, L. H. Philipson, and R. J. Miller, Departments of Pharmacology and Physiology and Medicine,University of Chicago, Chicago, IL 60637

To date partial or complete sequences of five mammalian Ca^{2+} channel α_1 subunits have been reported, including skeletal muscle and $\alpha_{1A,B,C,D}$. In addition, some have areas of alternative splicing creating further variability. Whether this constitutes the complete set of such genes is uncertain. Using PCR amplification with degenerate primers designed to amplify a well conserved region in the fourth repeat domain of Ca^{2+} channel α_1 subunits, we have cloned a 320 bp fragment of a putative novel α_1 subunit channel (α_{1x}) from rat brain cDNA. Subsequent screening of a mouse brain cDNA library (Clonetech) with the 320 bp fragment as probe yielded a 1.1 kb fragment which contained a single open reading frame. Nucleotide sequence comparisons in the fourth repeat domain show it to be 72% identical to the A type Ca^{2+} channel and 65% identical to the C type. The deduced amino acid sequence shows the IV H5-H6 region to be approximately 85% identical to both the A and B type α_1 subunits. This cDNA has also been found in abundance in cDNA rat superior cervical ganglion neurons in culture, using PCR amplification of cDNA with primers designed specifically for this channel. Investigations to date have not revealed the transcript in PC-12 cells (differentiated and undifferentiated), NG108-15 cells, nor IMR32 cells.

479.5

MOLECULAR CLONING AND REGIONAL EXPRESSION OF A RAT BRAIN CALCIUM CHANNEL ALPHA-2 SUBUNIT. S.J. Dubel*, W.J. Tomlinson, T.V.B. Starr, S.R. Vincent# and T.P. Snutch. Biotechnology Laboratory and #Division of Neurological Sciences, University of British Columbia, Vancouver, B.C., Canada V6T 1Z3.

Voltage-gated Ca channels are heteroligomeric complexes that include an alpha-1 subunit that serves both as a voltage sensor and ion conducting pore. In addition, exogenous expression studies demonstrate that other subunits (alpha-2, beta and gamma) can alter the physiological properties of the alpha-1 subunit. In the nervous system, the exact subunit composition of different neuronal Ca channel types has not been described and it is not yet known whether all Ca channel alpha-1 subunits are associated with ancillary subunits in vivo. Utilizing the skeletal muscle alpha-2 sequence as a probe we have isolated a 4.4 kb cDNA from a rat brain library. The cDNA encodes a 1091 amino acid protein (123 kDa predicted molecular mass) that shows 93% and 94% amino acid identity to rabbit skeletal muscle and human brain Ca channel alpha-2 subunits, respectively. The cellular localization of alpha-2 subunit expression was examined in adult rat brain using in situ hybridization with antisense oligonucleotides. Autoradiography reveals that the alpha-2 subunit is highly expressed in a subset of neurons in the cerebellum, olfactory bulb, hippocampus and cortex. The expression of the brain alpha-2 subunit does not correlate exactly with the expression of Ca channel alpha-1 subunits and suggests that only a subset of neuronal Ca channels are associated with an alpha-2 subunit.

479.6

cDNA CLONING OF A CALCIUM CHANNEL HIGHLY EXPRESSED IN THE HIPPOCAMPUS. T.-W. Soong and T.P. Snutch*. Biotechnology Laboratory, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1Z3.

Screening of a cDNA library constructed from whole rat brain RNA identified four major subtypes of Ca channel alpha-1 subunits (classes A, B, C, and D; Snutch et al, PNAS 87:3391-3395). A number of studies indicate that the class C and D alpha-1 subunits encode distinct L-type Ca channels while the class A and B alpha-1 subunits are likely to encode P and N type channels, respectively. To identify Ca channels that may be involved in processes such as long-term potentiation, we have utilized the polymerase chain reaction (PCR) to amplify Ca channel sequences expressed in the hippocampus. Degenerate oligonucleotides were used to amplify hippocampus RNA isolated from adult rats and the PCR products were subcloned and analyzed by DNA sequencing. Of 18 clones examined, 15 were identified as previously characterized Ca channel alpha-1 subunits, while 3 encode a new class of Ca channel (designated class E). The deduced primary structure of the class E protein is most closely related to the brain class A and B alpha-1 subunits. Limited amino acid identity to the brain class C and D alpha-1 subunits suggests it is unlikely that the class E Ca channel corresponds to an L-type Ca channel. Northern blot analysis shows that the class E Ca channel is encoded by an approx. 12 kb mRNA that is expressed throughout the rat CNS and is most abundant in the hippocampus. This pattern of expression is distinct from previously cloned Ca channels and may reflect a unique physiological role for the class E Ca channel in the CNS.

479.7

CHROMOSOMAL LOCALIZATION OF MURINE GENES ENCODING α1, α2, AND β-SUBUNITS OF THE DHP-SENSITIVE L-TYPE CALCIUM CHANNELS. H. Chin[1]*, B. Mock[2], H-L. Kim[1], and C.A. Kozak[3]. [1]LMB, NINDS; [2]LG, NCI; and [3]LMM, NIAID, NIH, Bethesda, MD 20892.

Recent molecular cloning has indicated that a heterogeneous family of voltage-sensitive Ca^{2+} channels are expressed in mammalian brain, providing structural bases for the functional diversity of neuronal Ca^{2+} channels. As a first step toward understanding genetic bases for diversity of brain Ca^{2+} channels, we have begun mapping the genes encoding the α1, α2-d, and β-subunits of the dihydropyridine (DHP)-sensitive L-type Ca^{2+} channels. Previously, we and others localized two of the α1 subunit genes (*Cchl1a1* and *Cchl1a2*) on human and mouse chromosomes (Power et al., Genomics, 10:835-839, 1991; Chin et al., Genomics, 11:914-191, 1991). Here, we have determined the chromosomal location of the third subunit gene, *Cchl1a3*, which encodes the isoform predominantly expressed in skeletal muscle, to mouse Chr 1. Analysis of the progeny of an inbred strain cross positioned *Cchl1a3* 1.3 cM proximal to the *Pep-3* locus on Chr 1. In contrast to the α1 subunit which are encoded by 3 distinct genes located on separate chromosomes, the α2 and β subunits are encoded by a single gene, located on Chr 5 and 11, respectively. Analysis of Chr 5 alleles for several genes in an intersubspecies cross between NFS/N and C58/J mice shows that the α2 subunit gene, termed *Cchl2a*, can be positioned at the centromeric end of Chr 5, with gene order centromere - *Cchl2a* - *Il-6* - *Pgm-1*. Similarly, the gene for the β-subunit is mapped on Chr 11 with gene order centromere - *Sparc* - *Cchlb* - Gfap - *Pkca*. Our mapping data indicate that the DHP-sensitive Ca^{2+} channels genes are apparently dispersed in the mouse genome, unlike the Na^{+} channel whose genes are clustered on Chr 2.

479.8

THE β-SUBUNIT OF THE DHP-SENSITIVE CALCIUM CHANNEL: cDNA CLONING AND IDENTIFICATION OF mRNA IN ADULT AND DEVELOPING RAT BRAIN. H-L. Kim[1]*, H. Kim[2], H. H. Jung[1], and H. Chin[1]. [1]LMB, NINDS, NIH, Bethesda, MD 20892; [2]Dept. Anat., Coll. Med., Korea Univ., Seoul, Korea.

The dihydropyridine (DHP)-sensitive, L-type Ca^{2+} channel from skeletal muscle consists of 5 polypeptide subunits (α1, α2, β, γ, and δ). The α1 subunit of the Ca^{2+} channel can form the functional Ca^{2+} channels in *Xenopus* oocytes. However, coinjection of skeletal muscle α2-δ and β subunit mRNAs with the α1 subunit mRNA drastically changed the electrophysiologic characteristics of the expressed Ca^{2+} channels. These findings suggest that α2-δ and/or β subunits may play a modulatory role in regulating Ca^{2+} channel function. Previously, we cloned and characterized the molecular properties of the DHP-sensitive Ca^{2+} channel α1 and α2 subunits. Here, we report the primary structure of rat brain DHP-sensitive Ca^{2+} channel β subunit and tissue distribution of its mRNA. Two cDNA clones, BT11 and BT8, encoding the β subunit were isolated and characterized. The deduced amino acid sequenceof BT11 cDNA is very similar to that of the rabbit skeletal muscle β subunit, showing 96% amino acid identity. The BT8 clone is a spliced variant of BT11 with deletion of a 45-amino acid fragment. The distribution of rat DHP-sensitive Ca^{2+} channel β subunit mRNA was examined in prenatal (E16, E19), postnatal (P0, P6, P12), and adult rat brains as well as whole-body sections of E19 embryos. In adult rat brain, large amounts of β subunit mRNA were found in the hippocampus, dentate gyrus, and medial habenula. A high level of DHP-sensitive Ca^{2+} channel β subunit transcript was already expressed at E16, and transcript levels significantly changed in several areas during development.

479.9

DIFFERENTIAL EXPRESSION OF BRAIN L-TYPE AND P-TYPE CALCIUM CHANNEL mRNAs IN ADULT AND DEVELOPING RAT BRAIN H. Kim[1]*, H-L Kim[2], and H. Chin[2]. [1]Dept. Anat., Korea Univ. Coll. Med., Seoul, Korea; [2]LMB, NINDS, NIH, Bethesda, MD, 20892.

The four major types of voltage-sensitive Ca^{2+} channels present in the central nervous system (CNS) are classified as T-, N-, L-, and P-types. Our earlier localization study indicated that L-type Ca^{2+} channels are predominantly expressed in those brain regions important for neuroendocrine function (Chin et al., FEBS Lett., 299:69-74, 1992). Several studies suggest that the P-type channels, initially identified in cerebellar Purkinje cells, are distributed more widely throughout the brain. Here, we investigated mRNA expression patterns of the α1 subunit of L- and P-type Ca^{2+} channels of adult and developing rat brains by *in situ* hybridization histochemistry, using the specific cRNA probes. The L-type α1 subunit transcript was abundantly expressed in the olfactory bulb, dentate gyrus, pituitary and pineal glands, superior colliculus, and facial nucleus; whereas the P-type α1 subunit was highly expressed in the hippocampus (CA3 region), geniculate bodies, inferior colliculus, and cerebellum. Resolution at the cellular level disclosed differential labeling of distinct cell types in various brain areas, suggesting that L- and P-type Ca^{2+} channels may be localized in specific neuronal subpopulations. Consistent with this conclusion, relative L-type Ca^{2+} channel mRNA contents in different brain regions varied during development, whereas the P-type Ca^{2+} channel transcript gradually increased from very low levels during early embryogenesis (from E16), peaked to the highest level at P12, and decreased thereafter in adults. The data reveal that spatial localization and temporal expression patterns of L- and P-type Ca^{2+} channel mRNAs change developmentally, and suggest physiologic role(s) for these channels in mammalian CNS ontogeny.

479.10

CLONING AND EXPRESSION OF A HUMAN NEURONAL N-TYPE CALCIUM CHANNEL. S.B. Ellis, D.H. Feldman, M.E. Williams, P.F. Brust, A. Maroufi, S. Patthi, S. Simerson, G. Velicelebi*, M.M. Harpold. SIBIA Inc., 505 Coast Blvd. So., La Jolla, CA 92037

At least four different α_1 subunit genes are expressed in the central nervous system, designated α_{1A} through α_{1D}. We report the primary structure of the α_{1B} subunit and the functional expression of a human N-type voltage-dependent Ca^{2+} channel mediated by this α_1 subunit. Functional expression was achieved by transient coexpression of α_{1B} with human neuronal α_{2b} and β_2 subunits [Williams *et al.* Neuron 8:71 (1992)] in mammalian cell culture. Whole cell recordings revealed a high-voltage activated, inactivating channel that was sensitive to holding potential, insensitive to Bay K 8644 (1 μM) and irreversibly blocked by ω-conotoxin (0.5 μM) (see abstract by Feldman *et al.*). Transfected cells expressed a single class of high-affinity ω-conotoxin binding sites (K_d = 55 $\pm$ 18 pM; B_{max} = 25,000 $\pm$ 10,000 receptors per cell).

The predicted α_{1B} subunit structure consists of the characteristic twenty-four transmembrane topology determined for α_1 subunits. The predicted α_{1B} amino acid sequence is 64.1% and 43.0% identical to the rabbit BI (α_{1A}) dihydropyridine-insensitive and human α_{1D} dihydropyridine-sensitive subunits, respectively. α_{1B} has a characteristic large putative cytoplasmic loop between the IIS6 and IIIS1 transmembrane domains like the rabbit BI subunit but is only 29.3% identical through a majority of the loop. The α_{1B} primary transcript is differentially processed to produce at least two isoforms, α_{1B-1} (calculated molecular weight = 262,494) and α_{1B-2} (calculated molecular weight = 251,757). α_{1B-1} and α_{1B-2} differ at their carboxyl termini.

479.11

PHYSIOLOGICAL CHARACTERIZATION OF A CLONED HUMAN N-TYPE CALCIUM CHANNEL. D.H. Feldman*, M.E. Williams, P.F. Brust, G. Velicelebi, S.B. Ellis, M.M. Harpold. SIBIA Inc., 505 Coast Blvd. So., La Jolla, CA 92037.

N-type Ca channels, found in neurons but not in muscle, are irreversibly blocked by ω-conotoxin and are insensitive to dihydropyridines. Activation requires strong depolarizations from negative holding potentials, and inactivation occurs over tens to hundreds of msec. We have now isolated cDNAs encoding a human neuronal α_{1B} subunit [see abstract by Ellis *et al.*] that, when transiently coexpressed in transfected mammalian cells with previously isolated cDNAs encoding human neuronal α_{2b} and β_2 subunits [Williams *et al.*, Neuron 8:71 (1992)], expresses Ca channels with the properties of N-type channels.

Whole-cell recordings from transfected cells revealed Ba^{2+} currents up to several nA that were irreversibly blocked by ω-conotoxin (0.5-10 μM) at a rate dependant on concentration, consistent with rates measured in neurons. Activation began near -20 mV, and peaked near 10 mV. The currents usually activated within 10 msec and inactivated with a time course fit by the sum of two exponentials ($\tau_{fast} \simeq 0.1$ sec; $\tau_{slow} \simeq 0.4$ sec). Whereas τ_{fast} dominated inactivation at test pulses of 0 or 10 mV, τ_{slow} dominated at >10 mV. The currents were sensitive to holding potential, with half inactivation occurring at -60 to -70 mV, and 90% inactivation near -40 mV. Currents were reversibly blocked by 50 μM Cd^{2+}, and were not affected by dihydropyridines. Two observations suggest that the expressed channel is under tonic inhibition in the host cells: currents typically increased in magnitude 2 to 20-fold during the first several minutes of recording, and currents increased in magnitude when the test pulse was immediately preceded by a brief strong depolarization (+70 mV).

Thus, many of the properties attributed to N-type channels observed in neurons are exhibited by the recombinantly expressed channel.

479.12

CLONING OF A cDNA FOR A SYNAPTIC PLASMA MEMBRANE Na^+/Ca^{2+} EXCHANGER. **M.L. Michaelis*, J. Walsh, K. Kumar, J. Foye and G. Hadwiger. Dept. of Pharmacology and Toxicology, Univ. Kansas, Lawrence, KS 66045.**

The Na^+/Ca^{2+} exchanger is a plasma membrane protein which is believed to participate in the regulation of Ca^{2+} fluxes, particularly in excitable cells. We recently reported on efforts to purify exchanger activity from brain synaptic membranes and the development of Ab's to a 36 kDa protein which immunoprecipitated exchanger activity from solubilized brain synaptic membranes (J. Neurochem., 58:147-157, 1992). The Ab's were used to screen a brain cDNA expression library (Lambda ZAP) and several positive clones identified. One of those clones has been found to have an insert of ~1.6 kb and, following autoexcision and transfer of the phagemid pBS to E. Coli, the transfected bacteria expressed an ~40 kDa fusion protein which was recognized by the Ab's. The initial sequencing of the insert revealed a novel nucleotide sequence and the presence of a short COOH-terminal region with a high degree of homology to the voltage gated Ca^{2+} channel. Work is underway to express the protein transiently in cells with little or no endogenous exchanger acitivty. (Supported by PHS grant #AA04732 and the American Heart Assoc., KS Affiliate, grant #KS-91-G-07.)

ACETYLCHOLINE: CNS II

480.1

AF64A (ETHYLCHOLINE AZIRIDINIUM ION) PRODUCES OXIDATIVE STRESS: RELATION TO CHOLINOTOXICITY AND FUNCTIONAL DEFICITS. T.J. Walsh*, G. Woertwein, R. W. Stackman and S.C. Bondy[1], Dept. Psychology, Rutgers Univ., New Brunswick, NJ, 08903, and 1 Univ. of California, Irvine, CA, 92717.

AF64A produces (i) a persistent decrease in presynaptic cholinergic function, (ii) a loss of cholinergic neurons in the medial septum, and (iii) cognitive impairments. While AF64A inhibits high affinity choline uptake (HAChU) the mechanisms responsible for its long-term neurotoxicity are not well-characterized. The following experiments examined the potential role of oxidative stress in the cholinotoxicity and functional deficits induced by AF64A. Male Sprague-Dawley rats were bilaterally injected icv with artificial CSF or 3.0 nmoles of AF64A. Rats were sacrificed two days following surgery and it was determined that AF64A increased the production of conjugated dienes, an index of lipid peroxidation and oxidative stress, and decreased the activity of choline ChAT in the hippocampus (HPC). Furthermore, there was a significant correlation between the degree of cholinergic toxicity and the extent of lipid peroxidation. In a subsequent study rats were pre-treated with saline or 50 mg/kg of the anti-oxidant Vitamin E acetate (VE) at 24 hrs and 15 min prior to icv injection of artificial CSF or AF64A (3 nmoles/side). Vitamin E prevented the AF64A-induced (1) deficits in acquisition and retention in a Morris-water maze task and (2) the decrease in HAChU in the HPC. Therefore, while the structure of AF64A leads to its accumulation in cholinergic neurons oxidative stress might significantly contribute to the cholinotoxicity and functional deficits induced by this compound. Supported by a Busch Grant to TJW.

480.2

CHRONIC EEG CHANGES IN RATS TREATED WITH THE CHOLINERGIC NEUROTOXIN ETHYLCHOLINE AZIRIDINIUM (AF64A). H.Hörtnagl, E.Groll-Knapp, G.Khanakah and O.Hornykiewicz*. Institute of Biochemical Pharmacology and Institute of Environmental Hygiene, University Vienna, A-1090 Vienna, Austria

The effect of a neurotoxic lesion of the cholinergic septo-hippocampal pathway on cortical EEG activity was studied. Male Sprague Dawley rats received bilateral stereotaxic infusions of 2nmol AF64A or vehicle into the lateral ventricle. Four months after AF64A Ag/AgCl electrodes were chronically implanted in epidural position over frontal and parietal regions of both hemispheres. One month after implantation various EEG parameters were analyzed. In the AF64A-treated rats a reduction of the amplitude of the spontaneous EEG in rest and activated conditions and of the main peak amplitude of visual and acoustic evoked potentials was observed (click evoked potentials: AF64A: -7.3 ± 3.0 μV, n=11; control: -32.1 ± 5.9 μV; n=9; $p < 0.01$; flash evoked potentials: AF64A: -29.6 ± 2.7 μV; control: -47.0 ± 5.6 μV; $p < 0.02$). Additionally, a diminution of the slow potential shifts evoked by physiologically meaningful vocalizations occurred. Neurochemical analyses of the brain revealed a specific reduction in the activity of choline acetyltransferase in the dorsal (42%) and ventral (52%) hippocampus. The data indicate that treatment with AF64A results in a chronic decrease in general central nervous activity and disturbance in higher nervous integrative processes, which appear to be linked to the specific deficit of the cholinergic transmission in the hippocampus.

480.3

EXCITOTOXIC LESIONS OF THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS IMPAIR RADIAL-ARM MAZE PERFORMANCE

Larry L. Butcher *, Justin D. Oh, and Cheryl Cesarz.
Laboratory of Chemical Neuroanatomy and Dept. Psychology, UCLA, Los Angeles, CA 90024-1563, U.S.A.

Lesions of the pedunculopontine tegmental nucleus (PPT) lead to reduced choline acetyltransferase (ChAT) immunoreactivity in cholinergic basal forebrain nuclei (Oh et al., *Soc. Neurosci Abstr.*, 15, 782, 1989). In order to assess possible effects of PPT lesion on spatial memory, 10 female Sprague-Dawley rats with excitotoxin lesions were trained and tested on an 8-arm radial maze. After rats (n=5) reached a 0% error rate defined as no entries into an arm already entered, 3.8 nmol kainate (0.1 μl, total injection time = 4.5 min) was injected unilaterally into the PPT. Five additional rats served as controls and were injected with 0.1 μl of saline into the PPT. Starting on the day after surgery, and for an additional 6 days, rats were tested on the maze and the behavioral data was statistically analyzed with a 2-way mixed-design ANOVA. Lesioned rats, but not controls, showed a significantly greater impairment in spatial memory performance [$F(10,2) = 12.143$, $MsE = 0.623$, $p<0.05$]. These data suggest that excitotoxic lesions of the cells in the PPT nucleus have a deleterious effect on the spatial memory of rats.

[Support: USPHS grant NS 10928 to L.L.B.].

480.4

RADIAL-ARM MAZE PERFORMANCE IN RATS IS IMPAIRED BY VESAMICOL [(-)-TRANS-2-(4-PHENYLDIPERIDINO) CYCLOHEXANOL], A POTENT INHIBITOR OF ACETYLCHOLINE ACTIVE TRANSPORT BY SYNAPTIC VESICLES.

Justin D. Oh*, Sonja Bockenhauer, Antoine Keller, Stanley M. Parsons, Gary A. Rogers and Larry L. Butcher. Laboratory of Chemical Neuroanatomy and Dept. Psychology, UCLA, Los Angeles, CA 90024-1563, U.S.A. and Dept. of Chemistry, University of Californis, Santa Barbra, CA 93106.

Effects on both choline acetyl-transferase (ChAT) immunohistochemistry and 8-arm radial maze performance were found for the drug Vesamicol, a potent inhibitor of acetylcholine (ACh) active transport by synaptic vesicles. Female rats (n=20) were randomly assigned either to vesamicol group or vehicle control group. Rats received bilateral injections of 1 (ng) vesamicol (2.0 ul X 0.5 mM) dissolved in PBS (pH=7.2) containing DMSO and acetic acid intraparenchymally in the nucleus basalis of Meynert (n=5) or intraventicularly (n=5). Rats injected with vesamicol showed significantly reduced numbers of ChAT-immunopositive cells in the basal forebrain 7 days following surgery. Staining for both Nissl and the nerve growth factor receptor revealed no actual cell loss or morphologic abberation in these cholinergic neurons. As measured by entries to repeat, radial-arm maze choice accuracy was significantly impaired in the vesamicol group compared to the control group ($p<0.05$). Paralleling the time-course of ChAT-immunoreactivity decrement, the effects of vesamicol on maze performance were significantly different from control 6 and 7 days post-surgery. [Support: USPHS grant NS 10928 to L.L.B.].

480.5

SELECTIVITY OF AN AFFINITY LIGAND, HEMICHOLINIUM MUSTARD, FOR THE HIGH AFFINITY CHOLINE TRANSPORT SYSTEM. K.H. Gylys* and D.J. Jenden *Department of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024.*

Hemicholinium mustard has been shown to be an irreversible inhibitor of high affinity choline uptake (HACU) in several preparations. (Smart, 1981, 1983; Gylys et al., 1990). In the present experiments the specificity of these irreversible effects was examined with respect to other cholinergic proteins and other sodium-dependent transport systems. To measure the effects of HCM on choline acetyltransferase (ChAT), synaptosomes were incubated in HCM, then washed. The synaptosomes were lysed and ChAT activity was measured. Treatment with 50 μM HCM, a concentration that inhibits 100% of synaptosomal HACU, results in a 24% decrease in ChAT activity. In other experiments, rat brain membranes were incubated with 1 μM HCM and then washed before saturation studies with the muscarinic receptor ligand [^{3}H]-NMS were carried out. HCM pre-incubation showed no significant effect on either the affinity or number of muscarinic receptors. Dopamine (DA) transport is also relatively unaffected by HCM pre-treatment: 10 μM HCM, which inhibits HACU in synaptosomes by >90%, inhibits DA transport by 11%. These results support the use of HCM as an affinity ligand for HACU. (Supported by MH 17691)

480.6

PRESYNAPTIC CHOLINERGIC MARKERS: CHANGES FOLLOWING DRUG ADMINISTRATION AND BEHAVIORAL TESTING IN RATS. L.K.Gorman*, V.Rodriguez, S.Golski & D.S.Olton. Psych Dept.,Johns Hopkins Univ., Balto., MD 21218.

Presynaptic functioning of the cholinergic system has been assessed by three different measures: choline acetyltransferase (ChAT) measures the activity of ChAT, the synthetic enzyme for acetylcholine; sodium dependent high affinity choline uptake (SDHACU) measures both the activity and the number of presynaptic HACU sites; hemicholinium-3 (HC-3) binding measures the number of HACU sites. CHAT, HC-3 and SDHACU were measured in dorsal and ventral hippocampus of the same rat following either systemic drug administration or behavioral testing. Preliminary data indicate that scopolamine decreased CHAT activity, whereas physostigmine increased CHAT activity; HC-3 binding and SDHACU activity were not changed by these drugs. In behaviorally tested rats, HC-3 binding was increased following performance on a spatial working memory task; ChAT was not altered by behavioral testing in previous experiments. These data indicate that different experimental manipulations differentially alter presynaptic cholinergic markers.

480.7

CHOLINE RESTORES BLOOD PRESSURE IN HYPOTENSIVE RATS: INVOLVEMENT OF VASOPRESSIN. V.Savcı, I.H.Ulus* S.Gürün, B.K.Kıran and L.R.Büyükuysal. Dept.of Pharmacology, Uludağ Univ. Medical School., Bursa, TURKEY.

Left carotid artery of rats (280-350 g) was cannulated with PE 50 tubing to monitor blood pressure and animals were made hypotensive by bleeding (2 ml/100 g of body weight) or by treatment with histamine (10 mg/kg; ia), hekzamethonium (15 mg/kg; ip), phentolamine (5 mg/kg; ia) or 6-hydroxydopamine (50 mg/kg;iv). Intracerebroventricular (ICV) injection of choline (50-150 ug; rat) to these animals increased and restored blood pressure within 1-5 minutes after the choline treatment. Choline's effect was abolished by pretreatment of rats with mecamylamine (50 ug). Pretreatment of rats with atropine (10 ug; ICV) failed to alter the effect of ICV choline. ICV choline (50 ug) also failed to increase and restore blood pressure when rats were pretreated with hemicholinium-3 (20 ug; ICV) 15 minutes before ICV choline. The increase in plasma levels of vasopressin was associated with the increase in blood pressure in ICV choline treated hypotensive rats. When animals were treated with an antogonist of vasopressin (10 ug/kg; ia) five minutes after ICV choline (150 ug) blood pressure decreased immediately to the pre-choline or to near pre-choline levels. These data indicate that ICV choline can increase and restore blood pressure in hypotensive rats by increasing the central cholinergic nicotinic neurotransmission. The increase in plasma levels of vasopressin involves in this effect of ICV choline.

480.8

EFFECTS OF APNEA ON BRAIN CHOLINE PRODUCTION IN RATS. O.U. Scremin* and D.J. Jenden. West L.A. V.A. Medical Center and UCLA School of Medicine, Depts of Physiology and Pharmacology. Los Angeles CA 90024.

Free choline (Ch) concentration in brain tissue results from a balance between synthesis and hydrolysis of acetylcholine (ACh), synthesis and degradation of Ch containing phospholipids and exchange with plasma. Since the ability of brain tissue to synthesize Ch *de novo* is negligible, losses of this base through the circulation can have serious consequences for phospholipid and ACh metabolism. We tested the hypothesis that the energy deprivation associated with apnea would enhance the loss of Ch. Experiments were performed in rats, mechanically ventilated with a N_2O/O_2 mixture. Ch in plasma of aorta (Ch_a) minus that of retroglenoid vein (Ch_v) multiplied by cerebral blood flow in the same vein represented the cerebral metabolic rate of Ch (CMRCh, nmoles/min) that reached -0.14±0.06 prior to apnea. Apnea of 1.5 min duration was followed by negative CMRCh values (-0.72±0.06 at 3 min post-apnea), returning to control after 16 min. Apnea of 3 min duration was followed by CMRCh values not different from zero during the initial 15 min followed by negative values (-0.52±0.11 at 60 min). Apnea of 6 min duration was followed by positive CMRCh initially (1.12±0.1 at 6 min) with negative values later (-0.74±0.23 at 64 min). Total Ch loss (nmoles) during 1 hr was related to the duration of the apnea episode that preceded it (1.5 min= -12.05 ±3.57; 3 min= -17.09±6.13; 6 min= -27.32±4.15; Controls (no apnea)= -6.99±2.25. This enhanced Ch loss took place in spite of increased Ch_a. Peak Ch_a (nmoles/ml) after apnea were: 1.5 min= 11.2±1.47; 3 min= 18.8±1.26; 6 min= 30.6± 1.07; Controls= 8.44±.21. It is concluded that apnea induces a significant loss of brain free Ch that could have metabolic consequences. Supported by the US Department of Veterans Affairs and USPHS MH 17691.

480.9

ONTOGENY OF CHOLINERGIC SENSITIVITY IN AN ANIMAL MODEL OF DEPRESSION: TOLERANCE DEVELOPMENT AND NEUROTRANSMITTER SYSTEM INTERACTION. L.C. Daws, G.D. Schiller, D.H. Overstreet*, School of Biological Sciences, Flinders University, Adelaide 5001, Australia.

The Flinders Sensitive and Resistant Lines (FSL, FRL-control line of rat) have been selectively bred for differences in cholinergic sensitivity. The FSL rat exhibits increased behavioural and physiological responses to cholinergic agonists. Thus, it is a an animal model of depression because an increased sensitivity of the cholinergic system has been postulated to be a trait marker of depression. The present studies aimed to investigate the development of responsiveness to the muscarinic agonist, oxotremorine, and its antagonist, scopolamine. FSL rats were consistently more sensitive to the hypothermic effects of oxotremorine by 20 days postpartum (dpp), whereas the development of sensitivity to scopolamine (as indexed by increased locomotor activity) was not different between the two lines. The early ontogenetic appearance of a substantial difference in sensitivity to muscarinic agonists suggests that cholinergic hyperfunction is a trait marker of predisposition towards depressive tendencies in FSL rats. However, since FSL rats are not different from FRL rats in their response to antagonists, it is suggested that the mechanisms underlying cholinergic supersensitivity are downstream from the muscarinic receptors. Subsequent studies showed that tolerance to oxotremorine emerged by 31 dpp when injected every third day from 10 dpp. Both lines exhibited tolerance suggesting that FSL rats have the capacity to maintain normal plasticity of the cholinergic system, although they are inherently more sensitive. A significant cross-tolerance to quipazine and salbutamol occurred in the FSL, but not the FRL rats. This strain-dependent cross-tolerance to noncholinergic drugs in FSL rats is consistent with the hypothesis advanced above that the mechanisms underlying their cholinergic supersensitivity are beyond the receptors.

481.1

AUTORADIOGRAPHIC LOCALIZATION OF MUSCARINIC CHOLINERGIC RECEPTOR (MCR) SUBTYPES IN CAT BRAIN STEM. H.A. Baghdoyan*, M.T. Roth, R.B. Duckrow, and D.C. Mash. Dept. of Anesthesia, Penn State Univ. Col. of Med., Hershey, PA 17033, and Dept. of Neurology, Univ. of Miami Sch. of Med., Miami. FL 33136.

Brain stem cholinergic mechanisms are involved in generating REM sleep, but the role of specific MCR subtypes is not clear. The goal of the present study is to map the distribution of MCR subtypes likely to be important for sleep cycle control. Using the technique of Flynn et al., (Neurosci. Abs. 17:586, 1991) which utilizes the kinetic properties of N-[^{3}H]-methylscopolamine to differentially label MCR subtypes, we are localizing M1, M2, and M3 subtypes throughout the cat brain stem. Receptor density is quantified using CCD-camera-based image analysis. Preliminary results show that the feline medial pontine reticular formation (mPRF), a non-cholinergic, cholinoceptive region involved in REM sleep generation, contains very few M3 receptors (4.0 fmol/mg tissue). The pedunculopontine tegmental nucleus (PPT), which provides cholinergic input to the mPRF, also has few M3 receptors (7.4 fmol/mg). In contrast, the laterodorsal tegmental nucleus (LDT) has 18.8 fmol/mg of M3 receptors. Relatively high levels of M3 receptors were also localized to the periaqueductal gray (15.4 fmol/mg) and the substantia gelatinosa (22.0 fmol/mg). M1 and M2 subtypes in mPRF, LDT, and PPT are being quantified. *Support: Dept. of Anesthesia, HL47749, MH45361 (HAB), NS24109 (RBD), NS25785 (DCM).*

481.2

COMPARISON OF CHOLINERGIC PROPERTIES AND MUSCARINIC RECEPTOR SUBTYPES PRESENT IN TWO NEURONAL CLONAL CELL LINES. M.A. BUCK,*L.A.TAYLOR, V. RUPERTO, C.E. TEDFORD and R.D. McQUADE, CNS Pharmacology, Schering Plough Research Institute, Bloomfield, NJ 07003.

Two cholinergic containing cell lines were investigated for various markers of cholinergic activity as well as characterized for muscarinic receptor subtypes. Muscarinic receptor characterization and identification in a neuronal cell model is of considerable interest to better understand muscarinic receptors and their mode of interaction in the central nervous system. Cells of the homogeneous hybrid line of mouse neuroblastoma X rat glioma (NG108-15) were compared with the PC12 phaeochromocytoma cell line derived from the rat adrenal medulla. PC12 and NG108-15 cells both demonstrated various levels of cholinergic activity. NG108-15 cells had higher levels of enzymatic activity for choline acetyltransferase (ChAT, 27.4 vs 13 nmoles/mg protein) and acetylcholinesterase (AChE, 1216 vs 376 nmol/mg protein) than PC12 cells. In contrast, functional measurements of high affinity choline transport (HAChT) indicated that PC12 cells contained almost double the activity. HAChT was sensitive to hemicholinium-3 (HC-3) and was modulated in both cell lines by muscarinic receptor agonists.

Muscarinic receptor characterization of the two cell lines was performed using saturation and competition binding studies with ^{3}H-N-methyl-scopolamine (^{3}H-NMS). Similar studies were performed in transfected Chinese hamster ovary (CHO) cell lines expressing the human muscarinic receptor subtypes, m1-5. Covalent labelling of the neuronal and transfected CHO cells with the muscarinic ligand, ^{3}H-propylbenzilylcholine mustard (^{3}H-PrBCM) and the mobilities of the mustard labelled species of these cells on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) confirm the existence and identity of muscarinic cholinergic receptor subtypes in these cell lines.

481.3

ALKALOIDS ISOLATED FROM TROPICAL MARINE SPONGES BIND TO MOLECULAR TARGETS IN RAT BRAIN MEMBRANES. G.E. de Motta, R. Rosa, A.D. Rodríguez, W. Silva and C. Jiménez*. Institute of Neurobiology and Depts. of Chemistry and Biology, U. of Puerto Rico and Dept. of Pharmacology, U. Central del Caribe.

Four structurally related $C_{11}N_5$ compounds isolated from sponges of the genus *Agelas* exhibited both stimulatory and inhibitory activities in frog skeletal and smooth muscles. Potential target sites for these compounds were evaluated for their interaction with muscarinic acetylcholine receptors (mAChR) and tetrodotoxin (TTX)-sensitive sodium channels using rat brain synaptosomal membranes and radioligand assays. Competition experiments using tritiated quinuclidinyl benzylate (^{3}H-QNB) to label mAChRs revealed the following rank order of potency : sceptrin > oroidin ≥ dibromosceptrin ≥ clathrodin. Competition experiments using tritiated saxitoxin (^{3}H-STX) as a sodium channel marker showed the following rank order of potency : sceptrin = dibromosceptrin > oroidin > clathrodin. Scatchard analyses demonstrated that sceptrin, the most potent member of this group, was a competitive inhibitor of both ^{3}H-QNB and ^{3}H-STX binding. (Supported by GM08102 and NS07464, NIH, and NOAA, UPR Sea Grant Program)

481.4

TOXIC EXTRACTS FROM THE BENTHIC DINOFLAGELLATE Ostreopsis lenticularis BIND TO RAT BRAIN MUSCARINIC RECEPTORS. J. Mercado, G.E. de Motta, W. Silva, T. Tosteson and R. Blanco*. Inst. of Neurobiology and Depts. of Biology and Marine Sciences, U. of Puerto Rico, and Dept. of Pharmacology, U.Central del Caribe.

Methanolic extracts prepared from clonal cultures of Ostreopsis lenticularis, a benthic dinoflagellate, were toxic to mice by i.p. injection. Reverse phase HPLC separation using isocratic methanol as the system solvent, produced two major fractions. Fraction I, with a retention time (R_t) of 2.04 min, contained apparently only one component while Fraction II consisted of a major component (R_t = 3.69 min) and several minors components. Using radioligand assay methods we studied the effect of the crude extracts and both HPLC fractions on muscarinic acetylcholine receptors (mAChR) in rat brain synaptosomal membranes. Preincubation of membranes with crude extracts (500 µg/mL), fraction I (500 µg/mL) and II (200 µg/mL) displaced binding of tritiated quinuclidinyl benzylate by 97.39 ± 3.7%, 64.285 ± 3.5% and 61 ± 1.51%, respectively. In frog gastric muscle strips, a preparation we have shown to contain M_3 mAChRs, crude extracts elicited contractions similar to those induced by acetylcholine in this muscle. (Supported by GM08102 and NS07464, NIH, and NOAA, UPR Sea Grant Program)

481.5

m1-TOXIN RECOGNIZES AND STABILIZES LIGANDED m1 MUSCARINIC RECEPTORS. S.I. Max*, J.S. Liang, and L.T. Potter. Molecular and Cellular Pharmacology, University of Miami School of Medicine, Miami, FL 33101.

m1-Toxin is the only ligand known which specifically identifies the extracellular face of m1 muscarinic receptors. By comparison, antagonists like pirenzepine show only partial selectivity, and antibodies recognize the third intracellular loop of m1 receptors. When applied before other ligands, m1-toxin blocks the binding of ^{3}H-antagonists only to m1 receptors (Max *et al.*, Neurosci Abstr 17:389). However, antagonists do not block the binding of m1-toxin. Membranes and soluble receptors were prepared from CHO-K1 cells transfected with the gene for m1 receptors. When applied after ^{3}H-N-methylscopolamine (NMS) or ^{3}H-pirenzepine, m1-toxin slowed the dissociation rates of these ligands from membranes at 25°C about four-fold. In digitonin, the dissociation of ^{3}H-NMS changed from a $T_{1/2}$ of about 100 minutes to zero. The implication of these results is that m1-toxin binds over the receptor pocket which holds traditional ligands, and does not project significantly into this pocket. Tetrahydroaminoacridine can similarly block m1 receptors and stop the dissociation of ^{3}H-NMS (Potter *et al.*, Mol Pharm 35:652), but this ligand is not specific for m1 receptors, and probably does not stabilize many of the extracellular loops of these receptors. Antibodies can bind to liganded m1 receptors (Levey *et al.* J Neurosci 11:3218; Wall *et al.* Mol Pharm 39:643), but have not been shown to stabilize the receptor pocket or the binding of other ligands. The specific, allosteric and nearly irreversible nature of the binding of m1-toxin may prove to have a number of uses. m1-Toxin can stabilize pure liganded (and unliganded) receptors for structural studies. Nonradioactive toxin can be used to increase the specific binding of ligands like ^{3}H-pirenzepine. Toxin-based affinity techniques may permit the isolation of pure already-labelled m1 receptors, even in detergents more effective and less expensive than digitonin. And m1-toxin may permit the study of agonist-receptor-G protein complexes which have so far proved too unstable to identify.

481.6

3-HEXYLOXY ANALOGS OF 3(1,2,5-THIADIAZOL-4-YL-1,2,5,6-TETRAHYDRO-1-METHYLPYRIDINE, TZTP) ARE POTENT AND SELECTIVE M1 AGONISTS IN VIVO. F. Bymaster*, D. Wong, C. Mitch, J. Ward, D. Calligaro, H. Shannon, B. Sawyer, S. Quimby, M. Sheardown, P. Olesen, P. Suzdak, P. Sauerberg. Lilly Research Laboratories, Indianapolis, IN 46285 and Novo Nordisk CNS Division, Malov, Denmark.

3-Hexyloxy-TZTP (TZTP-A) and 3-hexen-5-yl-TZTP (TZTP-B) are selective m1 agonists in vitro. In vivo, TZTP-A decreased the ex vivo binding (bdg) in cortex of 3H-pirenzepine (PZ), with an ED50 of 3 and 32 mg/kg, sc and po.; thus, TZTP-A penetrated into the brain. TZTP-A was a weaker inhibitor of bdg to M2 receptors with an ED50 of 14 mg/kg sc. TZTP-A and TZTP-B increased levels of the dopamine metabolite, dihydroxy-phenylacetic acid (DOPAC) in striatum up to 50%, presumably via M1 heteroreceptors. The increases in DOPAC were antagonized by M1-selective trihexyphenidyl. Both drugs transiently elevated acetylcholine (ACh) levels in striatum up to 60% and did not antagonize the large increases in ACh induced by oxotremorine, suggesting the increase in ACh was not via M2 receptors. TZTP-A inhibited PZ ex vivo bdg with a t1/2 of 3 hours and DOPAC levels were elevated up to 3 hours, but no changes were evident at 6 hours. Salivation via M3 receptor was not induced by TZTP-A up to 100 mg/kg po. Thus, TZTP-A entered the brain after systemic or oral administration, and was of moderate duration. Furthermore, TZTP-A and TZTP-B were efficacious M1 agonists in vivo, had low efficacy for M2-M3 receptors and are suitable for development as M1 agonists.

481.7

HEXYLOXY-TZTP:A POTENT AND SELECTIVE M1 AGONIST IN-VITRO. C.H. Mitch*, F.P. Bymaster, D.O. Calligaro, S. J. Quimby, B. D. Sawyer, H.E. Shannon, J.S. Ward, P.H. Olesen, P. Sauerberg, M.J. Sheardown, P.D. Suzdak. Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285 and Novo Nordisk, CNS Division, MålØv, Denmark.

Hexyloxy-TZTP (3-(4-hexyloxy-1,2,5-thiadiazole-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine) inhibited 3H-pirenzepine binding in rat hippocampus and 3H-oxotremorine-M binding in rat cortex with IC50 values of 7 nM and 10 nM, respectively. In cloned cells expressing human m1 receptors, hexyloxy-TZTP increased phosphoinositide turnover, and the potency and efficacy differed dependent upon the cell type in which the receptors were expressed (CHO>A9L>BHK). In cloned cells expressing m2, m3, m4 or m5 receptors, hexyloxy-TZTP was considerably less active. Hexyloxy-TZTP also was selective for M1 receptors in isolated tissues. At M1 receptors in rabbit vas deferens, it inhibited twitch height with an IC50 of 8 pM. Pirenzepine blocked the effects of hexyloxy-TZTP in rabbit vas deferens and had a Kb value of 10 nM. At M2 receptors in guinea pig atria, hexyloxy-TZTP had an IC50 of 5 μM. Hexyloxy-TZTP was a partial agonist in guinea pig ileum. In guinea pig bladder, it was neither an agonist nor an antagonist. The present results demonstrate that hexyloxy-TZTP is a potent, efficacious and selective M1 muscarinic agonist in-vitro.

481.8

EVIDENCE THAT M1 MUSCARINIC RECEPTOR SUBTYPE MEDIATES THE EFFECTS OF OXOTREMORINE ON MASCULINE SEXUAL BEHAVIOR.

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It has been shown that oxotremorine (OXO), a muscarinic receptor agonist, has a facilitatory effect on masculine sexual behavior in rats. Muscarinic receptors have been divided in several subtypes. This study analyzes the possible participation of M1 muscarinic receptor subtype in the mediation of OXO effects on masculine sexual behavior. Two groups of male rats received seven doses of the specific M1 antagonist trihexyphenidyl (TRI, 0.1, 0.2, 0.4, 0.8, 1.6 and 6.4 mg/Kg, i.p.) 30 min before assessing sexual behavior. Latency and frequency of mount, intromissions and ejaculation were recorded as well as hit rate and inter-intromission interval. No changes in these parameters of sexual behavior were observed following TRI administration. In a different group of male rats, five doses of TRI were administered before OXO (0.4 mg/Kg, i.p.). The facilitatory effect of OXO was completely prevented even with the smallest dose of TRI. These results strongly suggest the notion that cholinergic facilitation of masculine sexual behavior is mediated through the M1 muscarinic receptor subtype.

481.9

MODULATION OF ACETYLCHOLINE RELEASE BY MUSCARINIC RECEPTORS IS ALTERED FOLLOWING LESION OF CHOLINERGIC INPUTS WITH THE NEUROTOXIN AF64A.

B. Thorne* and P.E. Potter. Dept. Anesthesiology, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx NY 10467.

The effects of cholinergic agonists on the evoked release of ^{3}H-acetylcholine (ACh) were studied in male Sprague-Dawley rats in which hippocampal cholinergic terminals were lesioned with the neurotoxin AF64A (ethylcholine mustard aziridinium, 2 nmoles/ventricle). Two weeks after AF64A infusion, choline acetyltransferase activity was decreased by more than 40%. ACh release was evoked from superfused hippocampal slices by electrical stimulation (1 or 2 Hz, 2 min). The nicotine dose response curve was shifted to the left following treatment with AF64A. The EC_{50} was shifted from 40 μM (controls) to 3.7 μM (AF64A). This change in response to nicotine was not due to changes in nicotinic binding sites. When the muscarinic receptors were blocked by atropine the nicotine dose response curve of control tissue, was shifted to the left (EC_{50} 4.1 μM). Atropine had no effect on the nicotine dose response curve of AF64A treated rats. In contrast, the dose response curve for inhibition of ACh release by the muscarinic agonist oxotremorine, was shifted to the right following AF64A treatment. This change in response to muscarinic drugs was reflected in a change in muscarinic M_2 but not M_1 binding sites.

481.10

LIGHT AND ELECTRON MICROSCOPIC LOCALIZATION OF m2 MUSCARINIC RECEPTOR PROTEIN IN RAT SEPTUM AND HIPPOCAMPUS.

A. Levey*, S. M. Hersch and S. M. Edmunds. Dept. of Neurology, Emory University School of Medicine, Atlanta, GA 30322

Pharmacological studies indicate that muscarinic acetylcholine receptors presynaptically regulate the release of neurotransmitters. However, the precise identity of presynaptic genetic subtypes is unknown. The cellular and subcellular distribution of m2 receptor in medial septum and hippocampus was determined using subtype-specific antibodies (Levey et al., 1991), avidin-biotin-peroxidase immunocytochemistry, and light and electron microscopic analysis. In medial septum, m2 immunoreactivity was present in both noncholinergic and cholinergic perikarya, and was more dense and punctate in the neuropil. At the ultrastructural level, m2 immunoreactivity was associated with the cytoplasmic face of the plasma membrane in perikarya, and in dendrites and postsynaptic densities; m2 was also present in many terminals. In hippocampus, m2 was present in cell bodies and processes of large neurons in the stratum oriens and hilus. Neuropil immunoreactivity was abundant in the pyramidal neuron layer, the polymorph layer of dentate gyrus. At the ultrastructural level, immunoreactivity primarily was in dendritic spines and rare axon terminals. Labeled spines contained reaction product within cytoplasm and within their postsynaptic densities. Contrary to current dogma, these findings indicate that in the septal region, m2 receptors are presynaptic (as well as postsynaptic), and that in hippocampus, most m2 receptor immunoreactivity is postsynaptic. Supported by Alzheimer Association Faculty Scholar Award (AIL), NS 01387, and NS 30454.

481.11

MUSCARINIC RECEPTOR SUBTYPES INVOLVED IN CONTRACTION OF THE GUINEA PIG ILEUM.

E. A. Thomas* and F. J. Ehlert. Department of Pharmacology, College of Medicine, University of California, Irvine, CA 92717.

Muscarinic receptor subtypes involved in smooth muscle contraction were investigated in the guinea pig ileum. The muscarinic agonist oxotremorine-M (Oxo-M) caused concentration-dependent contractions of the isolated ileum (IC_{50}=38 nM) with pharmacology consistent with that of an M_3-mediated response. In order to inactivate the M_3 muscarinic receptors selectively, ilea were pre-treated with the irreversible M_1/M_3 selective muscarinic antagonist 4-DAMP mustard (N-(2-chloroethyl)-4-piperidinyl diphenylacetate) (40 nM) for 1 hr in the presence of the reversible M_2-selective antagonist AFDX-116 (1 μM), and then washed extensively. This treatment caused a 22-fold rightward parallel shift in the concentration-effect curve for Oxo-M. In separate experiments, ilea were pre-treated with histamine (0.3 μM) and isoproterenol (1 μM) before measuring oxo-M-induced contractions. The resulting concentration-effect curve was biphasic consisting of high (0-50 nM) and low (> 50 nM) affinity components. Similar treatment with 4-DAMP mustard and AFDX-116 only slighty shifted the high affinity component, while producing a much greater shift in the low affinity component of the curve. Treatment with AFDX-116 (1 μM) abolished the high affinity component producing a monophasic concentration-effect curve. 4-DAMP mustard (10 nM; 1 hr) also prevented M_3-receptor mediated stimulation of phosphoinositide hydrolysis in the longitudinal muscle of the rat ileum, resulting in a 6.6-fold increase in the EC_{50} value with a 65% reduction of the maximal response. In contrast, this treatment only blocked M_2-receptor mediated inhibition of adenylate cyclase by a 2-fold increase in EC_{50}, without affecting maximum inhibition. These results support the hypothesis that the M_2 muscarinic receptors present in smooth muscle may influence contraction, perhaps by inhibiting relaxation induced by other receptors. *Supported by N.I.H. Grant NS 26511*

481.12

THE (+) OPTICAL ISOMER OF [(Z)-2-PCE] SELECTIVELY BLOCKS M_3 (ILEAL) BUT NOT M_2 (CARDIAC) MUSCARINIC RECEPTORS,

E.B. Thompson, M. Lu, S.M. Vogel and N.P. Plotnikoff*. Depts. of Pharmacodynamics, and Med. Chem. College of Pharmacy; and Pharmacology, College of Medicine, U.I. Chgo, IL 60612.

Studies in our laboratory have utilized muscarinic antagonists as probes for exploring topographical areas of the muscarinic receptors . In these studies, direct clues as to the actual binding conformation of the hydrophobic partion of structurally flexible molecules (such as atropine and QNB) have been provided by use of synthetic analogues which are structurally locked into certain desired conformations. Our earlier studies (Lu et al., 1991) showed that the racemic compound 2-phenyl cyclohexyl diethylamino ethyl ether [(Z) -2-PCE] was more potent in ileal (PA_2 = 7.15) than in atrial (pA_2 = 4.96) preparations. This suggests that the Z isomer is one of the most ileal selective muscarinic antagonist reported to date. In this study the optical isomers of the above compound were synthesized and evaluated pharmacologically on isolated rat atria and ileum preparations. Ileal selectivity of the optical isomers was similar to that of the racemate. The (+) isomer was found to be a potent competitive antagonist by a factor >100 fold, for the ileal but not the atrial muscarinic receptors. Conversely, the (-) isomer appeared to be non-competitive in this ileum. The results indicate that the competitively antagonistic activity of the racemate is due to the (+) optical isomer.

481.13

PROPERTIES OF STRIATAL m4 MUSCARINIC RECEPTORS. S.L. Purkerson, H.E. Hanchett, and L.T. Potter*. Molecular and Cellular Pharmacology, University of Miami School of Medicine, Miami, FL 33101.

The rat striatum expresses an unusually high concentration of m4 muscarinic receptors, many m1 receptors, and a few m2 receptors (Levey *et al.* J Neurosci 11:3218). m1-Toxin quantitatively blocks the m1 receptors, and 95% of the residual receptors are m4 receptors (Purkerson *et al.* Neurosci Abstr 17:390). This approach permits the first binding studies of m4 receptors coupled to normal amounts of native G-proteins. The order of antagonist affinities was NMS, trihexyphenidyl, biperiden, silahexocyclium, hexahydrodifenidol, methoctramine, himbacine, pirenzepine, gallamine and AFDX 116. Five of these ligands bound with the same affinity to rat striatal m4 receptors and human m4 receptors expressed in CHO-K1 cells. Agonist binding curves showed equal populations of GppNHp-sensitive high affinity (K_H) sites and GppNHp-insensitive low affinity (K_L) sites in both striatal and CHO-cell membranes. These studies suggest that m4 receptors, like m1-m3 receptors (Potter *et al.* Mol Pharm 39:211), may be dimeric. The order of K_H affinities was oxo-M, oxotremorine, acetylcholine, pilocarpine, carbachol, methacholine and arecoline, and K_H and K_L affinities were very similar in striatal and CHO cells. The order of K_L/K_H ratios (which often correlate with agonist efficacies) was oxo-M, carbachol, acetylcholine, methacholine, oxotremorine, arecoline and pilocarpine, which would suggest that quaternary agonists work better than tertiary agonists. These data do not correlate well with the K_d values and efficacies found for agonists by McKinney *et al.* (Mol Pharm 40:1014) for the inhibition of cAMP levels in rat striatum. In their studies oxo-M showed high affinity and efficacy, but so did several tertiary agonists. Possible reasons for the different binding and functional data include effects of activating m1 receptors on cAMP levels, and the high activity of tertiary agonists on m2 receptors. Autoradiographic studies of m1 and m4 receptors confirm their wide distribution within the striatum, and indicate their distinct cellular locations.

481.14

ALLOSTERIC MODULATION OF MUSCARINIC RECEPTORS BY BASIC PROTEINS IN RAT CEREBRAL CORTEX AND HEART. J. Hu and E.E. El-Fakahany *. Division of Neuroscience Research in Psychiatry, University of Minnesota Medical School, Minneapolis, MN 55455.

Allosteric interactions of arginine or lysine-rich proteins, such as histones, myelin basic protein (MBP) and dynorphin A (1-13), with muscarinic receptors were investigated in rat cerebral cortex and heart using radioligand receptor binding assays. These basic proteins inhibited binding of the muscarinic ligand [^{3}H]NMS at equilibrium and altered kinetics of its dissociation from the receptors. Histone VIII (rich in arginine) showed an inhibition constant value of 1.4 μM and a cooperativity value (α) of 5.4 in cerebral cortex. It also decreased the rate of dissociation of [^{3}H]NMS with an IC_{50} of 0.9 μM and a maximal inhibition of 60%. No significant difference was observed between its effects in cerebral cortex and heart. MBP also inhibited [^{3}H]NMS binding at equilibrium in a concentration-dependent manner with a lower α value (3.3 in cortex and 1.9 in heart). Maximal inhibition of specific [^{3}H]NMS binding by MBP was reduced from 45% to 16% by increasing the concentration of [^{3}H]NMS from 0.04 nM to 0.8 nM. The allosteric nature of MBP was also demonstrated in kinetic studies. The small basic peptide dynorphin A also exerted an allosteric effect on muscarinic receptor which was dependent on the number of basic residues. Our data suggest that positively charged amino acid residues in endogenous proteins might play a role in the regulation of the conformation of muscarinic receptors.

481.15

PHOSPHATIDYLCHOLINE-SPECIFIC PHOSPHOLIPASE D IS COUPLED TO RECEPTOR OCCUPANCY IN A9 CELLS TRANSFECTED WITH THE M3-MUSCARINIC RECEPTOR. P.G. Holbrook*, J. Wess# and C.C. Felder, NIMH and #NINDS, National Institutes of Health, Bethesda, MD 20892

In the presence of ethanol, phospholipase D (PLD) catalyzes a transphosphatidylation reaction producing phosphatidyl-ethanol (PEt). PEt formed in A9 cells stably transfected with the rat *m3* muscarinic receptor and stimulated with carbachol (1mM) was isolated and analyzed by fast atom bombardment mass spectrometry. It had a molecular species profile identical to that of phosphatidylcholine (PC) from unstimulated cells. In cells labeled overnight in serum free media containing ^{3}H-palmitic acid, the carbachol (100 uM) stimulated formation of ^{3}H-PEt required extracellular calcium (1mM) and was blocked by atropine (10 uM). TPA stimulated formation of ^{3}H-PEt in the presence and in the absence of extracellular calcium. EC_{50}s for carbachol stimulated ^{3}H-PEt and ^{3}H-PA formation were respectively: 8.8×10^{-6} and 5.5×10^{-7} M. Experiments with receptor chimeras produced by switching the third cytoplasmic loops of the *m3* and *m2* receptors suggest that g-protein coupling is necessary for PLD-activation. Occupancy of muscarinic receptor subtypes that couple to PI-specific PLC leads also to activation of a PC-specific PLD.

481.16

DIFFERENCES IN CARBACHOL BINDING AND PI TURNOVER BETWEEN THE DORSAL AND VENTRAL HIPPOCAMPUS. H. Ladinsky[1]*, A. Garcia[2], M. Zambelli[2], G. Schiavi[1] and S. Consolo[2]. Department of Biochemistry, Boehringer Ingelheim Italia, Milan 20139 and Mario Negri Institute, Milan, Italy 20157.

The full agonist CARB was more effective in stimulating PI turnover in the ventral (VH) than in the dorsal hippocampus (DH) while the partial agonist oxotremorine was similarly weak in both regions. No differences in density or distribution (≈20% M1, 50% M3, 30% M4) of muscarinic receptor subtypes between DH and VH were found to explain the effect using several selective antagonists in competition experiments. From the affinities of pirenzepine (K_H 6.1 nM; K_L 757 nM) and DAU 6202 (4-hydroxy-3-(tropyl) oxycarbonyl-3,4-dihydro-1H- quinazoline-2-one)(Kb 2.5 nM) to antagonize CARB-stimulated PI turnover, it appeared that about 50% of the response was activated by M1 and the other 50% by M3 receptors in either region. In binding studies against [^{3}H]NMS, CARB recognized three agonist affinity states (SH, H, and L) in the VH and two (H and L) in the DH. Gpp(NH)p converted the SH and H to the L state in both regions. Thus, regional differences in types or concentrations of G proteins may account for the complexity with which CARB binds to muscarinic receptors and stimulates PI turnover in the areas.

481.17

EVIDENCE FOR INTERACTION BETWEEN THE PHOSPHOINOSITIDE AND ADENYLATE CYCLASE SIGNAL TRANSDUCTION PATHWAYS IN THE RAT PAROTID GLAND. E. H. Gerstin, Jr. and F. J. Ehlert* , Department of Pharmacology, College of Medicine, University of California, Irvine, CA 92717.

In this study, we investigated whether an increase in cyclic AMP levels could modulate muscarinic receptor-mediated phosphoinositide hydrolysis in the rat parotid gland. Slices of the gland were incubated with the muscarinic agonist, oxotremorine-M (OXO-M), in the absence and presence of the adenylate cyclase-stimulator, forskolin. OXO-M at concentrations of 10 and 100 μM caused 6.98- and 13.6-fold increases in phosphoinositide hydrolysis over basal values, respectively. Forskolin (75 μM) caused significant 19.3 ± 5.01% ($P < 0.01$) and 19.0 ± 7.05% ($P < 0.05$) inhibitions of the phosphoinositide hydrolysis elicited by OXO-M at concentrations of 10 and 100 μM, respectively In preliminary experiments, OXO-M stimulated PI hydrolysis with an EC_{50} of 2.76 μM; the maximal effect was a 4.36-fold increase over basal value. The effect of forskolin (75 μM) was to increase the EC_{50} of OXO-M to 23.83 μM, which represents an 8.6-fold increase. We conclude that the forskolin-mediated increase in c'AMP results in a decrease in the OXO-M mediated phosphoinositide hydrolysis. These results suggest that receptors which increase c'AMP levels in the parotid gland may dampen muscarinic receptor-mediated phosphoinositide hydrolysis.
Supported by NIH Grant NS 26511

481.18

COMPARISONS BETWEEN HUMAN MUSCARINIC RECEPTOR SUBTYPES COUPLED TO PHOSPHOLIPASE C AND THOSE COUPLED TO ADENYLYL CYCLASE: EFFECT OF RECEPTOR RESERVE. L.L. Lauffer, R.D. Schwarz*, R.D. . and C.J. Spencer.
Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.

Muscarinic receptors have been shown to exist as five distinct proteins which are functionally coupled through G proteins to phospholipase C (m1, m3, and m5) and adenylyl cyclase (m2 and m4). Stable expression of human receptors in CHO cells yielded cell lines with B_{max} values ranging from 210-2450 fmoles/mg protein and K_d values of 0.11-0.63nM (whole cell [^{3}H]-NMS binding). The use of these lines has allowed the pharmacological selectivity of various muscarinic agonists and antagonists to be determined using receptor binding and second messenger assays. Previous results obtained measuring PI hydrolysis, showed that both efficacy and potency of muscarinic agonists were markedly affected by receptor number, while antagonist results were not. However, it was not known whether similar effects occurred in cyclase linked receptors. In the present study, alkylation of Hm2 and Hm4 receptors was performed in order to determine the effect of receptor number on agonist/antagonist-induced changes in cAMP formation. These results are compared to those obtained measuring PI turnover under similar conditions in Hm1, Hm3, and Hm5 cells.

481.19

PROTO-ONCOGENE INDUCTION IN THE CNS OF LONG- AND SHORT-SLEEP MICE MEDIATED VIA MUSCARINE ACETYLCHOLINE RECEPTOR ACTIVATION. L. Tsiokas* and M. Watson. Dept. of Pharmacology, UMDNJ-N.J. Med. Sch., Newark, NJ 07103-2714.

Induction of several immediate early genes (IEGs) was studied in brains of long- and short-sleep (LS/SS) mice after activation of muscarinic receptors (mAChR) by oxotremorine (OXO;5mg/kg;ip) treatment (Rx). Brains were excised at t=0,15,30,45,60,90,120 and 180m after OXO Rx. Total RNA was extracted from LS/SS brains and processed for Northern analysis via cRNA probes for c-fos, c-jun, jun-B, jun-D and egr-1/NGFI-A as was described. c-fos mRNA (2.2 kb) shows no basal but a dramatic induction with peak levels at 60-90m in both strains. c-jun mRNA show rapid peak accumulation at 60m in both. Basal c-jun mRNA levels are significant with two transcripts of 2.9 and 3.6kb in murine CNS. The egr-1/NGFI-A gene was also induced by OXO and its mRNA (3.2kb) reaches a peak at t= 90 and 120m in LS and SS, respectively. Basal expression of the egr-1/NGFI-A gene is also seen in the murine CNS. jun-D expression is extremely robust and constitutive in nature, yet the significance of induction of jun-B is not certain. Induction of c-fos and c-jun genes show a characteristic dose-response with 1-5mg/kg OXO Rx producing increased c-fos and c-jun mRNA levels. Activation of mAChRs in vivo induce rapid and transient genomic responses via induction of several IEGs that might account for long-term changes in cellular phenotypes. (MH-43024).

EXCITATORY AMINO ACIDS: EXCITOTOXICITY V

482.1

USE OF A HERPES SIMPLEX VIRUS (HSV-1) VECTOR SYSTEM TO INTRODUCE GENES INTO CULTURED CORTICAL NEURONS WHICH ARE USED TO ASSESS EXCITATORY AMINO ACID NEUROTOXICITY. Dean Hartley*, Rachael Neve, Matthew During, and Alfred Geller. Div. Endocrinology, Childrens Hosp, McClean Hosp, and Harvard Med. Sch. Boston MA; Yale U. Sch. Med. New Haven CT.

Glutamate has been implicated in the pathophysiology of numerous neurodegenerative diseases, including Stroke, Huntington's disease and Alzheimers disease. Glutamate-induced neurotoxicity is mediated by the influx of calcium, however the biochemical pathways subsequent to calcium influx are poorly understood. Therefore, it would be useful to specifically alter calcium activated processes. Our laboratory has previously described a HSV-1 vector system which is capable of introducing genes into mature neurons (Science 241:166, 1988; TINS 14:428, 1991). We are exploiting this vector system to genetically alter signal transduction pathways to elucidate intracellular mechanisms mediating glutamate neurotoxicity. Our initial studies characterized the ability of the HSV vector system to deliver genes into cortical neurons which have been previously used to assess glutamate neurotoxicity (Hartley and Choi, JPET 250:752, 1989). The prototype vector pHSVlac expresses E.coli B-galactosidase from the constitutive HSV IE 4/5 promoter. Following infection of these cortical cultures with pHSVlac, B-galactosidase expression was readily detected in 30-50% of the neurons, as well as in glia cells. Because of the importance of calcium in mediating glutamate neurotoxicity, we are expressing genes that affect calcium mediated processes and assessing their ability to alter glutamate mediated neurotoxicity.

482.2

DELAYED ADMINISTRATION OF MEMANTINE (MTE) PREVENTS NMDA RECEPTOR-MEDIATED NEURONAL DEATH. James W. Pellegrini*, H.-S. Vincent Chen, Frances E. Jensen, and Stuart A. Lipton. Dept. of Neurology, Children's Hospital and Progr. in Neurosci., Harvard Medical School, Boston, MA 02115.

Increasing evidence indicates that escalating levels of excitatory amino acids are responsible for neuronal cell death in a variety of neurological conditions including hypoxia-ischemia. The predominant form of neurotoxicity appears to be mediated by the NMDA subtype of glutamate receptor. Recently, using rat retinal ganglion cell (RGC) cultures, we found that the anti-Parkinsonian drug MTE blocks NMDA-activated current by a mechanism of open-channel blockade (IC_{50} = 1 μM). Here, using single channel recording, we demonstrate that 12 μM MTE reduced the frequency of NMDA-elicited channel opening by 87%, shortened the mean open time by 30%, but did not change the unitary channel conductance. We had previously shown that MTE prevents NMDA receptor-mediated neurotoxicity when administered coincident with the insult *in vitro* or *in vivo*. We have now tested whether delayed administration of MTE can prevent neurotoxicity. Cultured RGCs were exposed to toxic (25 μM) glutamate levels in high Ca/low Mg medium and compared with control cells. By the next day, this insult resulted in neuronal survival of 35%. In sibling cultures, MTE (12 μM) was added at 0-7 h after glutamate exposure. Survival at 24 h was close to control levels when MTE had been administered at 0 or 1 h, and approached 80% after treatment at 4 h. Presently, we are using 7-10 day old Long-Evans rats in a bilateral carotid ligation stroke model. In 7/11 litter-matched pairs, animals treated 1 h after insult with MTE (20 mg/kg) had substantially smaller strokes by MRI and histopathology after 48 h.

482.3

HALOPERIDOL AND RIMCAZOLE PREVENT INDUCTION OF THE HSP70 HEAT SHOCK GENE IN NEURONS INJURED BY PHENCYCLIDINE AND MK801. F.R. Sharp*, S. Wang, M. Butman, J. Koistanaho, S. Graham, L. Noble, S.M. Sagar, P. Berger, F.M. Longo. Dept. Neurology, UCSF School Medicine, SFVAMC, San Francisco, CA 94121.

The non-competitive NMDA receptor antagonists, PCP and MK801, produce abnormal vacuoles in posterior cingulate and retrosplenial cortical neurons (Olney et al. Science 1989). We show that PCP and MK801 induce *hsp70* mRNA and HSP72 heat shock protein in these injured neurons, and PCP induces the hsp70 gene in injured layer 2,3,5, and 6 neocortical neurons. The drug induced injury in cingulate and neocortex: occurs in 30 day and older rats, but not in 0-20 day old rats; and is prevented by prior administration of the antipsychotic drugs haloperidol (>5mg/kg) and rimcazole (>60mg/kg). It is suggested that the vacuolar injury that occurs following blockade of NMDA receptors reflects denatured proteins that induce the *hsp70* gene. Since haloperidol, rimcazole, M1 antagonists, and GABA agonists (Olney et al., Science 1991) prevent the injury, this suggests multiple receptors might mediate the injury. Alternatively, NMDA receptor blockade might activate sigma receptors which mediate the injury since haloperidol, rimacazole, and M1 antagonists have all been reported to block sigma receptors.

482.4

A TRANSGENIC MOUSE MODEL TO ASSESS THE NEUROPROTECTIVE EFFECT OF HSP70 IN THE CA1 AND CA2 REGION OF THE HIPPOCAMPUS

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Heat shock proteins (HSPs) are induced rapidly in the mammalian brain following exposure to excitotoxins and ischaemia. Correlative evidence from several studies suggests that HSPs (particularly HSP70) may play a role in enhancing neuronal survival after stress. HSP70 proteins mediate the transport of newly synthesized precursor proteins across intracellular membranes. Cells expressing high concentrations of HSP70 may be more resistant to toxic insults due to the rapid replacement of damaged proteins. To test this possiblity, we have made a DNA construct in which the expression of HSP70 is driven by promoter 1 of the rhombotin gene. This promoter has previously been shown to direct LacZ expression to specific subpopulations of neurons in the hippocampus of adult mice, notably in the CA1 and CA2 regions. Thus far, injections of the contruct into the pronuclei of fertilized mouse eggs have resulted in the generation of 6 founder mice, which are currently being tested for expression. Lines of expressing mice will be used to assess the neuroprotective effects of HSP70 in specific hippocampal neurons following exposure to various insults, both in vivo and in vitro.

482.5

A SHORT EPISODE OF SEIZURE ACTIVITY PROTECTS CA3 NEURONS FROM PROLONGED SEIZURE ACTIVITY-INDUCED DEATH.

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Systemic administration of kainic acid (KA) produces recurrent seizure activity and the loss of selectively vulnerable neuronal populations, in particular pyramidal CA3 neurons. We investigated the effects of a short episode of seizure activity on CA3 neuronal death following subsequent prolonged seizure activity. One group of adult rats was subjected to 1 hr of KA-induced seizure activity which was terminated by pentobarbital anesthesia, and treated again 16 hrs later with KA. Another group received KA twice at 16 hrs interval, while a third group received only one injection of KA. Animals were sacrificed at 16 hrs or 5 days after the last KA administration. Animals which were subjected to a short seizure episode exhibited a marked protection against delayed neuronal death in CA3 neurons resulting from a subsequent KA-induced episode of seizure activity. Histological examination of the brains of animals sacrificed 5 days after the last KA administration indicated a total absence of neuronal loss in CA3 as compared to animals which had been treated with one or two KA injections and which showed extensive neuronal loss. Immunocytochemistry of heat shock protein 72 (HSP72) performed 16 hrs after a short episode of seizure activity indicated that this period was sufficient to cause an increase of HSP72 in CA3 pyramidal cells suggesting that HSP72 could participate in the neuronal protection observed under these conditions. This phenomenon of "seizure tolerance" might therefore be viewed as an analog of the phenomenon of " ischemic tolerance" described in the gerbil brains.

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482.6

CORTICAL NMDA TOXICITY *IN VITRO* IS DECREASED BY ETHANOL EXPOSURE.

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Among the many actions of ethanol on the central nervous system are interactions with the N-methyl-D-aspartate (NMDA) receptor complex leading to inhibition of cyclic GMP production, altered ion currents, decreased neurotransmitter release and diminished NMDA-induced intracellular Ca^{2+} increases. Cortical cultures obtained from gestational day 19 rat embryos were employed to investigate a possible ethanol inhibition of NMDA toxicity. Cultures exposed for 30 minutes to NMDA in Mg^{2+}-free buffer with and without ethanol were compared using release of lactate dehydrogenase (LDH) as an assay for cytotoxicity. Cultures exposed to NMDA in the presence of ethanol released significantly less LDH than those exposed without ethanol, although considerable LDH release was seen following both conditions. These findings are consistent with other ethanol-induced changes in NMDA receptor function and suggest a possible protective role for ethanol in NMDA toxicity *in vitro*.

482.7

SELECTIVE ANTAGONISM OF JORO SPIDER TOXIN (JSTX) AGAINST QUISQUALATE (QUIS)-INDUCED LESION IN THE HIPPOCAMPUS.

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QUIS, a non-NMDA agonist, is known to provoke seizures with a selective cell loss in the hippocampus, indicating a suitable neurotoxin for a specific experimental model of epilepsy. We reported that a JSTX analogue, 1-naphthyl-acetylspermine (1-NA-Spm), exerts a potent and selective suppression of hippocampal epileptic discharges induced by QUIS (Brain Res., in press).

The present study was conducted to evaluate whether 1-NA-Spm selectively antagonized against histological changes induced by QUIS as well. Male Wistar rats were injected icv with 1-NA-Spm followed by either QUIS or quinolinate (QUIN), a NMDA agonist. After behavioral changes were observed, the animals were subjected to histological examination. QUIS (30ug) resulted in the loss of CA3 pyramidal cells in the injection side, which was completely blocked by 1-NA-Spm (50 ug) pretreatment. Contra-lateral side remained intact irrespective of history of repetitive seizures. JSTX analogue alone (80 ug) had virtually no effect on the cellular architecture in the hippocampus. QUIN (30 ug) induced CA1/4 lesion which was refractory to JSTX pretreatment. In several rats, JSTX appeared to exacerbate QUIN-induced changes.

482.8

LOW POTENCY OF COMPETITIVE NMDA ANTAGONISTS AGAINST GLUTAMATE NEUROTOXICITY DUE TO ASTROCYTE UPTAKE.

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A puzzling feature of the pharmacology of glutamate neurotoxicity is the low potency of competitive NMDA antagonists compared to what would be expected on the basis of radioligand binding studies. These experiments were performed in order to examine the role of glutamate uptake in determining the apparent pharmacology of competitive antagonists in tissue culture models of glutamate neurotoxicity.

The potencies of APV against the transported agonist glutamate and the non-transported agonist NMDA in astrocyte-rich and astrocyte-poor cortical cultures were determined. Both agonists were used at approximately 5 times their EC50. The IC50 of APV against glutamate (1mM) in astrocyte-rich cultures was approximately 1500 μM compared to 25 μM against NMDA (200 μM). The difference in potency disappeared in astrocyte-poor cultures in which the IC50 for APV against both glutamate (20 μM) and NMDA (200 μM) was 60-70 μM.

These results are best explained by a model in which glutamate uptake plays an important role, and in which toxicity at the dendrite and at the cell body can be differentiated.

482.9

PICOLINIC ACID MODULATES KAINIC ACID INDUCED GLUTAMATE RELEASE FROM RAT STRIATAL SLICES.

L.C. Vrooman, K. Jhamandas*, R.J. Boegman, R.J. Beninger. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Picolinic acid (PIC), a pyridine monocarboxylic acid and metabolite of tryptophan, protects neurons from excitotoxic damage that is dependent on the presence of intact glutamatergic input. It is hypothesized that PIC may produce its effect by inhibiting glutamate (Glu) release. In the present study, we examined the effect of PIC on kainic acid (KA) (1 mM) induced Glu release from rat striatal slices. Endogenous Glu release was analyzed by HPLC with fluorescence detection. A dose response relationship was observed for the inhibition of KA-induced Glu release by PIC. PIC (100 μM) was found to maximally inhibit KA-induced Glu release by 65%, an effect similar to that produced by the selective non-NMDA receptor antagonist DNQX (500 μM). PIC only inhibited the calcium dependent component of releasable Glu. Nicotinic acid and isonicotinic acid, two structural analogues of PIC, showed similar profiles. PIC alone was found to significantly increase basal release of Glu by 44%. These results suggest that PIC has a stimulatory as well as an inhibitory action on striatal Glu release.

(Supported by the Medical Research Council of Canada)

482.10

SIGMA LIGANDS PROTECT CULTURED RAT SPINAL CORD NEURONS FROM NMDA-INDUCED TOXICITY.

J.B. Long[1]*, R.J. Gaspari[1], M.A. DeCoster[1], and B.R. de Costa[2]. [1]Neuropharm. Br., Dept. of Med. Neurosci., Div. of Neuropsych., Walter Reed Army Inst. of Res., Washington, D.C. 20307 and [2]Lab. of Med. Chem., NIDDK, Bethesda, MD 20892.

The NMDA receptor complex has been implicated as a primary mediator of excitotoxic neuronal injury due in part to the protective effects of a variety of competitive and PCP-related noncompetitive NMDA receptor antagonists. Recent evidence with neuronal ischemic injury in vivo suggests that salutary effects of several non-competitive NMDA receptor antagonists might involve actions at σ binding sites in addition to the PCP binding component of the NMDA receptor complex. We therefore examined the protective effects of several σ cross-reactive NMDA receptor antagonists [dextromethorphan (DM), ifenprodil (IF) and (+)-SKF 10,047 (SKF)] in comparison with several highly selective and potent σ receptor ligands [including (+)-pentazocine (PEN), DTG, (+)-PPP, and BD 1008 (BD)] during exposure of primary cultures of rat spinal cord neurons to excitotoxic concentrations of NMDA (50-200 μM). After 8-10 days in culture, neurons were treated for 1 hr with NMDA/drug combinations in Locke's solution from which $MgCl_2$ and glucose were omitted. The following day, cell damage was quantitatively assessed using a tetrazolium salt colorimetric assay. DM, IF and SKF dose-dependently (and at highest doses completely) prevented NMDA-induced loss of cell viability. Not all σ ligands were protective; however, at concentrations ranging from 0.1-100 μM, PPP, DTG, PEN, and BD (which by themselves had no effect on measures of cell viability), also significantly, dose-dependently protected against NMDA neurotoxicity. These results indicate a potential usefulness of σ receptor ligands in the treatment of CNS neuronal injury.

482.11

AMANTADINE INHIBITS EXCITOTOXICITY IN CEREBROCORTICAL CULTURES. H.S. Lustig, K.L. von Brauchitsch, J. Chan and D.A. Greenberg*. Department of Neurology, University of California, San Francisco, CA 94110.

Excitatory amino acids (EAAs) have been implicated in the pathogenesis of acute and chronic neurodegenerative processes, and EAA antagonists protect against excitotoxicity in a variety of *in vivo* and *in vitro* disease models. Certain antiparkinsonian drugs, including amantadine, inhibit EAA responses mediated through *N*-methyl-D-aspartate (NMDA)-preferring EAA receptors and compete for [^{3}H]MK-801 binding sites on NMDA receptor-gated ion channels. Therefore, such drugs might not only reduce parkinsonian symptoms, but also modify neurodegeneration. Using neuron-enriched cultures from embryonic rat cerebral cortex, we investigated the effect of amantadine on NMDA-induced toxicity, determined by lactate dehydrogenase (LDH) release, and on NMDA-stimulated elevation of intracellular Ca^{2+} (Ca^{2+}_i), measured by fluorescence video imaging with fura-2. LDH release from control cultures (25±2% of total LDH) was unaffected by amantadine alone at concentrations as high as 300 μM (23±3%). LDH release, measured 24 hr after exposure for 20 min to 100 μM NMDA, was increased to 46±3%. Pretreatment for 10 min with amantadine (10-1000 μM) inhibited the toxicity of 100 μM NMDA, with half-maximal inhibition at 30 μM and complete inhibition at 300 μM. Amantadine (100 μM) also reduced the rise in Ca^{2+}_i produced by NMDA. These findings indicate that amantadine and other antiparkinsonian drugs with NMDA receptor antagonist properties may protect against excitotoxic neuronal injury.

482.12

4-CHLOROKYNURENINE AS A PRECURSOR OF 7-CHLOROKYNURENIC ACID IN RAT AND HUMAN BRAIN. W. Schmidt*, H.-Q. Wu, R. Schwarcz, [1]F. Salituro, [1]B. Baron, [1]M. Palfreyman and [1]T. McDonald. Maryland Psych. Res. Center, Baltimore, MD 21228 and [1]Marion Merrell Dow Res. Inst., Cincinnati, OH 45215.

7-Chlorokynurenic acid (7-Cl-KYNA), a selective antagonist of the glycine site associated with the NMDA receptor, is neuroprotective in experimental test systems. Since the compound penetrates poorly into the brain, it is difficult to explore its therapeutic potential for excitotoxic brain diseases. We have now examined if 4-chlorokynurenine (4-Cl-KYN), which should have easier access to the brain, can serve as a bioprecursor of 7-Cl-KYNA. Purified preparations of rat (Brain Res., 534:37, 1990) and human (Brain Res., 542:307, 1991) kynurenine aminotransferase (KAT) were used to investigate the conversion of 4-Cl-KYN to 7-Cl-KYNA. Enzymatic production of the latter was confirmed in all cases. In kinetic experiments with rat KAT, 4-Cl-KYN behaved like an effective competitive inhibitor, thus further indicating its ability to serve as a substrate of KAT. Preliminary evidence suggests that conversion of 4-Cl-KYN to 7-Cl-KYNA can also take place in the rat brain in vivo. Since (rat) brain KAT is predominantly localized in astrocytes which are often seen in apposition to excitatory synapses, 4-Cl-KYN administration may provide a means to produce 7-Cl-KYNA in situ in anatomically distinct and pharmacologically relevant foci.

Supported by grants NS 16102 and NS 28236 (to RS).

482.13

EXCITOTOXIC HIPPOCAMPAL DAMAGE AND NEUROPROTECTIVE AGENTS. G. Wolf, G. Keilhoff, S. Fischer, P. Hass and W. Schmidt.* Inst. of Biology, Med. Acad., D(O)-3014 Magdeburg, Germany.

Glutamate agonists, such as quinolinate, kainate, N-methyl-D-aspartate (NMDA), quisqualate, and AMPA were injected intracerebroventricularly in rats to induce convulsive reactions and hippocampal damage in order to model glutamate-mediated brain injury. Animals showed typical convulsive effects and heavy lesions of hippocampal tissue. In rats treated systemically with non-competitive glutamate agonists magnesium sulfate, MK 801, ketamine, or the adamantane derivative memantine, a reduction in neuropathological signs was observed. Depending on the dose and the application schedule even complete protection was observed. Factors of the glutamate transmitter metabolism were found to be affected in lesioned areas, most prominent the activity of aspartate aminotransferase. The vulnerability of neurons containing nitric oxide synthase (NADPH-diaphorese stained) was observed to be related to the type of glutamate agonist used as a noxious stimulus.

482.14

REPEATED EXPOSURE TO GLUTAMATE ALTERS BOTH ITS POTENCY AND EFFICACY AS AN EXCITOTOXIN. J.M. Dubinsky.* Department of Physiology, University of Texas Health Sciences Center, San Antonio, Texas 78284-7756.

Transient increases in extracellular glutamate (GLU), associated with ischemic events *in vivo*, are thought to contribute to the eventual neuronal loss observed several days later. One hypothesis explaining this delayed excitotoxicity states that normal synaptic release of GLU becomes toxic following the initial ischemic event. To test this hypothesis, in vitro toxicity experiments were performed on cultured hippocampal neurons experiencing two successive GLU insults. In the first toxic exposure, all cultures received 5 min of mildly toxic concentrations of GLU. Dose-response curves for the percentage of surviving neurons were constructed for varying doses of GLU applied during the second 5 min exposure, 2 hr after the first. Control dose-response curves following simple solution changes as a first exposure were characterized by an EC_{50} of 55 μM. When 30 μM GLU was used as the first exposure, the EC_{50} for the second exposure dropped slightly to 49 μM. When 100μM GLU was applied during the first exposure, the EC_{50} decreased to less than 5 μM. However, the amount of survival in both experiments was greater than expected from the control dose-response curve. The increase in both efficacy and potency indicate complex interactions may be involved in understanding GLU toxicity following an ischemic event. This work was supported by NIH #AG10034.

482.15

N-METHYL-D-ASPARTATE (NMDA) EXPOSURE BLOCKS GLUTAMATE TOXICITY IN CULTURED CEREBELLAR GRANULE CELLS. D.-M. Chuang*, X.-M. Gao and S.M. Paul. Biological Psychiatry & Clinical Neuroscience Branches, NIMH, Bethesda, MD 20892

Exposure of cultured cerebellar granule cells to glutamate results in a concentration-dependent (EC_{50}=22.7 ± 0.04 μM) and delayed (24-72 hr) neurotoxicity, which is blocked by the NMDA receptor antagonists APV and MK-801, but is unaffected by the non-NMDA receptor antagonists CNQX and DNQX. Pretreatment of cerebellar granule cells with subtoxic concentrations of NMDA markedly antagonizes the neurotoxic actions of glutamate with an IC_{50} of 55 ± 4 μM. The NMDA-induced neuroprotection requires a preincubation time of approximately 120 min to be fully manifest and does not require the presence of NMDA during glutamate exposure. These data demonstrate that NMDA receptors mediate both neurotoxicity and neuroprotection in cerebellar granule cells. Among four glutamate receptor agonists tested, only NMDA was able to provide a complete neuroprotection against glutamate toxicity. Quisqualate was neither neurotoxic nor neuroprotective. Ibotenate, which was also nontoxic, induced a small degree of neuroprotection. In contrast, exposure to kainate resulted in a weak neurotoxicity but also resulted in significant neuroprotection. Since preincubation of these neurons with NMDA fails to alter NMDA receptor-mediated phosphoinositide hydrolysis or the specific binding of [^{3}H] MK-801 to NMDA receptors, it appears that the neuroprotective effects of NMDA are not due to NMDA receptor desensitization.

482.16

CONTRIBUTION OF Na^+/Ca^{2+} EXCHANGE AND Na^+/K^+ ATPase IN Ca^{2+} HOMEOSTASIS: ROLE IN GANGLIOSIDE PROTECTION AGAINST GLUTAMATE NEUROTOXICITY. L. Kiedrowski*, G. Brooker, E. Costa, and J. T. Wroblewski. Fidia-Georgetown Institute for the Neurosciences, Georgetown University, Washington, D.C. 20007.

In cerebellar granule cells the concentration of free intracellular calcium ($[Ca^{2+}]_i$) and sodium ($[Na^+]_i$) was studied using the respective fluorescent probes Fura-2 and SBFI. Application of glutamate (1 μM), in the absence of Mg^{2+}, led to the increase of both $[Ca^{2+}]_i$ and $[Na^+]_i$. In the presence of extracellular Na^+ (154 mM) the increase of $[Ca^{2+}]_i$ after glutamate (1 μM) withdrawal was always followed by a rapid return to normal level and no neurotoxicity was observed. When extracellular Na^+ was replaced with *N*-methyl-D-glucamine, neurotoxicity and persistent elevation of $[Ca^{2+}]_i$ was observed with 1 μM glutamate. These results indicate that the activity of Na^+/Ca^{2+} exchanger might be operative in maintaining $[Ca^{2+}]_i$ homeostasis following glutamate receptor stimulation by nontoxic glutamate concentrations (1 μM). Application of excitotoxic doses of glutamate (50 μM) led to a sustained elevation of $[Ca^{2+}]_i$ which persisted after glutamate removal. In contrast, in cells pretreated for 2h with ganglioside GM_1 (100 μM), glutamate (50 μM) was not toxic, and $[Ca^{2+}]_i$ homeostasis was restored after glutamate removal. Additionally, GM_1 pretreatment attenuated glutamate-evoked $[Na^+]_i$ increase and accelerated the restoration of the basal $[Na^+]_i$ after glutamate removal. These effects of GM_1 were not observed in the presence of the Na^+/K^+ ATPase inhibitor, ouabain (1 mM). These data indicate that the pretreatment with GM_1 might facilitate Na^+/K^+ ATPase activity which, in turn, improves the function of the Na^+/Ca^{2+} exchanger. Moreover, these data suggest that in calcium-dependent glutamate neurotoxicity the elevation of $[Na^+]_i$ may impair the Na^+/Ca^{2+} exchange leading to $[Ca^{2+}]_i$ homeostasis destabilization.

482.17

AGE DEPENDENCY OF NMDA ANTAGONIST NEUROTOXICITY NB Farber, MT Price, J Labruyere, TA Fuller* and JW Olney, Washington Univ., St. Louis MO 63110.

Antagonists of the NMDA subtype of glutamate receptor, including phencyclidine (PCP), MK-801 and ketamine, protect neurons against excitotoxic injury in conditions such as ischemia, hypoglycemia and status epilepticus. However, in rats these agents cause neurotoxic side effects (pathomorphological changes in cerebrocortical neurons), and in humans, both PCP and ketamine are known to cause acute schizophrenia-like psychotic symptoms. The psychotic reaction associated with ketamine anesthesia, known as an "emergence" reaction, is suppressed by certain drugs (barbiturates and benzodiazepines) that also block the neurotoxic reaction in rat cortex, suggesting a relationship between the neurotoxic and psychotomimetic actions of these agents. The ketamine "emergence" reaction, like schizophrenia, is peculiarly age-dependent – typically, susceptibility is greatest in early to mid adulthood, and pediatric populations are not vulnerable. Here we report that susceptibility to the cerebrocortical neurotoxic reaction is also age-dependent with rats being non-susceptible to MK-801 neurotoxicity at 1 month of age, weakly susceptible at 2 months and not fully susceptible until 3 months. Thus, a similar age-dependency profile characterizes: 1) the neurotoxicity of NMDA antagonists in rats, 2) the psychotomimetic effects of NMDA antagonists in humans, 3) onset of schizophrenic psychosis, and 4) susceptibility to other adult-onset neurological disorders. Our findings suggest the possibility that some or all of these phenomena may be mechanistically related, and that NMDA antagonists may be safer for use as neuroprotectants in infancy than in adulthood. Supported by DA 06454, DA 05072, AG 05681 and RSA MH 38894 (JWO).

482.18

THE NMDA RECEPTOR ANTAGONIST MK-801 IS TOXIC TO VENTRAL HORN NEURONS IN ORGANOTYPIC CULTURES OF SPINAL CORD. D.M. Saroff AND J.R. Delfs*. Laboratory of Neurodegenerative and Aging Studies, New England Deaconess Hospital, Geriatric Medicine Program, Boston, MA. 02215.

We have previously demonstrated that the competitive N-methyl-D-Aspartic Acid (NMDA) antagonist D-2-amino-5-phosphonovaleric acid (D-AP5) has only a partial protective effect against NMDA-mediated neurotoxicity. These findings appeared to be due to a neurotoxic effect of D-AP5 itself on ventral horn acetylcholinesterase-positive neurons (VHANs) in organotypic rollertube cultures of spinal cord (OTC-SCs) (Saroff et al., Soc. Neurosci. Abstr., Vol. 17, Part 1, p. 787, 1991). In vivo studies have reported the specific non-competitive NMDA antagonist (+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d]cyclohepten-5,10-imine maleate (MK-801) to have toxic effects on neurons in cingulate cortex (Labruyere et al.,Soc. Neurosci. Abstr., Vol. 15, Part 1, p. 761, 1989).

In the present study we examined the effects of MK-801 on VHANs in the OTC-SC system. Dose-response studies with MK-801 were carried out on OTC-SCs after 2 weeks in vitro. Cultures were exposed for 72 hours to either control media or media with MK-801 at concentrations of 1μM, 10μM, 100μM, or 1mM. MK-801 aplication produced a marked toxic effect on VHANs in culture. This toxicity was evident even at the lower concentrations studied. These results suggest that antagonists of excitation at the NMDA receptor, whether competitive or non-competitive, may cause neurotoxic effects on ventral horn acetylcholinesterase-positive neurons (VHANs). These studies support caution in the use of NMDA antagonists in clinical trials. (Supported by NIH Grant NS27685-03)

482.19

CALPAIN I INHIBITION DOES NOT BLOCK EXCITATORY AMINO ACID-INDUCED NEUROTOXICITY IN MURINE CORTICAL CULTURES. V.M.G. Bruno* and R.G. Giffard. Dept. of Anesthesia, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Activation of the Ca^{2+}-dependent protease calpain I has been implicated in neuronal responses to excitatory amino acids in such diverse settings as long term potentiation and ischemic brain damage. Spectrin breakdown subsequent to Calpain I activation by increased intracellular calcium has been related to neuronal death, but reports of its involvement in the development of excitatory amino acid neurotoxicity has varied in different systems. We investigated the effect of Calpain I inhibitors in cortical cultures injured by exposure to maximal and submaximal concentrations of the glutamate receptor agonists N-methyl-D-aspartate (NMDA), kainate and AMPA. Injury was assessed by release of lactate dehydrogenase. We studied both calpain inhibitor I and MDL 28170 which has high specificity for the enzyme, both up to 100μM. Neither inhibitor protected neurons from brief exposure to NMDA (100μM-500μM) or 24 hr exposure to NMDA (12.5μM-500μM), kainate (30μM-300μM) or AMPA (10μM-100μM). MDL 28170 has been shown to inhibit CA^{2+} ionophore A23187-induced proteolysis of spectrin in erythrocyte ghosts. We then investigated the effect of the drug on A23187-induced toxicity in our cultures. Cells exposed to the ionophore for 20 minutes were moderately injured when assessed at 24 hr. MDL 28170 reduced the neuronal damage by about 30%. Activation of calpain I does not appear to be a major source of injury in excitatory amino acid-induced neurotoxicity in neocortical cultures.
Sponsored in part by NS 01425

482.20

L-trans-2,4-pyrrolidine dicarboxylate (L-PDC), an Inhibitor of High Affinity Glutamate Uptake (HAGU), is Neurotoxic in Neonatal Rat Brain. John D.E. Barks* and Faye S. Silverstein. University of Michigan, Departments of Pediatrics and Neurology, Ann Arbor, MI 48109

Strong evidence of the neurotoxicity of endogenous glutamate (GLU) in mammalian brain was provided by the observation that DL-threo-3-hydroxyaspartate (THA), an HAGU inhibitor, was neurotoxic in adult rodent striatum (J Neurochem 44:247); however, THA did not elicit neuropathologic changes in neonatal brain. The absence of injury was interpreted as evidence that immaturity of glutamatergic innervation limited the potential toxicity of endogenous GLU at this developmental stage. Yet, there is considerable support for the hypothesis that endogenous GLU can be neurotoxic in the developing brain. To address this issue, we assessed the neurotoxicity of the selective HAGU inhibitor L-PDC (J Med Chem 34:717) in 7 day old rats. L-PDC (pH 7.4) was injected into right anterior striatum (STR) (568 nmol, n=2) or through dorsal hippocampus into posterior STR (568 nmol, n=4, 150 nmol, n=2). Neuropathology was assessed in animals killed 5 days later. After anterior injections, focal neuronal necrosis was evident in dorsal STR. High-dose posterior injections caused prominent hippocampal lesions with CA_{1-3} pyramidal layer thinning and focal necrosis in dorsal thalamus; 150 nmol produced small foci of pyramidal cell loss. Focal cortical necrosis and callosal cysts were apparent adjacent to the injection track. Preliminary autoradiographic 3H-GLU binding assays were also done; L-PDC (10^{-7} or 10^{-5} M) did not displace 3H-GLU, suggesting that L-PDC lacked intrinsic agonist properties. Thus, L-PDC-induced brain injury provides direct support for the hypothesis that endogenous GLU may be neurotoxic in the developing brain.

EXCITATORY AMINO ACIDS: ANATOMY AND PHYSIOLOGY III

483.1

DISCOVERY OF A HIGH AFFINITY, SODIUM-DEPENDENT L-PROLINE TRANSPORTER EXPRESSED IN SUBPOPULATIONS OF PUTATIVE GLUTAMATERGIC NEURONS

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We have used PCR with degenerate oligonucleotides derived from two conserved regions of the norepinephrine and GABA transporters to identify novel sodium-dependent transporters expressed in rat brain. One PCR product hybridized to a 4 kb RNA concentrated in subpopulations of putative glutamatergic neurons. Prominent *in situ* hybridization signals were observed over mitral cells of the olfactory bulb, pyramidal cells of layer V of the cerebral cortex, pyramidal cells of the piriform cortex, and pyramidal cells of field CA3 of the hippocampus. In contrast, background labeling was observed over granule cells of the dentate gyrus, caudate-putamen, white matter tracts, choroid plexus, and ependymal cells of the cerebral ventricles. Transient expression of the cognate cDNA conferred sodium-dependent, high affinity (K_m=9.7 μM) L-proline uptake in HeLa cells which exhibited a pharmacological profile similar to that for high affinity L-proline transport in rat brain slices. The cloned transporter cDNA predicts a 637 amino acid protein with 12 putative transmembrane domains and exhibits 44-45% amino acid sequence identity with other members of the emerging family of neurotransmitter transporters. These findings support a synaptic role for L-proline in specific excitatory pathways in the CNS and provide the basis for a direct molecular analysis of the presynaptic components of these excitatory projections.

483.2

CHANGES OF pH DURING GLUTAMATE UPTAKE. M. Bouvier*, M. Szatkowski, A. Amato and D. Attwell. Dept of Physiology, University College London, Gower St, London WC1E 6BT, UK.

Uptake of glutamate into glial cells and neurones ultimately terminates the post-synaptic action of the neurotransmitter. The glutamate uptake carrier is known to be powered by the co-transport of an excess of Na^+ ions (at least 2) and it also counter-transports one K^+ ion.

When L-glutamate or D-aspartate uptake was activated in whole-cell clamped Müller cells from the salamander, a pH sensitive electrode outside the cell detected an alkalinization of the extracellular medium, and intracellular BCECF detected an internal acidification. If intracellular Cl^- was replaced by ClO_4^-, the magnitude of the uptake current evoked by glutamate was increased but the external alkalinization was reduced. Using microelectrodes sensitive to perchlorate, it was possible to monitor ClO_4^- efflux when uptake was activated with ClO_4^- inside the cell. These data suggest that a pH changing anion such as OH^- or HCO_3^- is transported out of the cell by the uptake carrier and that ClO_4^- can be transported in place of this anion. Inhibition of carbonic anhydrase by acetazolamide had no effect on the intracellular acidification nor on the extracellular alkalinization, suggesting that HCO_3^- is not counter-transported on the carrier.

We therefore propose an uptake stoichiometry in which one glutamate ion is transported into the cell together with 2 Na^+ ions, and one K^+ ion and one OH^- are counter-transported, per cycle of the carrier.
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483.3

DIFFERENTIAL LOCALIZATION OF THE METABOTROPIC (mGluR1) AND NMDA RECEPTORS IN THE BRAIN. M. Fotuhi*, A.H. Sharp, S.H. Snyder, and T.M. Dawson. Depts. Neuroscience and Neurology, Johns Hopkins Univ. Sch. Med., Baltimore, MD, 21205.

The G-protein-linked mGluR1 and the ion-channel-complex of the NMDA receptor mediate slow and fast responses of neurons to glutamate, respectively. We have compared the distribution of the two forms of mGluR1, alpha and beta, with NMDA receptors in the rat brain by immunohistochemistry and by in situ hybridization. We find that both mGluR1 and NMDA receptors are widely distributed. However, in a number of brain regions, they occur in distinctly different neuronal populations.

For instance, mGluR1 predominates in the non-pyramidal neurons of the cerebral cortex, stratum oriens of CA1 and CA3 of the hippocampus, islands of Callaje, subthalamic nucleus, lateral hypothalamus, mammillary bodies, and the molecular layer of the cerebellum. In contrast, the NMDA receptor predominates in the pyramidal neurons of the cerebral cortex and the hippocampus, medium spiny neurons of the striatum, paraventicular nucleus of hypothalamus, and the granule cell layer of the cerebellum.

These findings, in combination with reports of the distribution of other glutamate receptors, suggest that while many neurons are equipped to mediate both slow and fast responses to glutamate, many others are speciallized to have either a slow or fast response.

483.4

DISTRIBUTION AND MORPHOLOGY OF NEURONS EXPRESSING THE GLUR 2/3 SUBUNIT IN FOUR NEOCORTICAL AREAS OF MONKEYS. Fiorenzo Conti*, and Andrea Minelli. Institute of Human Physiology, University of Ancona, Via Ranieri, I-60131 Ancona (Italy).

In the last few years, several non-NMDA ionotropic glutamate (Glu) receptor subunits (GluR) have been cloned. Four of these, GluR1-4 (or GluR A-D), have pharmacological properties of AMPA receptors and appear to form heterometric receptor complexes. Recent data show that the GluR 2 subunit appears to be dominant in determining the properties of the receptor complex, since its presence is required for Ca^{++}-impermeability. Here we report on the distribution and morphology of neurons expressing the GluR 2/3 subunit in four neocortical areas of the monkey brain as studied using a subunit-specific antibody produced and characterized in Dr. Wenthold's lab (*Wenthold et al., J. Biol. Chem., 267: 501-507, 1992*). Under deep barbiturate anaesthesia, animals were perfused transcardially with phosphate buffer followed by 4% paraformaldehyde, the brains were removed, and postfixed. Small blocks from the primary somatic sensory (areas 3a, 3b, 1, and 2), the primary visual (area 17), the primary motor (area 4), and the posterior parietal (area 5) cortices were cut on a Vibratome in 15-μm thick sections, and processed for GluR-ICC (Ab 25) using the ABC method. Immunostaining was present both on cell bodies (excluding the nucleus) and on the major dendrites, and in the neuropil (mostly on dendritic processes).The pattern of GluR 2/3-positive neurons was basically similar in the four areas studied and was characterized by the presence of numerous small and medium sized pyramidal neurons in layer II and upper layer III, sparse non-pyramidal neurons in lower layer III and in layer IV, and some pyramidal and non-pyramidal neurons in layers V-VI. Some differences were, however, present when comparing the four areas: the number of positive neurons in layer IV was highest in areas 17 and 5, whereas the number of pyramidal neuron in layers V-VI was highest in area 4. The identity of immunoreactive non-pyramidal neurons could not be determined at the light microscope, and experiments are in progress to define the ultrastructural features of these neurons.

483.5

EVIDENCE FOR GLUTAMATERGIC NONPYRAMIDAL NEURONS IN THE RAT HIPPOCAMPUS: GOLGI STAINING COMBINED WITH POSTEMBEDDING IMMUNOCYTOCHEMISTRY. E. Soriano[1], H.-D. Hofmann[2]* and M. Frotscher[2]. [1]Unit of Cell Biology, Faculty of Biology, Univ. Barcelona, Spain 08028; [2]Inst. Anat., Univ. Freiburg, D-7800 Freiburg, FRG.

Nonpyramidal neurons of the hippocampus are known to be inhibitory using GABA as a transmitter. They control the principal cells, granule cells and pyramidal neurons, which are excitatory. By using postembedding immunocytochemistry in combination with Golgi staining, we provide evidence here that at least two types of nonpyramidal neurons in the rat hippocampus use glutamate as transmitter.

Mossy cells in the hilus and a novel type of spiny nonpyramidal cell in the mossy fiber termination zone of CA3 were Golgi-stained, gold-toned, and processed for postembedding immunocytochemistry using glutamate and GABA antibodies. At the electron microscopic level, both cell types were seen to receive massive input from the mossy fibers. When adjacent semithin sections through the cell body region of Golgi-stained neurons were immunostained, both cell types reacted for glutamate, like the pyramidal neurons and granule cells, but did not stain for GABA. In contrast, many other local circuit neurons were GABA-positive but could not be immunostained with the glutamate antibody. Excitatory glutamatergic nonpyramidal cells that are integrated in the main excitatory pathway of the hippocampal formation may play a hitherto underestimated role in hippocampal circuitry.

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483.6

PHOSPHATE-ACTIVATED GLUTAMINASE mRNA EXPRESSION IN RAT SPINAL CORD. B. Srinivasan* and K.E. Miller. Dept. Anatomical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190

Phosphate-activated glutaminase (PAG) is the enzyme responsible for converting glutamine to glutamate in glutamatergic and GABAergic neurons. We have examined PAG mRNA in rat spinal cord using RNA blots and *in situ* hybridization. Spinal cords, kidneys, and brains from male rats were removed, total RNA was extracted, and slot blots were prepared. ^{32}P-labelled PAG cDNA (ATCC; Banner et al.,Molec.Br.Res 3:247,'88) was used to probe RNA blots. PAG mRNA was expressed in spinal cord similar to brain and kidney. To localize PAG mRNA to neurons, *in situ* hybridization was performed on sections from cervical, thoracic, and lumbar spinal cord and dorsal root ganglia (DRG). ^{32}P-labelled PAG cDNA was used for autoradiographic localization and digoxigenin-labelled PAG cDNA or 20mer PAG oligoprobes were used for nonradioactive localization. In the DRG, most neurons were labelled. In addition to intensely labelled motor neurons, other labelled spinal neurons were found in the intermediolateral cell column, lateral spinal & cervical nuclei, marginal zone, substantia gelatinosa, and central gray region (lamina X). The results from this study indicate that PAG mRNA is expressed in specific neurons of rat DRG and spinal cord, including some neurons not traditionally considered glutamatergic or GABAergic. Supported by NS27213 (KEM).

483.7

THREE SUBPOPULATIONS OF CORTICAL GABAERGIC INTRINSIC NEURONS IN THE RAT. Y. Kubota* and E.G. Jones Laboratory for Neural Systems, Frontier Research Program, RIKEN, Wako, Saitama 351-01, Japan and Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Recent studies have revealed that the cortical GABAergic neurons contain a wide variety of other neuroactive substances and specific calcium binding proteins. In this study we demonstrated the subpopulations of GABAergic neurons in different cortical area of the rat with double immunohistochemical methods. In all areas of the neocortex, intrinsic GABAergic neurons are subdivided into at least three types based on the selective colocalization of parvalbumin, calbindin D 28kDa or choline acetyltransferase (ChAT). All calbindin D 28kDa/GABA immunoreactive cells show somatostatin immunoreactivity and a small number of calbindin D 28kDa containing cells also show neuropeptide Y immunoreactivity and NADPH-diaphorase staining. All ChAT/GABA immunoreactive cells also show VIP immunoreactivity. In the piriform cortex and entorhinal cortex, these three subpopulations of GABAergic intrinsic neurons have slightly different characteristics. GABAergic parvalbumin containing neurons always contain calbindin D 28kDa but about half the calbindin D 28kDa containing neurons do not show parvalbumin immunoreactivity.

483.8

GLUTAMATE, GABA AND SUBSTANCE P IN THE PARAVENTRICULAR NUCLEUS OF THE THALAMUS (PV): AN IMMUNO-EM STUDY IN THE RAT. G. Balercia, S. De Biasi°, A. Amadeo° and M. Bentivoglio (SPON:ENA). Institute of Anatomy, University of Verona and °Dept. of General Physiology and Biochemistry, University of Milano, Italy.

PV is part of the midline group of thalamic nuclei and its synaptic organization and neurochemical features are still largely unknown. Immunopositivity for GABA and glutamate (Glu) in the rat PV was here revealed by a post-embedding immunogold procedure, and that for substance P (SP) by a pre-embedding method, using polyclonal antisera. Glu-, GABA, SP-immunoreactive (ir) terminals were observed, whereas only Glu-immunopositivity was also detected in neuronal perikarya, thus confirming the extrinsic origin of the GABAergic and SPergic innervation of the rat PV. Glu-ir terminals contain round clear vesicles and occasionally large granular ones; they make asymmetric synaptic contacts with proximal and distal dendritic arborizations. GABA-immunolabeling is in small to medium-sized terminals that contain clear pleomorphic vesicles and make symmetric synapses on dendrites of different caliber and frequently on cell bodies; some of them also contain several large granular vesicles. SP-immunoreactivity is present in small, dome-shaped terminals contacting distal dendrites, and in medium- to large-sized terminals contacting proximal dendrites and their spines. SP-ir terminals make asymmetric synaptic contacts and contain round clear vesicles and a variable number of large granular vesicles.

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483.9

GLUTAMATE-IMMUNOREACTIVE CLIMBING FIBERS IN THE RAT CEREBELLAR CORTEX. P. Grandes, F. Ortega, L. Hennequet, J. Gondra and P. Streit*[1]. Dept. of Neuroscience, Basque Country University, E-48080 Bilbao; [1]Institut für Hirnforschung, University of Zürich, CH-8029 Zürich.

The nature of the climbing fiber transmitter is still a matter of debate. To determine whether glutamate-immunoreactive profiles with the morphological characteristics of climbing fibers could be found in the rat cerebellar cortex, an immunocytochemical study was performed at the levels of light (LM) and electron microscopy (EM). A monoclonal 'anti-glutamate' antibody was used in combination with postembedding staining. At the LM level, strongly labeled fibrous profiles and chains of interconnected varicosities appeared, often in close apposition to principal Purkinje cell dendrites. In EM preparations, some immunoreactive presynaptic elements were varicosities and elongated profiles that were 3-4 times more heavily labeled than their postsynaptic partners. These results show that a subset of climbing fibers and their terminals contain relatively high levels of glutamate-like immunoreactivity and provide additional evidence for a role of glutamate as transmitter in these cerebellar afferents.

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483.10

[3H]D-aspartate labeling of Calbindin immunoreactive striato-pallidal and striato-nigral projection neurons. H.D. Hodges, L.E. White, K.M. Carnes, J.M. Dubinsky, & J.L. Price* Dept. Anatomy & Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO and Dept. of Physiology, Univ. Texas Health Science Ctr., San Antonio, TX.

The medium spiny neurons that project from the neostriatum to the globus pallidus and substantia nigra are immunoreactive for gamma-aminobutyric acid (GABA) and its synthetic enzyme glutamic acid decarboxylase (GAD). In addition, a subpopulation of these cells are co-labeled by retrograde transport of [^{3}H]D-aspartate injected into the globus pallidus or substantia nigra. [^{3}H]D-aspartate is taken up at axon terminals through the high affinity glutamate/aspartate uptake system found in neurons that use these excitatory amino acids as neuro-transmitters. Thus, both inhibitory and excitatory neurotransmitters may be present in the same striatal neurons (Carnes et al., Soc Neurosci Abs., 1991).

In this study, we tested whether cells that can be labeled with [^{3}H]D-aspartate are also immunoreactive for calcium binding proteins, parvalbumin and calbindin. Parvalbumin has been shown to be present in medium to large aspiny interneurons that are scattered throughout the striatum. Our findings show that parvalbumin does not colocalize with cells labeled by injections of [^{3}H]D-aspartate, even in cases in which the injection involved the striatum directly. Calbindin immunoreactivity is present in medium spiny projection neurons and defines the matrix compartment in the patch/matrix organization within the neostriatum. Most of the striatal cells labeled by [^{3}H]D-aspartate were also immunoreactive for calbindin. However, some [^{3}H]D-aspartate positive, calbindin negative cells are also found possibly because [^{3}H]D-aspartate cells are found in both matrix and patch compartments. Supported by the National Parkinsons Foundation and NIH grants DC00093 and GM07200.

483.11

AMINO ACID IMMUNOCYTOCHEMISTRY OF PRIMARY AFFERENT TERMINALS IN DEEP LAMINAE OF THE RAT DORSAL HORN

K.D. Phend*, R.J. Weinberg, J.G. Valtschanoff and A. Rustioni. Dept. of Cell Biology & Anatomy, University of North Carolina, Chapel Hill, NC 27599.

As part of a broader investigation we tested whether terminals of large fibers mediating low-threshold cutaneous input are enriched in glutamate or aspartate. Four to 7 days after injection of HRP conjugated to B subunit of cholera toxin in the sciatic n., rats were perfused with 2.5% glutaraldehyde/0.5% paraformaldehyde/0.1% picric acid. Vibratome sections of lumbar spinal segments were cut, reacted for TMB and embedded in plastic. Blocks from lamina IV were cut out; thin sections were stained with glutamate and aspartate antisera separately or in combination using different sizes of gold particles. Quantitative EM methods were employed to compare the density of gold particles coding for either antiserum in labeled terminals. To help define background, sections were also stained for GABA.

Mean staining level for glutamate was above background in primary afferent terminals in lamina IV compared with staining in dendrites, glia and GABA-positive terminals. Staining for aspartate was only marginally higher than background in afferent terminals and was elevated in dendrites as well. Density of staining for both amino acids in primary afferent terminals in lamina IV was lower than that in terminals of primary (unmyelinated and small myelinated) fibers in lamina II (Phend et al., Neurosci. Abst.1991).

483.12

AN ELECTRON MICROSCOPIC STUDY OF GLUTAMATE-LIKE IMMUNOREACTIVE (GLU) PERIKARYA AND PROCESSES IN THE DORSAL RESPIRATORY GROUP (DRG) OF THE FELINE MEDULLA. **V.J.Massari*, Z.Z. Song, H.M. Rhee# and P.J. Gatti. Dept. Pharmacology, Howard Univ. Coll. Med., Washington, DC and #FDA, Rockville, MD.**

We have previously observed GLU neurons and processes in the ventrolateral subnucleus of the tractus solitarius (vlNTS) using light microscopy. In this study, we have attempted to define the types of immunoreactive processes which are found in the vlNTS. Adult mongrel cats were anesthetized and perfused through the descending aorta with a combination of 4% paraformaldehyde and 0.3% glutaraldehyde. Vibratome sections were processed by an avidin-biotin based immunoperoxidase method utilizing a mouse anti-glutamate antiserum (INCSTAR). We have observed several small GLU perikarya with a large invaginated nucleus and a thin rim of immunoreactive cytoplasm. In addition, we have seen a few large GLU neurons with similar characteristics. Numerous large GLU dendrites receiving primarily asymmetric synapses from unlabeled nerve terminals were also seen. GLU axons were observed and unmyelinated. As yet, we have not observed any GLU nerve terminals in the vlNTS. These data suggest that some of the inspiratory neurons which predominate in the DRG utilize glutamate as an excitatory neurotransmitter, however, as yet the data do not indicate a role for glutamatergic nerve terminals in the regulation of the activity of DRG neurons. Supported by NIH HL 1RO1-44922 and the American Heart Association.

483.13

GLUTAMATE AND ASPARTATE IMMUNOREACTIVITY IN CORTICOSTRIATAL NEURONS OF THE RAT - R. Giuffrida* - Ist. Fisiologia umana, University of Catania, I-95125, Catania ITALY

The aim of the present work was to test whether corticostriatal neurons are immunostained by antisera raised in rabbits against glutamate and aspartate conjugated to hemocyanin by glutaraldehyde (Hepler *et al.*, J. Histochem. Cytochem, 1988, 36: 13-22). Corticostriatal neurons were identified by the retrograde transport of a tracer (colloidal gold-labeled WGA-apoHRP; Basbaum and Menétrey, J. Comp. Neurol., 1987, 261: 306-318) pressure-injected in the caudate-putamen; antisera were visualized by peroxidase immunocytochemistry using the ABC method. The bulk of corticostriatal neurons was observed in layer V of frontal and parietal areas, mainly in the ipsilateral hemisphere. In a sample of 1012 corticostriatal neurons in sections processed for Glu-antiserum, 595 (or 59%) were immunostained; in a sample of 1470 corticostriatal neurons in sections processed for Asp-antiserum, 837 (or 57%) were immunostained. Since it has been previously demonstrated that the two antisera largely reveal distinct populations of cortical neurons, results obtained combining Glu- and Asp-immunocytochemistry indicate that the entire population of corticostriatal neurons contains elevated concentrations of Glu or Asp in their cell body.

483.14

GLUTAMATERGIC RAPHESPINAL EXCITATION OF SPINAL MOTOR OUTPUT IN CATS. S.J. Fung*, R.H. Liu, V.K. Reddy and C.D. Barnes. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

The nucleus raphe pallidus (NRP) has been described to evoke, upon electrical stimulation, a rapid depolarization and action potential discharges in cat hindlimb motoneurons of both flexor and extensor origins (Fung and Barnes, 1989, *Neurosci. Lett. 103:* 185-190). We sought to answer two fundamental questions pertaining to such findings: (1) whether the motor excitation is caused by raphespinal neurons or other descending systems traversing through or adjacent to the medullary sites of stimulation; (2) is the fast, excitatory glutamate (Glu) transmitter involved in the bulbospinal excitation of hindlimb motoneurons?

Using the simultaneous, dual (Glu and serotonin) immunofluorescence method in combination with retrograde tract-tracing technique (by unilaterally injecting FITC-labeled microspheres to L7 ventral horn), spinally projecting neurons arising from NRP and nucleus raphe obscurus (NRO) were frequently found to contain either or both antigens, with more triple-labeled compared to double-labeled cells. Electrical stimuli (4 pulses of 50 μs duration, 500 Hz, 50-150 μA) applied to these medullary sites, in decerebrate cats, potentiated the L7 monosynaptic reflex (MSR). That the NRO or NRP neurons (instead of adjacent fiber systems) were the main source of the MSR enhancement was confirmed by a similar potentiation of the MSR upon chemically stimulating these raphespinal neurons with 0.2-0.5 μl of 0.5 M glutamate. Microinjections of the vehicle to the same sites were without effect on the MSR. Electrical stimulation of the NRP or NRO also induced ventral root responses signifying motoneuron discharges. These effects were reversibly antagonized by intraspinally injecting a non-NMDA antagonist CNQX (8x0.2 μl injections of 0.1M solution along the L7 ventral horn). Vehicle injections caused no changes on the ventral root responses. These data support a role of glutamate in raphespinally induced excitation of lumbar cord somatomotor outflow in cats.

483.15

EVIDENCE FOR A DESCENDING GLUTAMATERGIC CONTROL OF LUMBAR MOTONEURONS BY LOCUS COERULEUS COMPLEX NEURONS IN THE CAT. R.H. Liu, V.K. Reddy, S.J. Fung and C.D. Barnes*. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

Glutamate (Glu) is considered to be a major excitatory neurotransmitter in the central nervous system. Previously reported presence of Glu-like immunoreactive (Glu-LI) somata in the rodent catecholaminergic nucleus locus coeruleus (LC) raises two questions: (1) Are there any Glu-LI neurons in the catecholaminergic coerulospinal system of the cat? (2) What is the physiological role, if any, of Glu in LC control of spinal motoneurons?

In this study we used simultaneous immunofluorescence technique to identify tyrosine hydroxylase (TH) and Glu immunoreactivities in neurons that were retrogradely labeled by spinal (L7 ventral horn) injections of fluorescent microspheres (Zhuo et al., 1992, *J. Chem. Neuroanat.* 5:1-10). We found a widespread coexistence of Glu and TH in most (>80%) descending neurons and they were distributed randomly throughout the rostrocaudal extent of the LC complex. Electrical stimulation (4 pulses of 50 *us* duration at 500 Hz, intensity = 50-200 *uA*) of the LC complex, in decerebrate cats, consistently induced L7 motoneuron discharges recordable ipsilaterally as ventral root responses. The LC evoked ventral root responses were reversibly reduced (>50%) upon intraspinal injecions of the non-NMDA antagonist CNQX (in a total of 4 pene trations along the L7 ventral horn, each penetration consisted of 2 injections of 5 ng/0.2 *ul*). Vehicle injections were without any significant effect on the LC evoked ventral root responses. These results support that Glu has a pivotal role in mediating coerulospinal excitation of lumbar motoneurons in cats.

EXCITATORY AMINO ACIDS: PHARMACOLOGY V

484.1

DISTRIBUTION OF THE NMDA RECEPTOR ANTAGONISTS [3H]-CGS 19755 AND [3H]MK-801 AFTER INTRATHECAL INJECTION IN MICE. J. Näsström*, É. Böö, M. Ståhlberg and O.-G. Berge. Astra Pain Control, Preclinical Research, Södertälje, Sweden.

The tissue distribution of [3H]CGS 19755 and [3H]MK-801 was investigated for up to 6 h after single lumbar spinal i.t. injection. [3H]CGS 19755 redistributed slowly from its injection site towards brainstem and cortex. In the cortex, the radioactivity peaked 3-4 h after injection. At no time did the level in frontal cortex exceed 10 % of the level in lumbar spinal cord. The highest peripheral level of [3H]CGS 19755 was found in kidneys. [3H]MK-801 redistributed rapidly from the spinal cord injection site to peripheral organs. The highest peripheral levels of [3H]MK-801 were found in lungs and liver, where the radioactivity peaked between 10 and 30 min after the injection. The levels of [3H]CGS 19755 were consistently higher in CNS tissues (except for the first 15 min in frontal cortex) and blood than the corresponding levels of [3H]MK-801. The opposite relationship was true in liver, lungs, kidneys, stomach, intestine, spleen and heart. The effect of these antagonists on response latency in the hot-plate test was quantified in the same animals immediately prior to sacrifice for the distribution study. For the first hour after the injection, the effect of CGS 19755 in the hot-plate followed the temporal distribution of this antagonist to the lumbar region of spinal cord. MK-801 was substantially ineffective in the hot-plate test even at 100 times the effective equimolar dose of [3H]CGS 19755. The marked difference in the distribution profile of [3H]MK-801 and [3H]CGS-19755 in the spinal cord does not appear to explain the lack of effect of MK-801 in the hot-plate test.

484.2

EFFECTS OF GLYCINE ON DIZOCILPINE-INDUCED CHANGES IN ACOUSTIC STARTLE. R.S. Mansbach* Dept. of Pharmacology & Toxicology, Medical College of Virginia, Richmond, VA 23298-0613.

Noncompetitive N-methyl-D-aspartate (NMDA) antagonists such as dizocilpine (MK-801) block central glutaminergic neurotransmission by binding to a site within the NMDA receptor-associated cation channel. Application of exogenous glycine has been shown to improve NMDA-stimulated binding of ligands to the noncompetitive antagonist (PCP) site and to potentiate NMDA-induced responses. These effects are thought to be mediated by a strychnine insensitive glycine binding site associated with the NMDA receptor complex. In the present study, effects of glycine and a lipophilic glycine prodrug, milacemide, were examined on the acoustic startle reflex in rats, both alone and in combination with a dose of dizocilpine (0.5 mg/kg) known to alter startle responses. Following injections, rats received 40 ms, 122 dB broad-band acoustic stimuli. These startle-inducing stimuli were presented alone or were preceded by 80 dB prepulses. When vehicle alone was administered, significant decreases in startle amplitude were produced by prepulses (pre-pulse inhibition). Dizocilpine significantly elevated startle magnitude and eliminated prepulse inhibition, but glycine (17 and 56 mg/kg) and milacemide (10-100 mg/kg) had no effect. Moreover, administration of these compounds did not affect changes in response magnitude and pre-pulse inhibition produced by dizocilpine. A higher dose of glycine,170 mg/kg, had no effect on startle magnitude but in combination with dizocilpine killed 42% of the subjects tested. Except for the lethal effects of glycine-dizocilpine combinations, these preliminary results do not indicate that behavioral effects of NMDA antagonists acting at the PCP receptor are modified by exogenous glycine. Further work with glycine antagonists and selective agonists at the strychnine-insensitive glycine binding site will explore other possible interactions with PCP-like drugs. Supported by PHS grant MH-46631.

484.3

NOVEL ANALOGS OF DEXTROMETHORPHAN ANTAGONIZE NMDA CONVULSIONS IN RATS. L. Robles, J. Taylor, +A. Newman* and F. Tortella. Walter Reed Army Inst. Res., Washington, DC 20307 and +NIDA Addiction Res. Ctr., Baltimore, MD 21224.

Dextromethorphan (DM) and its major metabolite dextrorphan (DX) possess significant anticonvulsant activity. We have previously reported on the anticonvulsant effects of a novel series of 3-substituted DM analogs to protect rats against maximal electroshock-induced convulsions. To date, three analogs have been synthesized which are more potent anticonvulsants than DM. In this study we explored the potential anticonvulsant nature of these DM analogs in a rat model of NMDA (12.5 nM, i.c.v.) convulsions. Pretreatment with DM (20-80 μg, i.c.v.) delayed the onset to NMDA-induced "popcorn" convulsions, while having little effect on the incidence of convulsions. DX (2.5-20 μg, i.c.v) attenuated both latency and incidence. Of the three analogs tested, the ethylether derivative of DM, AHN1036 (20-80 μg, i.c.v.), was the most potent anticonvulsant affording complete protection against NMDA convulsions. The isopropyl ether and primary amine derivatives were at least as potent as DM. These results will be discussed relative to receptor mechanism of action, behavioral side effects and possible clinical usefulness.

484.4

CONANTOKIN PEPTIDE FROM *CONUS GEOGRAPHUS* MODULATES [^{3}H]THIENYLPIPERIDINE (TCP) BINDING TO THE NMDA CHANNEL AND REDUCES NMDA RECEPTOR CURRENT. L. L. Coughenour, D. M. Rock, C. Hanchin, J. Hawley and G. W. Campbell*. Parke-Davis Pharmaceutical Res. Div., Warner-Lambert Co., Ann Arbor, MI 48106-1047.

Conantokin-G (CT-G) is a seventeen amino-acid peptide extracted from the venom of the marine snail *Conus geographus*. It previously has been shown to interact with the NMDA subtype of glutamate receptors. In well washed Triton-treated rat brain membranes the binding of [^{3}H]TCP was markedly enhanced upon the addition of the NMDA channel agonists, glutamate and glycine. The maximal enhancement caused by glycine (no added glutamate) was reduced in the presence of CT-G (2 μM). The maximal enhancement by glutamate (no added glycine) was unaffected, but the concentration-response curve for glutamate enhancement was shifted to the right by CT-G. In addition, CT-G did not affect the binding of [^{3}H]AMPA or [^{3}H]kainate to non-NMDA receptors. This data suggests a selective effect on the glycine site of the NMDA channel.

In cultured rat cortical neurons CT-G (2-20 μM) reduced the amplitude of whole-cell NMDA receptor currents and the frequency of NMDA-evoked single-channel openings in excised outside-out patch clamp recordings. Onset of the reduction of whole-cell and single-channel current by CT-G was slow, and the reductions were not readily reversible.

484.5

KINETIC ANALYSIS OF NMDA MODULATORS ON [^{3}H]MK-801 BINDING. D.P. Carrozza*, K. Williams and P. B. Molinoff. Dept. of Pharmacol., Univ. of Penn., Phila., PA 19104.

The NMDA receptor complex contains a distinct binding site for polyamines, including spermine, which enhance the binding of [^{3}H]MK-801, to a site within the ion channel. In the present studies, analysis of the kinetics of association of [^{3}H]MK-801 was used to investigate potential mechanisms of action of polyamines. Experiments were performed to determine whether specific modulators alter the conformation or dynamics of the receptor, which might be detected as an increase in the observed association rate (K_{obs}). The observed association rates were biphasic in the presence of glutamate and glycine (gg) or gg plus spermine and were not altered by preincubation of membranes with these modulators before addition of [^{3}H]MK-801. This suggests a mechanism that involves a change in the duration of opened and closed states rather than a persistent change in the conformation of the receptor. In other experiments, the rates of association (fast and slow) were independent of the concentration of [^{3}H]MK-801 in the presence of gg plus spermine (30 uM), a condition which results in maximal changes in equilibrium binding. These results, which were an unexpected finding, suggest that the limiting factor of [^{3}H]MK-801 binding even under these conditions, involves access of ligand to its binding site. This phenomenon may involve changes in the dynamics of channel opening initiated by activation of the polyamine site (supported by USPHS grant GM34781 and NS30000).

484.6

DIAMINES INHIBIT NMDA RECEPTOR RESPONSES THROUGH A CHANNEL-BLOCKING MECHANISM. S. Subramaniam*, S.D. Donevan and M.A. Rogawski. Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892.

It has been proposed that the inhibitory actions of the diamines diaminodecane (DA-10) and diaminododecane (DA-12) on NMDA-receptor responses is due to their actions as inverse agonists at the polyamine site. Recently, we and others have observed that the polyamines spermine and spermidine can block the NMDA receptor through an open channel mechanism. In this study we examined whether diamines could affect NMDA channels through a similar mechanism. In whole-cell recordings from cultured rat hippocampal neurons (V_H = −60 mV), the diamines inhibited NMDA currents in a concentration-dependent fashion. The potencies of the diamines increased with increasing length of the carbon chain. Thus, DA-12, the longest diamine tested, was the most potent (IC_{50} = 7 μM), whereas diaminopropane (DA-3), the shortest diamine tested, produced only partial inhibition (~70% at 10 mM). The blocking action of the polycationic diamines decreased at positive holding potentials supporting a channel blocking mechanism. In outside-out patches, DA-12 produced a flickery block of NMDA-induced single-channel currents whereas the shorter diamine putrescine (DA-4) produced an apparent reduction in channel amplitude. The ability to resolve flickering with DA-12 but not with the less potent shorter chain diamine DA-4 may reflect differences in binding kinetics but we cannot rule out the possibility of a distinct mechanism of block for the shorter diamines. These observations indicate that diamines antagonize NMDA currents through an open channel mechanism, and therefore it may not be necessary to invoke inverse-agonist properties of these compounds to explain their inhibitory actions on NMDA receptor responses.

484.7

NEUROCHEMICAL TOLERANCE TO MK-801 (DIZOCILPINE) IN THE MOUSE. P.H.Hutson*, L.J. Bristow, K. Flatman, L. Thorn and M.D. Tricklebank, Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex CM20 2QR, U.K.

In addition to anticonvulsant and neuroprotective effects, acute administration of the non-competitve NMDA receptor antagonist, MK-801 (dizocilpine) stimulates the turnover of dopamine in corticolimbic brain. We now show that chronic treatment with MK-801 decreases the neurochemical response to the compound in the nucleus accumbens, but not in the medial prefrontal cortex (mPFCX).

Dihydroxyphenylacetic acid (DOPAC) was determined in mouse brain regions by HPLC and electrochemical detection. In mice killed 60 min after drug administration (n = 5 per group), MK-801 (0.4 mg/kg, s.c.) increased the concentration of DOPAC (ng/g tissue) by 66 % and 85 % in mouse nucleus accumbens and medial prefrontal cortex respectively (Table 1). Forty eight hours after the last of 21 daily injections of 0.4 mg/kg MK-801, this same dose given 60 min before death again markedly increased DOPAC concentration in the medial prefrontal cortex (89 %), but led to a much reduced response in the nucleus accumbens (18 %). Neither acute nor chronic drug treatment altered striatal DOPAC concentration.

Table 1

	Chronic saline		Chronic MK-801	
	saline	MK-801	saline	MK-801
N. Accumbens	727	1206	813	956
s.e.m.	±24	±86	±55	±47
mPFCX	39	72	39	74
s.e.m.	±5	±11	±2	±8

Thus, mesolimbic and mesocortical dopaminergic neurones appear to be differentially affected by chronic treatment with MK-801.

484.8

IFENPRODIL SELECTIVELY POTENTIATES NMDA-INDUCED DEPOLARIZATIONS IN THE RAT CORTICAL WEDGE *IN VITRO*. *P.L.Herrling* and D.A. Lowe*. Sandoz Research Institute, P.O. Box,CH 3006 Bern, Switzerland.

Ifenprodil, an agent thought to interact with the polyamine site of the NMDA receptor (Carter et al. Europ. J. Pharmacol. 164:611, 1989), was studied in cortical wedges as described by Lowe et al.(Neurosc.Letters 113:315, 1990). Excitatory amino acids (EAAs) were added to ACSF perfused into the chamber containing the cortical layers. Effects were quantified by determining the area under the curve of EAA-induced depolarizations. In Mg^{++}-free ACSF Ifenprodil (10 to 100uM) potentiated NMDA-induced depolarizations by 50 to 187% over controls (mean±SD: 97±52). This potentiating effect was not observed in ACSF containing 1mM $[Mg^{++}]_o$ and was attenuated at 0.2 mM $[Mg^{++}]_o$. The potentiation was fully reversed by 5uM 7-chloro-kynurenic acid (7Cl-KYAC). If the potentiation was established before 7-Cl-KYAC application it reappeared after washout of the antagonist. Depolarizations elicited by non-NMDA EAA agonists such as AMPA and quisqualate were not affected by Ifenprodil. The potentiating effect of Ifenprodil is therefore NMDA specific and the experiments with Mg^{++} and 7Cl-KYAC suggest a site of action deep within the NMDA operated channel.

484.9

PUTRESCINE OVERPRODUCTION EFFECTS ON THE LEARNING & MEMORY AND THE SEIZURE THRESHOLD. [1]T. Halonen*, [1]J. Sivenius, [1]R. Miettinen, [2]L. Alhonen, [2]M. Halmekytö, [3]S. Syrjänen, [3]K. Syrjänen, [2]J. Jänne, and [1]P.J. Riekkinen. Depts of [1]Neurol., [2]Biotechnol., and [3]Pathol., A.I.Virtanen Institute, Univ. of Kuopio, 70211 Kuopio, Finland.

Polyamines appear to modulate excitatory synaptic transmission mediated by N-methyl-D-aspartate (NMDA) reseptor. We tested the effect of increased putrescine levels in transgenic mice line that overexpresses human ornithine decarboxylase on the spatial learning/memory and on the epileptic seizure threshold. Concentrations of putrescine in the different brain areas of transgenic mice increased from undetectable (limit 5 μmol/g) to more than 60 μmol/g compared with their nontransgenic littermates. Contents of spermidine and spermine remained unchanged. Transgenic mice had impared acquisition in water maze task (expressed using escape latency). Transgenic mice had also increased seizure threshold to clonic phase of pentylenetetrazol-induced seizures and to tonic phase of corneal electroshock convulsions. The effect against electroshock convulsions was potentiated when putrescine content was further increased by adding 5-fluoromethyl ornithine to drinking water. These results suggest that putrescine may play an important role in the excitatory neurotransmission.

484.10

EFFICACY OF SYNTHETIC POLYAMINES IN MODULATING NMDA-MEDIATED INCREASES IN INTRACELLUALR CALCIUM. J.M. Fahey*, G.A. Pritchard, L.G. Miller. Dept. of Pharmacology and Experimental Therapeutics and Neuroscience Program, Tufts Univ. School of Medicine Boston, MA 02111.

The endogenous polyamines spermine and spermidine potentiate N-methyl-D-aspartate (NMDA) receptor ionophore-mediated events. We have previously shown that both spermine and spermidine are able to potentiate NMDA-mediated increases in intracelluar calcium (Ca_i) of cultured chick cortical neurons using the calcium sensitive fluorescent dye Fura2. The present study characterizes structurally related synthetic polyamines. IBPA (3,3' imino-bis-propylamine) and AEPD (N-(2-aminoethyl)-1,3 propanediamine) are triamines which differ from spermidine in the length of one aliphatic chain between central terminal amines. IBPA potentiated NMDA-medited increases of Ca_i at concentrations of 100 , 250, 500 and 1000 μM. Its effects at these concentrations were also approximately 40% greater than effects of spermidine. AEPD produced an increase of Ca_i only at1mM. Diaminodecane(DA10) is a putative inverse agonist at the NMDA receptor ionophore and in this system decreases NMDA-mediated increases of Ca_i. No other diamine tested(DA5-DA8) had a similar effect. Only DA5(cadaverine) at 1 mM significantly altered NMDA-mediated responses by increasing, not decreasing, Ca_i. As previously reported, the presence of a proapnediamine moiety may predict the functional efficacy of a synthetic polyamine but it is strongly influenced by the properties of other sidechains.

484.11

MK-801-INDUCED IMPAIRMENT ON A MEMORY TASK IS COUNTERACTED BY SCOPOLAMINE. DF Wozniak*, M McEwen, G Brosnan-Watters and JW Olney. Washington Univ, St. Louis, MO, 63110.

NMDA antagonists, such as MK-801, block induction of hippocampal long term potentiation (LTP) and impair the performance of rats on various behavioral tasks, especially acquisition of spatial learning/memory tasks. NMDA antagonists also have neurotoxic side effects. Two hours after subcutaneous injection of a single low dose of MK-801, pathomorphological changes appear in neurons of the posterior cingulate cortex. A muscarinic cholinergic receptor is implicated in this neurotoxic reaction in that the reaction is blocked by certain antimuscarinic drugs, including scopolamine. Since this neurotoxic reaction affects cingulate but not hippocampal neurons, the question arises whether the memory impairing effects of MK-801 might reflect a functional disturbance in cingulate rather than hippocampal neurons. If this were the case, scopalamine might block the memory-impairing action just as it blocks the neurotoxic action of MK-801 on cingulate neurons. Using a hole board test and cross-over design, we found that MK-801 (0.05 mg/kg ip), compared to scopolamine (0.025 mg/kg ip) or saline, significantly impaired the ability of male rats to learn which of 4 holes contained a food reward. However, when scopolamine (0.025 mg/kg ip) was coadministered with MK-801 (0.05 mg/kg ip), rats performed like saline controls and better (marginally nonsignificant; $p = .077$) than MK-801-treated rats. Moreover, significantly more animals were unable to learn the task under the influence of MK-801 alone (7/23) compared to MK-801 + scopolamine (0/23). These findings suggest, contrary to popular assumption, that the locus of action of NMDA antagonists in causing a performance deficit in learning tasks may be the cingulate cortex rather than the hippocampus. If so, it may be necessary to reinterpret prior behavioral studies in which performance deficits in learning tests have been attributed either implicitly or explicitly to an action of the drug on LTP-linked mechanisms in the hippocampus. Supported by RSA MH 38894 (JWO), DA 05072, DA 06454 and AG 05681.

484.12

SIGMA RECEPTOR LIGANDS REDUCE NMDA-EVOKED DEPOLARIZATIONS AND ELEVATIONS OF INTRACELLULAR CALCIUM IN HIPPOCAMPAL NEURONS E.J. Fletcher, [1]J. Church* and J.F. MacDonald. Dept. Physiology, Univ. Toronto, Toronto, ONT. M5S 1A8 and [1]Dept. Anatomy, Univ. British Columbia, Vancouver, B.C. V6T 1Z3

Magnesium and the dissociative anaesthetics ketamine and phencyclidine block the ion channel associated with the NMDA-subtype of glutamate receptor in a characteristically voltage-dependent manner. The psychosis induced by the anaesthetics is similar to that induced by *sigma* receptor ligands and a common site of action has been debated for some time. The *sigma* ligands DTG and 3-PPP were reported to potentiate NMDA-evoked responses and may interact with polyamine binding sites. Interestingly, the polyamine antagonist ifenprodil also has affinity for *sigma* ligand binding sites.

The *sigma* receptor ligands tested here were potent and selective antagonists of NMDA-evoked responses in whole-cell, voltage-clamped hippocampal neurons in culture (V_h=-60mV). The potency of the compounds as NMDA antagonists was (Kd [μM]): (+)-SKF(10,047) [0.8] > ifenprodil [2.3] > dextromethorphan [2.8] > (+)-pentazocine [8.4] > DTG [35] ≈ (-)-44 [35] > caramiphen [98] > carbetapentane [188] > (+) and (-)-29 [200] > 3-PPP [360]. The antagonism was not competitive in nature, nor could it be reversed with 10μM glycine or 100μM spermine, but it was voltage-dependent (except for ifenprodil). The *sigma* receptor ligands also reduced NMDA- and high K^+-evoked increases in intracellular calcium in Fura-2 loaded hippocampal cells at similar potencies and reduced voltage-activated calcium currents. As *sigma* receptor ligands can reduce calcium influx into cells through two distinct mechanisms they may have great value as therapeutic agents in disease states involving over-activity of neurons and potentially damaging elevations of intracellular calcium.

484.13

INTRACEREBRAL INJECTION OF DOPAMINE IN A DENERVATED STRIATUM REVERSES CIRCLING INDUCED BY THE NMDA ANTAGONIST MK-801. J.A. St-Pierre, L. Grégoire and P.J. Bédard*. Centre de Recherche en Neurobiologie, Hôp. de l'Enfant-Jésus, Laval University , Québec City, Canada.

Carlsson and Carlsson (*J. Neural. Transm.* 77: 65-71, 1989) have shown that systemic administration of a dopamine agonist potentiates the behavioral response to the NMDA antagonist MK-801. The goal of the present study was to investigate whether a similar potentiation would be observed after direct intrastriatal injection of dopamine.

Ovariectomized female rats were lesioned with 6-OHDA in the left medial forebrain bundle. Fifteen days later, they were tested with apomorphine (0.25mg/kg s.c.). Microinjections of different doses of dopamine (1, 5, 25 or 50 μg/1.0 μl) were performed by guide-cannulae implanted stereotaxically into the striatum. Dopamine induced a contralateral circling response, in a dose-dependent manner, when injected into the lesioned side. Similarly, microinjection of dopamine into the lesioned side followed by systemic injection of MK801 (100 μg/kg i.p.) 20 minutes later, produced a contralateral circling response. However, this circling response persisted with a duration 4-fold that of the circling response induced by dopamine alone.

As previously reported in unilaterally lesioned rats, MK801 alone, produce an ipsilateral circling response (St-Pierre *et al., Soc. for Neurosc.* 1991) whereas microinjection of dopamine combined with MK801, reverses the circling response. Taken together, our results suggest that the potentiation seen in the present experiment cannot be explained by increased release of dopamine. In addition, the circling response induced by the suppression of the glutamatergic neurotransmission seems to be facilitated by dopamine receptor activation.

Supported by MRC of Canada.

484.14

ELECTROPHYSIOLOGICAL EVIDENCE FOR THE EXISTENCE OF NMDA, KAINATE (KA) AND AMPA RECEPTORS ON VENTRAL TEGMENTAL (VTA) DOPAMINE NEURONS IN THE RAT. T.Wang*and E.D.French. Dept.Pharmacology, Univ. Arizona, Coll. Med. Tucson, AZ 85724.

Three ionotropic receptors (NMDA, KA and AMPA) which bind L-glutamate are present in various CNS structures. However, their existence on mesocorticolimbic dopamine (DA) neurons is less clear. Information on the factors controlling the excitability of these neurons may provide some insight into the neurobiology of schizophrenia, and the reinforcing properties of drugs of abuse. In midbrain slices extracellular recordings were made from VTA DA neurons identified by location, pacemaker-like firing, a rate of < 5 spikes/s, and action potential durations >2 ms. Superfusion with NMDA, KA and AMPA produced dose-dependent reversible excitations of all 52 neurons tested. The threshold for NMDA (N=17) activation was 1-3 μM, with 10 and 30 μM increasing rates by 1.7 and 3.0 spikes/s. The threshold for KA (N=25) was 300 nM-1 μM, with increases of 1.5 and 2.4 spikes/s occurring at 3 and 10 μM. AMPA (N=10) was the most potent excitant, with a threshold at 100 nM and increases of 3.4 spikes/s at 1 μM. Also, none of the agonists changed the firing pattern from regular to bursting activity. The effects of NMDA were antagonized by 30 μM CGS 19755, while KA and AMPA were selectively blocked by 10 and 1 μM NBQX. The antagonists did not affect spontaneous firing. These data indicate that the excitability of VTA neurons is under the influence of the three glutamate receptor subtypes with a rank order of potency of AMPA > kainate > NMDA.

484.15

THE ROLES OF EXCITATORY AMINO ACID (EAA) RECEPTORS IN THE CONTROL OF EXCITABILITY OF SYMPATHETIC PREGANGLIONIC NEURONES (SPN) D. Spanswick, A.E. Pickering, I.C. Gibson and S.D. Logan. (SPON:Brain Research Association) Dept. of Physiol., Univ. of Birmingham, B15 2TT, U.K.

Intracellular and whole-cell recordings were obtained from SPN in neonate rat spinal cord slices. Focal electrical stimulation of the dorsal horn or laternal funiculus evoked an EPSP which was both voltage-dependent and potentiated in magnesium-free ACSF. The EPSP was partly reduced by the selective NMDA receptor antagonists APV, CPP or MK801 and was completely abolished by the non-NMDA receptor antagonist CNQX . Superfusion of the selective EAA receptor agonists AMPA, kainate, quisqualate, NMDA and ACPD induced concentration-dependent depolarisations in all neurones tested. AMPA, kainate and NMDA-induced responses were blocked by a cocktail of both NMDA (APV, CPP or MK801) and non-NMDA (CNQX or DNQX) antagonists, whereas responses to quisqualate and ACPD persisted. Depolarising responses to quisqualate and ACPD were associated with an increase in neuronal input resistance and decreased in amplitude with increased membrane hyperpolarisation. In a subpopulation of cells, quisqualate and ACPD induced oscillations in membrane potential which gave rise to rhythmic burst firing at higher agonist doses .

We conclude that both AMPA and NMDA subtypes of receptors mediate synaptic transmission in SPN. Glutamate can also act via a metabotropic receptor to excite SPN by reducing a potassium conductance, and in some SPN to induce rhythmic activity.

This work is supported by the British Heart Foundation, the Wellcome Trust and the MRC

484.16

KYNURENIC ACID MODULATES EXCITATORY AMINO ACID-INDUCED EXCITATION OF DOPAMINE-CONTAINING NEURONS IN RAT SUBSTANTIA NIGRA. H.-Q. Wu*, R. Schwarcz and P.D. Shepard. Maryland Psychiatric Research Center, Baltimore, MD 21228

Kynurenic acid (KYNA), an endogenous antagonist of ionotropic excitatory amino acid (EAA) receptors, was tested for its ability to modulate NMDA- and AMPA-induced excitation of dopamine (DA)-containing neurons in the rat substantia nigra (SNc). Experiments were conducted using conventional extracellular recording techniques in conjunction with an *in vitro* brain slice preparation. Bath application of NMDA (1-20 μM) or AMPA (1-10 μM) produced a concentration-dependent increase in the firing rate of SNc DA neurons. The highest concentration of both agonists produced a rapid and reversible cessation of activity that was attributed to acute induction of depolarization block. KYNA (10 μM - 1 mM) inhibited the excitatory effects of NMDA in a concentration-dependent fashion (EC_{50} ≈ 100 μM) without affecting basal firing rate. KYNA (100 μM) proved slightly more potent in inhibiting the effects of AMPA. Perfusion of tissue slices with low Mg^{2+} Ringers (0.12mM) increased the NMDA-induced excitation of DA neurons but failed to affect the ability of KYNA to antagonize the effects of NMDA. Addition of glycine (up to 100 μM) or kynurenine (3 mM), the immediate precursor of KYNA, to the bathing solution had no effect on either basal firing rate or the increase in firing produced by concomitant application of NMDA. However, co-application of D-serine (100 μM) partially attenuated the ability of KYNA to block the excitatory effects of NMDA. Thus, the ability of KYNA to block NMDA-induced excitation of SNc DA neurons may be mediated by an interaction with the glycine allosteric site on the NMDA receptor. Taken together, these data suggest that endogenous KYNA may play an important role in regulating DA cell excitability by modulating EAA neurotransmission.

484.17

INFLUENCE OF MK-801 AND NBQX ON ACUTE RECOVERY OF CEREBRAL METABOLISM IN PIGLETS AFTER HYPOTHERMIC CIRCULATORY ARREST. M. Aoki, F. Nomura, R.A. Jonas, M. Stromski, M.K. Tsuji, J.C. Fackler, P.R. Hickey, D.H. Holtzman*, Children's Hospital and MIT, Boston, MA 02115

Neuroprotective effects of MK-801 and NBQX were evaluated in 34 4-week old piglets (10:MK-801, 10:NBQX, 14:controls) undergoing cardiopulmonary bypass (CPB) and hypothermic circulatory arrest at 15°C nasopharyngeal for 1 hour as used clinically for repair of congenital heart defects. MK-801 (0.75 mg/kg) or NBQX (25 mg/kg) were given I.V. before CPB. Equivalent doses were given to the CPB prime plus infusions after reperfusion (0.15 mg/kg/hr or 5 mg/kg/hr). High energy phosphate (HEP) metabolism was analyzed by magnetic resonance spectroscopy (4.7 Te, horizontal bore) in 17 animals and cerebral blood flow by microspheres and cerebral metabolic rate (CMR) by oxygen and glucose extraction were studied in 17 until 225 min after reperfusion. CMR was depressed relative to control by both MK801 and NBQX at baseline. Recovery of phosphocreatine ($p = 0.01$), ATP ($p = 0.03$) and pH ($p = 0.004$) was accelerated by MK-801 and retarded by NBQX over the first hour of normothermic reperfusion. Final recovery of ATP at 3 hours 45 minutes reperfusion was significantly ($p = 0.025$ [ANOVA]) reduced by NBQX (44 ± 30% baseline, mean±S.D.) vs control (81 ± 19%) and MK-801 (75 ± 10%). CMR02 recovered to 105 ± 30% baseline in group MK-801 but only to 61 ± 22% in group NBQX (control 94.0 ± 32%) ($p = 0.07$). **Conclusion:** MK-801 accelerates recovery of CMR and HEP metabolism after CPB and hypothermic circulatory arrest in the immature animal. At the dose used, NBQX exerts an adverse effect.

474.18

EFFECTS OF ANIRACETAM AND BMS 181168 (BMY 21502) ON EXCITATORY AMINO ACID RESPONSES. S.I. Dworetzky*, M.C. McKay, C.G. Boissard and V.K. Gribkoff. Dept. of Biophysics and Molecular Biology, Bristol-Myers Squibb Pharm. Res. Inst., 5 Research Parkway, Wallingford, CT 06492.

Modulation of NMDA and non-NMDA EAA receptors by the substituted pyrrolidinones BMS 181168 and aniracetam was studied using the Xenopus oocyte receptor expression system, whole cell patch clamping of hippocampal neurons in culture, and extracellular recording of evoked hippocampal synaptic potentials in hippocampal slices. Hippocampal poly(A)$^+$mRNA was isolated from 2-3 week old rats and microinjected into defolliculated Xenopus oocytes. After 3-5 days of incubation, cells were tested for receptor expression using two electrode voltage clamp. The results showed that aniracetam significantly enhanced a fast component of the AMPA-induced inward current in oocytes, and potentiated EPSC's in hippocampal neurons, but did not effect responses to kainate and NMDA. This confirms observations seen by other laboratories (Vyklicky et al., Neuron 7, 1991:971-984). However, BMS 181168 was seen to have a qualitatively different effect on EAA-induced inward current, in comparison to aniracetam. BMS 181168 had only a small effect on the fast AMPA-induced current component, but greatly potentiated and prolonged a late, slow inward current component. BMS 181168 had little direct potentiating action on hippocampal synaptic potentials, but has been shown to prolong hippocampal LTP (Gribkoff et al., Neuropharm. 29, 1990:1001-1009).

EXCITATORY AMINO ACIDS: RECEPTORS VI

485.1

GLUTAMATE AND GLYCINE ACT SYNERGISTICALLY TO STIMULATE [^{3}H]MK-801 BINDING TO NMDA RECEPTORS. J. C. Marvizón* and M. Baudry. Neuroscience Program, University of Southern California, Los Angeles, CA 90089-2520.

In well-washed membranes, glutamate and glycine increased each other's efficacies to stimulate [^{3}H]MK-801 binding to NMDA receptors, with no detectable effects in their potencies. Glutamate was virtually unable to enhance [^{3}H]MK-801 binding in the absence of glycine or in the presence of glycine antagonists, confirming the idea that occupancy of the glycine site is an absolute requirement for NMDA receptor activation. Conversely, the efficacy of glycine to stimulate [^{3}H]MK-801 binding was greatly reduced in the absence of glutamate and in the presence of glutamate antagonists. However, [^{3}H]MK-801 binding was stimulated by high concentrations (1 mM) of glutamate or glycine acting independently of each other. These low affinity components for glutamate and glycine are probably due to an agonist action of glutamate at the glycine site and of glycine at the glutamate site, respectively. In agreement with this idea, glutamate inhibited [^{3}H]glycine binding, and glycine inhibited [^{3}H]glutamate binding, each at millimolar concentrations. Moreover, the potency of the low affinity component for glycine was reduced by glutamate antagonists, but not by glycine antagonists. These results indicate that 1) simultaneous occupancy of the glutamate and glycine sites is necessary to activate NMDA receptors, and 2) glutamate and glycine at millimolar concentrations can act as agonists at the high affinity site for the other compound in the NMDA receptor.

485.2

Magnesium interacts with the NMDA receptor by two distinct mechanisms. **J.O.McNamara*, G.C.Yeh and D.W.Bonhaus. Duke and V.A. Med. Ctr. Durham, NC 27710. Taipei Med.Col. Taiwan, Syntex Res. Palo Alto CA.**

Depending upon concentration, magnesium can either inhibit or potentiate binding of the NMDA channel blockers [^{3}H]MK-801 or [^{3}H]TCP. To determine how magnesium produces these effects, we examined the action of magnesium on ligand binding to three distinct sites of rat hippocampal NMDA receptor: glycine, glutamate, and the [^{3}H]TCP binding sites (a.k.a. phencyclidine receptor). As previously reported by others, magnesium produced biphasic effects on [^{3}H]TCP binding; magnesium exerted potentiating effects at lower concentrations and inhibitory effects at higher concentrations. Both effects required agonist occupancy of the glutamate and glycine binding sites. The inhibitory effect of magnesium on [^{3}H]TCP binding was paralleled by an inhibitory effect on the apparent affinity of the [^{3}H]TCP binding site. The potentiating effects of lower concentrations of magnesium were associated with a concentration dependent increase of both the association and dissociation rate of [^{3}H]TCP without change in either the K_D or B_{max} of [^{3}H]TCP binding. Magnesium also increased the affinity of [^{3}H]glycine at concentrations comparable to those that increased [^{3}H]TCP binding. Magnesium did not modify NMDA-displaceable [^{3}H]glutamate binding. These findings support the idea that magnesium has two distinct effects on the NMDA receptor: 1) the apparently competitive interaction between magnesium and [^{3}H]TCP binding which likely corresponds to the well characterized channel blocking action; 2) a potentiation of NMDA channel activation likely mediated by the increased affinity of glycine binding.

485.3

Polyamine interacts with NMDA receptor by two distinct mechanisms. **G.C.Yeh*, D.W.Bonhaus and J.O.McNamara. Taipei Med.Col, Taiwan, Syntex Res. Palo Alto CA, Duke and V.A. Med.Ctr Durham, NC 27710.**

NMDA receptor mediated neurotransmission is regulated by polyamines. To examine the direct interaction of polyamines with the NMDA receptor we determined the effects of the polyamines spermine (SPM) and spermidine (SPD) on the binding of the NMDA channel blocker, [^{3}H]TCP. Both SPM and SPD produced biphasic effects on nonequilibrium binding of [^{3}H]TCP. Both the potentiating and inhibitory effects of polyamines required agonist binding at both the glutamate and glycine binding sites. The potentiating effect, detected at concentrations of polyamines less than 30 uM, was associated with increases of both the association and dissociation rates of [^{3}H]TCP binding. The inhibitory effect of polyamines, detected at concentrations of polyamines greater than 100 uM, was associated with a decrease of the apparent affinity of [^{3}H]TCP binding. This study suggests that SPM and SPD both have two distinct actions on the NMDA receptor: 1) the apparently competitive interaction between polyamines and [^{3}H]TCP binding which likely corresponds to the voltage-dependent block of NMDA channels described by MacDonald et al (Mol. Pharmacol.1992); 2) a potentiation likely due to an increase of NMDA channel activation which may correspond to the increased channel open frequency described by MacDonald et al.

485.4

THE NMDA ANTAGONIST [^{3}H]IFENPRODIL BINDS TO HETEROGENOUS POLYAMINE-SENSITIVE SITES IN RAT BRAIN. **H. Schoemaker, S. Pigasse and B. Zivkovic*. Department of Biology, Synthélabo Recherche, 31 Av. P.Vaillant-Couturier, 92220 Bagneux (France).**

The noncompetitive NMDA antagonist and cerebral anti-ischaemic properties of ifenprodil have been ascribed to its blockade of polyamine-stimulatory effects on the NMDA receptor complex. In support of this hypothesis, a polyamine-sensitive [^{3}H]ifenprodil binding site was demonstrated in the rat cerebral cortex (Schoemaker et al; Eur. J. Pharmacol. 176, 249-250, 1990). The present study further examined the pharmacology of polyamine-sensitive [^{3}H]ifenprodil binding in rat brain.

Specific [^{3}H]ifenprodil binding to the rat cerebral cortex, determined as described by Schoemaker et al. (1990) in 5 mM Tris-HCl buffer, is fully sensitive to inhibition by unlabeled ifenprodil (IC_{50}= 0.015 µM), SL82.0715 (IC_{50}= 0.041 µM), spermine (IC_{50} = 3.3 µM) and spermidine (IC_{50}= 8.0 µM). The competitive NMDA antagonist CGP 37849 partially inhibits [^{3}H]ifenprodil binding to 17 % of control values with an IC_{50} of 3.8 µM. L-Glutamate fails to affect [^{3}H]ifenprodil binding under control conditions, but reverses the inhibition of binding produced by CGP 37849.

In the adult rat cerebellum, [^{3}H]ifenprodil binding is only inhibited by higher concentrations of unlabeled ifenprodil (IC_{50}= 0.22 µM), SL82.0715 (IC_{50}= 1.8 µM), spermine (IC_{50}= 28 µM) and spermidine (IC_{50}= 63 µM) and is not affected by CGP 37849 (100 µM). Conversely, in the immature cerebellum, [^{3}H]ifenprodil binding is inhibited by low concentrations of ifenprodil (IC_{50}= 0.029 µM) and spermine (IC_{50}= 5.9 µM), and partially sensitive to CGP37849 (IC_{50}= 1.5 µM).

The present data demonstrate that polyamine-sensitive [^{3}H]ifenprodil binding sites in the rat cerebral cortex and immature cerebellum form part of the NMDA receptor complex and are allosterically coupled to the glutamate/NMDA recognition site. Polyamine-sensitive [^{3}H]ifenprodil binding sites in the adult rat cerebellum present a different pharmacology and may not be part of the NMDA receptor complex.

485.5

7-CK-DEPENDENT AND -INDEPENDENT EFFECTS OF POLYAMINES ON NMDA RECEPTOR ACTIVATION. S.R. Zukin*, D.C. Javitt, R. Sircar, M. Frusciante, Depts. of Psychiatry and Neuroscience, Albert Einstein College of Medicine and Bronx Psychiatric Center, Bronx, NY 10461.

Activation of *N*-methyl-D-aspartate (NMDA) receptors is regulated at distinct sites by polyamines and glycine. In order to determine the differential effects of polyamines and glycine on NMDA receptor activation, kinetics of [^{3}H]MK-801 binding were measured in the absence and presence of L-glutamate, the polyamine agonist spermidine and the putative glycine antagonist 7-CK. Two components of association were found: a fast component (not observed under control conditions) reflecting binding to the activated conformation of the NMDA receptor complex and a slow component reflecting binding to agonist-associated closed conformations. Addition of L-glutamate (0.3 - 100 mM) alone led to a dose-dependent 3-fold increase in total [^{3}H]MK-801 binding but did not significantly increase binding manifesting fast kinetics. Spermidine significantly potentiated the degree to which L-glutamate stimulated total steady-state binding and also led to a large increase in the percentage of total steady-state binding that was attributable to the fast component of association. 7-CK attenuated the degree to which spermidine increased the percentage of binding attributable to the fast component without affecting the spermidine-induced increase in total binding. These findings suggest that the glycine site primarily regulates the interconversion between open and closed conformations of the NMDA receptor complex, whereas the spermidine site regulates both the total number of non-desensitized complexes and the interconversion between open closed conformations.

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485.6

SUBCELLULAR DISTRIBUTION OF [^{3}H]IFENPRODIL BINDING IN RAT BRAIN K. Hashimoto, C.R. Mantione,* M. Spadda[1], J.L. Neumeyer[1] and E.D. London, Neuropharmacology Laboratory, NIDA Addiction Research Center, Baltimore, MD 21224 and [1] Research Biochemicals Inc., Natick, MA 01760.

Ifenprodil has been shown to be cytoprotective in animal models of focal ischemia. It has been suggested that ifenprodil exerts this action by antagonism at polyamine modulatory sites on the N-methyl-D-aspartate (NMDA) receptor. In the presence of 3 μM GBR 12909 to mask σ receptor binding, [^{3}H]ifenprodil labels polyamine-sensitive binding sites in rat cerebral cortex at 0-4°C (Schoemaker H. et al., *Eur. J. Pharmacol.* 176, 249 (1990)). However, at 37°C, [^{3}H]ifenprodil binding to cortical membranes has a pharmacological profile similar to that of a σ receptor ligand (Schoemaker H. et al., *Br. J. Pharmacol.* 100s, 316 (1990)). σ receptors, labeled with (+)-[^{3}H]SKF-10,047, exhibit a unique distribution in rat brain subcellular fractions (McCann D.J. & Su T.P., *Eur. J. Pharmacol.* 188, 211 (1990)). The present study examined the subcellular distribution of [^{3}H]ifenprodil binding at 4°C in the presence of 3 μM GBR 12909 and at 37°C in the absence of 3 μM GBR 12909.

In the presence of GBR 12909, [^{3}H]ifenprodil binding at 4°C was enriched in synaptosomal and mitochondrial fractions, suggesting that polyamine binding sites occur in these fractions. This distribution of [^{3}H]ifenprodil binding at 4°C was similar to that of [^{3}H]MK-801 binding. In contrast, in the absence of GBR 12909 [^{3}H]ifenprodil binding at 37°C is enriched in microsomal and myelin fractions, consistent with subcellular distribution of σ receptors. These finds support the view that, at 4°C, [^{3}H]ifenprodil labels polyamine sites on the NMDA receptor. Subcellular distribution at 37°C indicate binding to σ receptors, therefore, effects of ifenprodil *in vivo* appear to involve σ receptors. Thus, it is likely that [^{3}H]ifenprodil may label σ sites in rat brain at 37°C. Therefore, ifenprodil may exert its pharmacological effects through the σ sites *in vivo*.

485.7

SPERMINE MAY MODULATE PRESYNAPTIC N-METHYL-D-ASPARTATE RECEPTORS. F. Della Vedova*, L. Facheris, R.G. Fariello, A. Bianchetti and C. Speciale. Farmitalia Carlo Erba-Erbamont Group, CNS/CV Research Group, 20014 Nerviano, Italy.

Previously, we showed a dual effect of spermine (SPM) on the N-methyl-D-aspartate (NMDA)-evoked [^{3}H]noradrenaline (^{3}HNA) release in hippocampal slices (Facheris et al., Soc. Neurosci. 17:108.13). This was possible by distinct evaluation of the release bursted directly by NMDA, in fractions with the agonist (NMDA fractions), and indirectly, in later fractions in the absence of the agonist (tail fractions), collected up to 25 min. We now demonstrate that tetrodotoxin (TTX), a blocker of synaptic transmission, discloses the enhancing SPM effect on the NMDA fractions, while the tail fractions-related effect is insensitive to TTX. In all experiments sliced hippocampi from Wistar rats (200-225 g) were used. Fractions (5 min each) were collected and fractional release (FR) expressed as percent of the ^{3}HNA present in the tissues at the corrispondent times. 500 μM NMDA evoked 7.08±0.86% FR. TTX (2 μM) inhibited NMDA fractions of approximately 90% ($P<0.01$). 1 mM SPM, per se inactive, restored FR to 3.71±0.37% ($P<0.01$). Moreover, the increasing effect of SPM on the tail fractions was not modified by TTX. 400 μM D(-)2-amino-7-phosphonoheptanoic acid significantly prevented SPM effect (1.62±0.26% FR, $P<0.01$), in the presence of TTX. Our data suggest that SPM may modulate the NMDA-induced ^{3}HNA hippocampal release by acting on presynaptic NMDA receptors.

485.8

INTERACTIONS BETWEEN THE GLUTAMATE AND GLYCINE RECOGNITION SITES ON THE NMDA RECEPTOR COMPLEX. T. Priestley, L.L Iversen* and J.A. Kemp. Merck Research Laboratories, Terlings Park, Harlow, Essex, U.K., CM20 2QR.

Concentration-jump experiments on voltage-clamped rat cortical neurones in culture have previously indicated an allosteric interaction between the glycine- and glutamate-recognition sites on the NMDA-receptor complex (Kemp & Priestley, Mol. Pharmacol., 39, 1991, 666-670) Thus, glutamate dissociates more slowly from its recogition site on the NMDA-receptor complex when glycine is bound to the glycine recognition site than when the low efficacy partial agonist, (+)-HA 966, is bound. We have extended these observations of interactions between these two co-agonist recognition sites by evaluating the effect of a range of agonists with differing levels of efficacy and affinity binding at one of the sites on the kinetics of agonists acting at the other.

The dissociation rate for glutamate in the presence of (+)-HA 966 is significantly faster than that seen in the presence of glycine. Similar, but progressively smaller effects were seen with D-cycloserine, ACPC (Aminocyclopropanecarboxylic acid) and L-alanine. A reciprocal effect on glycine kinetics was found to occur with several NMDA receptor agonists. Thus, the dissociation rate for glycine was slowest in the presence of glutamate and became progressively faster in the presence of NMDA, quinolinic acid and cis-2,3-PDA (piperidine dicarboxylic acid).

These results suggest that co-agonists acting at the NMDA and glycine recogition sites on the NMDA receptor complex influence each others affinity by an allosteric interaction the extent of which appears to correlate with the level of efficacy of the agonist rather than its affinity.

485.9

OPPOSITE EFFECTS OF ZINC ON AGONIST AND ANTAGONIST BINDING TO THE NMDA RECEPTOR RECOGNITION SITE IN RAT HIPPOCAMPAL FORMATION. D.D. Savage, L.L. Paxton and S.A. Queen* Dept. Pharmacology, University of New Mexico School of Medicine, Albuquerque, NM, 87131-5316.

Monaghan *et al.* (PNAS 85:9836, 1988) have shown that glycine alters the ability of NMDA receptor agonists and antagonists to bind to the NMDA-glutamate recognition site on the NMDA channel complex. Glycine produces a dose-dependent increase in NMDA-sensitive [3H]-glutamate binding and decreases binding by tritiated NMDA receptor antagonists. We conducted similar studies investigating the effect of zinc on NMDA agonist and antagonist binding.

Histological sections of brain containing dorsal hippocampal formation were incubated with either [3H]-glutamate (150 nM) or [3H]-CGS 19755 (33 nM) in the presence of 18 different concentrations of zinc ranging from 20 nM to 1 mM. NMDA-sensitive [3H]-glutamate binding was defined as the difference between [3H]-glutamate binding in the absence and presence of 100 μM unlabelled NMDA. Specific [3H]-CGS 19755 binding was defined as the difference between [3H]-CGS 19755 binding in the absence and presence of 10 μM unlabelled CGS 19755.

NMDA-sensitive [3H]-glutamate binding was increased in the presence of zinc concentrations between 50 nM and 5 μM. Maximum zinc-stimulated increase in NMDA-sensitive [3H]-glutamate binding (30%) occurred at 5 μM. At concentrations above 5 μM, zinc stimulation of NMDA-sensitive [3H]-glutamate binding diminished. Zinc produced an opposite effect on NMDA antagonist binding. Specific [3H]-CGS 19755 binding was decreased between 50 nM and 5 μM zinc. At 5 μM zinc, specific [3H]-CGS 19755 binding was decreased to about 25% of [3H]-CGS 19755 binding in the absence of added zinc. At concentrations above 5 μM, the inhibitory effect of zinc on [3H]-CGS 19755 binding diminished.

These data suggest that zinc, like glycine, may affect the function of the NMDA receptor-operated ion channel complex by altering the affinity of the NMDA receptor recognition site for glutamate. Increasing concentrations of zinc up to 5 μM may enhance the ability of glutamate to activate the NMDA channel. At concentrations above 5 μM, zinc may diminish the ability of glutamate to activate the NMDA channel. (Supported by AA06548)

485.10

GLYCINE BINDING TO CEREBRAL CORTICAL MEMBRANES FROM DEVELOPING AND ADULT MICE: INTERACTIONS WITH ß-ALANINE. P. Saransaari* and S.S. Oja. Tampere Brain Res. Ctr, Dept. Biomed. Sci., Univ. Tampere, Finland.

It is well established that glycine potentiates responses mediated by *the N-methyl-D-aspartate (NMDA) subtype of the glutamate receptor.* We have characterized with [^{3}H]glycine these strychnine-insensitive binding sites in cerebral cortical membranes isolated from adult and 7-day-old mice. The binding was saturable, consisting of only one component in both age groups studied. The maximal binding capacity B_{max} was significantly higher in immature mice than in adults. The binding constant K_D was greater in immature mice, indicating lower affinity of the binding sites for glycine. The binding was most strongly inhibited by glycine itself, followed by serine and ß-alanine in both immature and mature cerebral cortex. ß-Alanine was found to cause a mixed type of inhibition in glycine binding. The binding of ß-[^{3}H]alanine to cerebral cortical membranes was also saturable, consisting of one component. The binding constant K_D for ß-alanine was of the same order of magnitude as K_D for glycine, whereas the binding capacity B_{max} was smaller. The binding of ß-alanine was inhibited by glycine, NMDA and glutamate but not by strychnine. The results point to the possibility that ß-alanine could act at the glycine modulatory site in the NMDA receptor complex in both developing and adult cerebral cortex. (Supported by the Emil Aaltonen Foundation, Finland).

485.11

DIFFERENTIAL EFFECTS OF L-GLUTAMATE ON [^{3}H]MK-801 BINDING IN PCP-TREATED WEANLING RATS COMPARED TO SALINE-TREATED CONTROLS. R. Sircar*, Depts. of Psychiatry and Neurology, Albert Einstein College of Medicine, Bronx, NY.

The developing rat brain is more susceptible to *N*-methyl-D-aspartate (NMDA) receptor-mediated neurotoxicity compared to adults. We have earlier shown that Scatchard analyses of [^{3}H]MK-801 binding isotherms indicate lower density of binding sites in weanling rats treated postnatally with phencyclidine (PCP) compared to saline-treated rats; [^{3}H]MK-801 binding was used as a marker for NMDA channel activation. Here we report characterization of L-glutamate-induced NMDA receptor activation on [^{3}H]MK-801 binding in postnatal PCP-treated rats. Rat pups were treated with PCP (5 mg/kg) for eleven days. Controls received saline injections. [^{3}H]MK-801 bindings were measured in forebrain synaptosomal membranes prepared from PCP and saline-treated animals, both in the absence and presence of increasing concentrations of L-glutamate (0.1-100 μM) with or without added glycine (10 μM). In the absence of added glycine, incubation in the presence of L-glutamate lead to a dose-dependent increase in specific [^{3}H]MK-801 binding both in the PCP- and saline-treated rats but neither mean EC_{50} values nor maximum [^{3}H]MK-801 bindings were different in the two groups. When binding was carried out in the added presence of glycine, maximal binding in the PCP-treated rats were lower than in the saline-treated controls. The mean EC_{50} value for L-glutamate-stimulated [^{3}H]MK-801 binding was reduced in both experimental and control rats but there was no apparent difference in the mean EC_{50} values between the two groups. These findings suggest that PCP treatment during early postnatal period in rat alters the agonist-induced regulation of the NMDA channel activation.

Support: NARSAD; NAMI

485.12

Probing the Molecular Interactions between PCP-like Ligands and the PCP Binding Site of the NMDA Receptor Complex: Evidence for the Heterogeneity of PCP Binding Sites. Y.-P. Pang and A. P. Kozikowski*, Neurochem. Research, Mayo Clinic, 4500 San Pablo Rd., Jacksonville, FL 32224.

To further delineate the topography of the phencyclidine (PCP) binding site of the *N*-methyl-D-aspartate (NMDA) receptor complex, six oxygenated analogues of (+)-1,2,3,4,4a,9a-hexahydro-4a-fluorenamine (HFA) were designed and synthesized to serve as probes of the hydrogen bonding interaction postulated to exist between a substituent located at the *meta*-position of the phenyl group of the PCP-like ligand and its binding site. In addition, these oxygenated HFA analogues can serve as probes of topography to provide information about the possible heterogeneity of the PCP binding sites (for the conjecture that there are two subclasses of PCP-like ligands binding sites, namely the MK-801 site and the PCP site, on the NMDA receptor complex, see A. P. Kozikowski and Y.-P. Pang, *Mol. Pharmacol.*, 37:352, 1990; for the synthesis of these analogues, see Y.-P. Pang and A. P. Kozikowski, *J. Org. Chem.*, 56:4499, 1991). From the binding affinities measured for these HFA analogues, the existence of the putative hydrogen bonding interaction between the phenyl ring substituent of the HFA analogues and their binding sites appears unlikely, a result which stands in marked contrast to the increased binding affinities reported for the *meta*-hydroxy and *meta*-methoxy substituted derivatives of PCP. This discrepancy can be explained by assuming that the structural requirements for the binding of the MK-801 types of ligands (or HFA and its analogues) with smaller molecular volumes to the "MK-801 site" are different from those of the PCP-site preferring ligands with larger molecular volumes. The new data serve to support our previous conjecture that the PCP site and the MK-801 site are co-localized but are not identical. In addition, the binding results reveal the existence of unoccupied space which can accommodate certain functional groups in the vicinity of the 6-position of the phenyl ring of HFA, which therefore allows for the incorporation of an electrophilic alkylating group or a photoaffinity labeling group capable of irreversible labeling of the binding site. A more detailed "enzyme-excluded" volume of the MK-801 binding site has thus been defined.

485.13

DEVELOPMENT OF PHOTOAFFINITY PROBES FOR THE NMDA-ASSOCIATED GLYCINE BINDING SITE. *A.C. Nichols*, K.L. Yielding. and L.D. Snell. Dept. of Pharmacology, Univ. of Texas Medical Branch, Galveston, TX 77555.*

The photolabile acyl azide function was placed at the 2-position of 7-halogenated kynurenic acid derivatives to determine their usefulness as photoaffinity probes for the glycine modulatory site on the NMDA receptor complex. Similar derivatives were synthesized from 5,7-dichlorokynurenic acid and 4-(methylamino)-5,7-dichloroquinoline-2-carboxylic acid. The acyl azide of 7-chlorokynurenic acid was the most promising derivative. In dark experiments this compound could displace ^{3}H glycine binding from rat cortical membranes with relatively high affinity (K_i = 2.4 +/- 0.2 uM) in an apparently competitive fashion (nH = 0.90 +/- 0.08). When membranes were UV irradiated in the presence of 10 uM of the acyl azide, washed, and then assayed for glycine enhancement of ^{3}H- TCP binding, the maximal enhancement was reduced by 30%. This was taken as evidence of irreversible binding of the probe to the glycine receptor. Use of a radioactive precursor in the synthesis can provide a probe capable of radiolabeling NMDA-associated glycine binding sites irreversibly.

485.14

GLUTAMATE SENSITIVE BINDING OF [^{125}I]MK-801 TO INTACT CEREBELLAR GRANULE CELLS. T.F. Murray* and V.J. Caldwell. Oregon State Univ., College of Pharmacy, Corvallis, OR 97331.

Efforts to label NMDA receptors in membrane preparations derived from rat cerebellum have yielded conflicting results. Although unsuccessful attempts to detect specific binding of [^{3}H]TCP and [^{3}H]MK-801 in rat cerebellar membranes have been reported, a demonstration of specific [^{3}H]MK-801 labeling of cerebellar membranes has recently appeared (Ebert et al., Europ. J. Pharmacol.-Mol. Pharmacol. Sect. 208, 49, 1991). These cerebellar binding sites are distinct inasmuch as the affinity for [^{3}H]MK-801 is substantially lower than that of NMDA receptors in forebrain structures. To further explore the characteristics of cerebellar NMDA receptors, we have investigated the binding of [^{125}I]MK-801 in intact cultured cerebellar granule cells. The binding of [^{125}I]MK-801 was inhibited in a concentration-dependent manner by glutamate (1-300μM). The glutamate inhibition of [^{125}I]MK-801 binding to intact granule cells was reversed by Mg^{++} in concentrations ranging from 1 to 10,000μM. Similarly, the glutamate-induced inhibition of binding was antagonized by the competitive NMDA receptor antagonist AP-5. Noncompetitive NMDA receptor antagonists such as MK-801, phencyclidine and dextrorphan produced biphasic effects on [^{125}I]MK-801 binding with low concentrations stimulating and high concentrations inhibiting binding. Equilibrium saturation analysis of [^{125}I]MK-801 binding resulted in a K_D value of 77nM and a B_{max} value of 5.3pmol/plate. Companion experiments indicated that glutamate exerted similar negative modulation of cerebellar NMDA receptors labeled with [^{3}H]dextrorphan. These results suggest that intact cerebellar granule cells in culture express functional NMDA receptors. (Supported by PHS Grant DA07218)

485.15

DIFFERENTIAL REGULATION OF [^{3}H]DEXTRORPHAN AND [^{3}H]MK-801 BINDING TO SLIDE-MOUNTED RAT BRAIN SECTIONS BY MODULATORS OF NMDA RECEPTOR-CHANNEL FUNCTION. P.H. Franklin* and T.F. Murray, College of Pharmacy, Oregon State University, Corvallis OR 97331

Although structurally unrelated to either MK-801 or arykycloalkylamines such as PCP, the analgesically inactive dextrorotatory morphinan, dextrorphan(DX), has been found to possess significant NMDA antagonist efficacy in vivo and in vitro. Although DX clearly antagonizes NMDA-mediated excitation in a noncompetitive manner in the hippocampal slice, in contrast to the prototypical noncompetitive NMDA antagonist MK-801, DX blocks NMDA-mediated excitation in a use-independent manner(Cole *et al.*, Neuropharmacol., 28:249 '89). We have found that [^{3}H]DX, like [^{3}H]MK-801, labels a site in rat brain membranes in the domain of the NMDA-operated cation channel (Franklin and Murray, Mol. Pharmacol., 41:134-146, '92). Accordingly, [^{3}H]DX binding is modulated by the level of activation of the NMDA receptor-channel and thus is increased under "open channel" conditions and, conversely, inhibited by pharmacological antagonists of NMDA receptor function. Unique aspects of the regulation of [^{3}H]DX binding by Mg^{2+} and the polyamines, however, suggest that [^{3}H]DX labels a site in the channel which does not coincide with those of either [^{3}H]MK-801 or [^{3}H]TCP. In order to address this issue in the context of the apparent heterogeneity of NMDA receptors in the CNS we have compared the regulation of [^{3}H]DX and [^{3}H]MK-801 binding in slide-mounted sections of rat brain binding as a prelude to quantitative autoradiographic analysis of the binding of these radioligands. In HEPES/EDTA(1mM/10mM; pH 7.4) treated coronal sections of rat brain, taken rostral to the anterior commisure, we find that glutamate(1-100μM) stimulates both specific [^{3}H]MK-801 and [^{3}H]DX binding in a concentration-dependent manner. Glycine(1-1000μM), in contrast, stimulated the binding of [^{3}H]MK-801 only moderately, while exerting a concentration-dependent inhibition of specific [^{3}H]DX binding. The basis for these observations will be addressed using quantitative autoradiography.

485.16

EVIDENCE THAT HIGH AND LOW AFFINITY AMPA BINDING SITES REFLECT MEMBRANE-DEPENDENT STATES OF A SINGLE RECEPTOR. R. Hall, M. Kessler* and G. Lynch. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717.

AMPA-type glutamate receptors are the primary mediators of excitatory neurotransmission in mammalian forebrain and are also thought to be central to the form of synaptic plasticity known as long-term potentiation (Staubli *et al., Psychobio.* 18:377, 1990). Binding of [^{3}H]AMPA (DL-α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid) to lysed rat brain membranes in the presence of potassium thiocyanate resulted in curvilinear Scatchard plots which could be resolved by regression analysis into a large low affinity component (K_D=200-1000 nM; approx. 90% of the total number of sites) and a small high affinity component (K_D=10-40 nM; approx. 10% of the sites). Treatment with 0.4% Triton X-100 resulted in a solubilized fraction which contained 74% of the sites recovered after solubilization. These sites were uniformly of the high-affinity type with a K_D of 29 ± 5 nM. The total number of these sites was three to four times higher than the number of high affinity sites which was present in the starting material. Solubilization thus appears to convert low affinity into high affinity receptors; it follows from this that the two affinity states represent interconvertible forms of the same receptor rather than separate receptor types. Moreover, high-affinity sites were found to be greatly reduced in number in lysed synaptic plasma membranes: they accounted for only 1% of total binding in SPMs as compared to 10% in regular lysed membranes. This suggests that it is some factor in the synaptic environment which keeps receptors in a low affinity state and that it is the loss of this factor during solubilization which allows receptors to revert to a high affinity state. (Supported by NS 21860 and AFOSR 89-0383).

485.17

RAPID VISUALIZATION OF NMDA RECEPTORS; CHARACTERIZATION OF (+)-3-[^{125}]IODO-MK801 BINDING TO THIN SECTIONS OF RAT BRAIN. W. Jacobson* and G.A. Cottrell Department of Obstetrics and Gynecology and Department of Pharmacology, University of Toronto, Toronto, Ontario, M5G 1L7.

We have developed and characterized a method for the rapid autoradiographic determination of receptor sites for the non-competitive NMDA receptor antagonist MK801, using an iodinated form of the compound, (+)-3-[^{125}]-Iodo-MK801. The binding site was shown to exhibit those criteria necessary for its definition as a receptor site, i.e. the binding was saturable, of high affinity, easily reversible, and stereospecific. Saturation analysis of binding to thin brain sections revealed a B_{max} of 108.1±10.5 fmole/mg protein and a K_d of 383±67 pM. The pharmacology of the interaction of the ligand with the binding site yielded good correlation between the potency of various substances to compete for the binding site and their ability to act as antagonists of NMDA. Autoradiographs of thin coronal brain sections using (+)-3-[^{125}]-Iodo-MK801 yielded high quality images in 24-48 hours with a distribution of binding sites paralleling that reported for the tritiated form of the ligand, i.e. with high densities in the hippocampus, cerebral cortex and lateral septum. Other areas with significant binding included parts of the thalamus, the amygdala and the olfactory tubercules. Furthermore, due to its high specific activity, this ligand lends itself to the study of regions not rich in MK801 binding sites, such as the diencephalon. Supported by the Medical Research Council of Canada, MA-10911.

485.18

DEVELOPMENTAL CHANGES IN HYPOTHALAMIC EXCITATORY AMINO ACID RECEPTORS IN THE RAT. G.A. Cottrell * and W. Jacobson. Department of Obstetrics and Gynecology & Department of Pharmacology, University of Toronto, Toronto, Canada, M5G 1L7.

Using an iodinated form of the non-competitive NMDA receptor antagonist MK-801 (IMK) which labels a site on the NMDA receptor, as well as [^{3}H]-Kainic acid (KA), we have examined the development of excitatory amino acid (EAA) binding sites in the hypothalamus of young male and female rats during the pubertal transition. Coronal brain sections were obtained from rats of both sexes at d15, d25, d32 and d37 of life, and autoradiograms were generated using both ligands. In the female, IMK binding increased in all regions examined (MPOA, Lat Sept, BNST, VMH, and Striatum) over the course of development. Such was not the case in males, where increases were not seen in the BNST, VMH or Striatum. The ontogeny of KA binding sites in the median eminence was also sexually dimorphic, in that males demonstrated a transient elevation at d25 and d32 of life, while females did not show an increase until d32, and this was maintained at d37 of life. Significant KA binding was not measured in any other hypothalamic region in either males or females. These findings are considered in light of suggestions that EAA play a role in the control of the onset of puberty. Supported bty the Medical Research Council of Canada, MA-10911.

485.19

MATERNAL VITAMIN B-6 DEFICIENCY ALTERS POSTNATAL DEVELOPMENT AND ZINC REGULATION OF ^{3}H-MK801 BINDING TO CORTICAL NMDA RECEPTOR-ION CHANNELS. T.R. Guilarte* and R.C. Miceli Dept. of Environmental Health Sciences, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD.

Vitamin B-6 (B6) deficiency during development results in reduced function of NMDA receptor-coupled ion channels in the cerebral cortex (CTX) of 14 day old rats (Guilarte TR. *Neurosci. Lett.* 121: 207-210, 1991). The present investigation extends the previous findings and indicates that B6 deficiency during development reduces the number of ^{3}H-MK801 binding sites and increases the level of zinc (Zn) required to inhibit ^{3}H-MK801 binding.

Long Evans female rats (125-150 g) were fed either a normal (7.0 mg/kg pyridoxine.HCL [PN.HCl]) or marginally deficient B6 diet (0.7 mg/kg PN.HCl) two weeks prior to mating. Dams and progeny (weaned at 21 days of age) were fed the same diet throughout the study. ^{3}H-MK801 binding to cortical membranes was assayed at postnatal day (PN) 7, 14, 21, 28, and 56 as described (Guilarte TR. *Ibid*). Rats fed a normal diet, had a transient increase in the Glu or Gly-dependent ^{3}H-MK801 binding to PN14 CTX, relative to all other ages. This transient increase in ^{3}H-MK801 binding sites was significantly reduced in rats fed the marginally deficient B6 diet. The effect of the NMDA receptor endogenous modulators Zn and magnesium (Mg) on ^{3}H-MK801 binding was also measured as a function of age. Cortical membranes from PN7 rats were significantly more sensitive (IC50= 0.91 mM) to the inhibitory effects of Mg than any other age tested (combined IC50= 2.93 mM). There were no significant effects of diet. In normal rats, Zn showed a transient increase in the IC50 (112 µM) at PN14 relative to PN7, 21, and 56. (IC50= 27.3, 30.7, and 45.9 µM, respectively). However, B6 deficient membranes at PN21 and 56 required greater amounts of Zn to produce the same inhibitory effect on ^{3}H-MK801 binding relative to age matched controls. These results suggest that vitamin B-6 deficiency can influence the postnatal development and regulation of NMDA receptor-coupled ion channels in the rat cerebral cortex (Grant # HD20939).

485.20

NMDA-RECEPTOR mRNA EXPRESSION IN THE CORTEX AND HIPPOCAMPUS OF CYCLING AND LACTATING RATS. R. Abbud,* B. Attardi, G.E. Hoffman, and M.S. Smith. Department of Physiology, University of Pittsburgh, Pittsburgh, PA 15261.

Using cFos expression as a marker of neuronal activation, we observed an inhibition of cortical and hippocampal activation in response to NMA, but not kainate, in lactating rats. This lack of responsiveness to NMA in lactating rats could be due to a decrease in the number of NMDA receptors (R) in these areas. To examine this hypothesis, we examined the expression of NMDA-R mRNA in cycling and lactating rats. *In situ* hybridization was performed using a ^{35}S-labeled riboprobe recognizing 1.4 kb of the NMDAR1 mRNA. Areas of silver grains were analyzed in the parietal cortex, the piriform cortex, the CA1 region of the hippocampus, and the dentate gyrus, using the Optimas Image Analysis System. NMDA-R mRNA was abundant in all areas of the brain examined. Differences in NMDA-R mRNA expression between cycling and lactating rats were observed only in the CA1 region of the hippocampus (18% decrease in the lactating rats). Differences in NMDA receptor expression were not detected by Northern Blot assays of tissue isolated from the cortex or hippocampus. These data suggest that the changes in NMDA-R mRNA expression in the lactating rat are very subtle, suggesting that alterations in NMDA receptors in the regions examined might not explain the functional deficits in cortical activation in response to NMDA. Other possible explanations are that a small but key subpopulation of neurons is altered in the cortex, or that changes in NMDA receptors occur within the brainstem or spinal cord rather than in the cortex. It is also possible that suckling may inhibit activation of brainstem pathways necessary for cortical activation. Supported by NIH Grant HD14643.

GABA RECEPTORS: FUNCTION IV

486.1

BACLOFEN INHIBITS THE INCREASE IN CYCLIC AMP INDUCED BY HIGH FREQUENCY ELECTRICAL STIMULATION IN THE DENTATE GYRUS OF RAT HIPPOCAMPAL SLICE. M.J. Bonnet* and J.M. Sarvey, Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814.

Activation of second messenger systems is implicated in the induction and maintenance of long-term potentiation (LTP), a form of synaptic plasticity. A high-frequency train (HFT; 100 Hz, 2 sec) of electrical stimuli was applied to the medial perforant path in the dentate gyrus of rat hippocampal slices. After HFT the dentate gyrus was cut from the rest of the slice, and cAMP levels in the dentate were determined by a protein binding assay. Each treatment group consisted of 3 slices from each of 6 rats. One min after HFT, cAMP levels increased to 240% of levels in slices that did not receive an HFT. Immediate post-treatment exposure of slices to baclofen (10, 100, 1000 µM) for 1 min resulted in concentration-dependent inhibition of HFT-stimulated cAMP levels. Levels of cAMP were reduced by 50% by 10 µM baclofen compared to levels produced post-HFT without baclofen. A high concentration (1000 µM) of baclofen post-HFT suppressed cAMP to basal levels. Pre-treatment of slices with 100 µM baclofen for 20 min before HFT also inhibited the HFT-induced increase in cAMP to a similar degree to post-HFT baclofen. However, pre-HFT treatment with 10 µM baclofen did not produce any significant suppression of HFT-stimlated cAMP. These results suggest that the baclofen-sensitive GABA-B receptor can regulate stimulated cAMP levels in the dentate gyrus.

486.2

***IN VITRO* AND *IN VIVO* EFFECTS OF BACLOFEN ON cAMP OVERFLOW IN RAT CEREBRAL CORTEX.**

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The dual effect of baclofen upon cAMP production in brain tissue *in vitro* is well documented. The purpose of the present study is to elucidate and compare its effects *in vivo*.

In vitro experiments where performed by incubating aliquots of rat cross chopped cortical slices for 10 min with either 10 µM forskolin or 100 µM norepinepherine to stimulate adenylate cyclase (AC) activity. *In vivo* experiments were performed by implanting a concentric dialysis probe into rat frontal cortex, 100 µM forskolin was administered in the dialysing medium as two pulses, the first (S1) to standardize the preparation, the second (S2) for experimental manipulation. Radioimmunoassay was used to quantify the cAMP accumulated in the supernatant or the dialysate as a measure of AC activity. *In vitro* baclofen (0.01 to 100 µM) had no effect on base line cAMP production but was found to dose dependently inhibit forskolin stimulated AC activity (pIC_{50} 6.07 ± 0.29) and to augment the maximum stimulation obtained with norepinepherine (pIC_{50} 5.04 ± 0.17). *In vivo*, 10 µM baclofen also failed to alter base line cAMP. However, when baclofen (10µM) was administered simultaneously with forskolin during S2, cAMP overflow was not reduced when compared to control S2, but instead appeared to increase the stimulation produced by forskolin (S2/S1 ratio = 1.59 ± 0.27, control = 0.57 ± 0.07). The reasons for this are currently under investigation.

486.3

EFFECT OF (-)BACLOFEN ON cAMP FORMATION AND SUBSTANCE P RELEASE FROM RAT SPINAL CORD.

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It has been proposed that baclofen may induce antinociception in rodents through an action on $GABA_B$ receptors localized in the spinal cord. In this study we have investigated the effect(s) of (-)baclofen on stimulated cAMP formation in rat spinal cord slices (350x350 μm) and on substance P release evoked by electrical stimulation (15 V, 10Hz, 1ms) of dorsal roots attached to rat spinal cord slices. (-)Baclofen, dose dependently (10-100 μM), inhibited forskolin (10 μM)-induced formation of cAMP (maximal inhibition 58±9.5% at 100 μM, n=3 rats) but this was not prevented by CGP 35348 (concentrations up to 1.5 mM). By contrast with cerebral cortical slices (-)baclofen (1-100 μM) failed to enhance noradrenaline (100 μM)-induced formation of cAMP. Evoked release of substance P-LI was completely inhibited by (-)baclofen (IC_{50} ~ 4.5 μM). Whilst $GABA_B$ mediated analgesia may stem from a reduction in primary afferent neurotransmitter release it seems unlikely that an alteration in cAMP production is implicated.

486.4

DIFFERENTIAL DISCRIMINATIVE-STIMULUS EFFECTS OF ANXIOLYTICS AND HYPNOTICS IN RATS TRAINED TO DISCRIMINATE LORAZEPAM (LRZ), DIAZEPAM (DZP), OR PENTOBARBITAL (PB).

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Under two-lever drug vs. no-drug discrimination procedures, the LRZ training condition previously was shown to be unique among benzodiazepine (BZ) training conditions studied to date in that rats did not generalize to pentobarbital reliably, even when training dose and tolerance to its rate-decreasing effects were manipulated (Ator and Griffiths, Psychopharm., 1989, 98, 20). Subsequently, generalization profiles for LRZ, DZP, and PB training conditions have been directly compared in Long-Evans rats in tests with a range of BZ agonists and partial agonists, barbiturates, and other anxiolytics or sedatives. Rats in all three training groups generalized to BZ, but greater selectivity in generalization to barbiturates and other sedative/anxiolytic compounds occurred in rats trained to discriminate LRZ than DZP. Rats trained to discriminate LRZ failed to generalize reliably to any barbiturate or other non-BZ sedative/anxiolytic, but the profiles for rats trained to discriminate DZP and PB were comparable. Among novel BZ-receptor ligands, abecarnil occasioned drug lever responding under all conditions; zolpidem did so in the PB and DZP conditions but not as reliably in the LRZ condition; and bretazenil occasioned the drug response in the DZP and PB, but not the LRZ, conditions. (Supported by PHS Grant DA01433)

486.5

ALTERATIONS IN TBPS BINDING IN THE LIDOCAINE-KINDLED RAT MODEL OF EPILEPSY.

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This autoradiographic study examined regional $GABA_A$ receptors in chemically kindled rats. [^{35}S]t-Butyl bicyclophosphorothionate (TBPS), which binds in or near the $GABA_A$ receptor chloride channel, was used as a ligand to identify $GABA_A$ receptor complexes. Male Sprague-Dawley rats were injected daily with lidocaine (65 mg/kg, i.p.). Control rats received an equal volume of saline vehicle. Seizure activity was evaluated using the Racine Scale of 5 behavioral stages. The animals displayed a gradual increase in the severity of behavioral indices and by day 20 greater than 50% were in Stage 4 or 5. Midway through the injection regimen some animals exhibited a gradual regression toward less severe behavioral stages; these animals were considered 'compensated'. After 25 injections the animals were killed, the brains removed and 30 μm sections incubated with 2 nM [^{35}S]TBPS. Brain paste standards were prepared and included in the autoradiographic process. After appropriate exposure, the developed films were analyzed using a computer based imaging system. Binding was decreased in the subiculum, posterior lateral thalamic nuclei, hippocampal regions CA_1 and CA_3, and cerebellar nuclei in sections from kindled animals as compared to controls. Sections from rats that 'compensated' during the kindling process had normal or slightly elevated [^{35}S]TBPS binding in those regions. These data support the hypothesis that alterations in the $GABA_A$ receptor occur during the kindling process.

486.6

GABA EFFECTS ON ASCENDING AND DESCENDING TRACTS IN ISOLATED DORSAL COLUMNS OF NEONATAL RAT SPINAL CORD.

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We compared the effects of GABA and related drugs on axonal conduction in of neonatal rat spinal cords *in vitro*. In addition, we evaluated the effect of GABA-evoked extracellular K^+ concentration changes on axonal conduction in these spinal tracts, using double-barrelled K^+-sensitive microelectrodes. The compound action potential evoked by supramaximal stimuli was recorded with glass micropipettes or reference barrels of K^+-sensitive microelectrodes. GABA (10^{-4} M) reversibly depressed the compound action potential amplitude and prolonged the latency of the ascending tracts. In contrast, GABA had little or no significant effects on the compound action potential of the descending tract. The GABA-A receptor agonist isoguvacine (10^{-5}-10^{-4} M) reduced compound action potential amplitudes and increased response latencies of both ascending and descending tracts. However, these effects were far less pronounced in the descending tract. GABA (10^{-4}-10^{-3} M) increased extracellular K^+ concentrations in the ascending tracts with a depression of the compound action potential in a concentration-dependent manner. The GABA-induced compound action potential changes correlated only partially with the extracellular K^+ concentration changes. Furthermore, compound action potential changes induced by elevation of extracellular K^+ concentrations were far less than those induced by GABA. GABA (10^{-3} M) also caused a similar discrepancy of extracellular K^+ concentrations and compound action potential changes in the descending tract. These results suggest that extracellular K^+ concentration increases induced by GABA play a minor role in GABA actions on axonal conduction in the ascending and descending tracts.

The present results indicate that GABA-A activation modulated axonal conduction of both ascending and descending tracts in the neonatal rat spinal cord. However, the effects were much less on the descending tract compared to the ascending tracts. In addition, GABA-evoked extracellular K^+ concentration changes contributed only partially to the GABA-A mediated conduction changes.

486.7

PHASE SHIFT OF LLD IN DIFFERENT AREAS OF THE HIPPOCAMPUS: MULTI SITE RECORDING OF SPONTANEOUS AND EVOKED LLD FROM AN IN VITRO RODENT SLICE.

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A depolarizing bicuculline sensitive response can be elicited in slices of hippocampus and neocortex under various conditions. This response is not effected by the antagonism of excitatory amino acid receptors and seems to occur throughout the tissue. This response also occurs spontaneously in the presence of 4-AP, and does not seem to propagate but occurs simultaneously throughout the tissue. It can be shown that there is some delay in the occurrence of the response between the distal CA3 dendrites and the subiculum. The delay across 2mm of tissue is approximately 150 msec suggesting there may be a synaptic component to the response and/or that there may be a loci where the response originates.

The ability of GABAergic cells to produce depolarizing responses may be important in stabilizing GABAergic networks as well as limiting seizure-like activity. Whether activation of the postsynaptic cell through this mechanism can change the efficacy of synaptic contacts is a possibility, since calcium can enter the cell through these channels. When this occurs intracellular calcium increases, even when not paired with synaptic activation which produces synaptic modifications. As this response is most prominent in neonates, this would be a good mechanism for laying down a framework of GABAergic connections.

486.8

DIFFERENTIAL LAMINAR EFFECTS OF BACLOFEN ON DEVELOPING RAT VISUAL CORTEX.

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Baclofen application has resulted in facilitation, depression and no effect in various CNS structures. These disparate results have led to the speculation that baclofen effects on $GABA_B$ receptors demonstrate regional heterogeneity. We utilized a 16 channel rake electrode with subsequent CSD analysis and intracellular recordings to examine the laminar effects of baclofen following white matter stimulation in 16-22 day old rat visual cortex. Monosynaptic EPSPs in both layers II/III & V were unaffected by low dose (0.5-2.0 μM) baclofen and equally attenuated by high doses (10-40 μM). Polysynaptic EPSPs were selectively attenuated in layer II/III at low doses while layer V EPSPs were facilitated. Low dose baclofen selectively attenuated IPSPs in both layers. We conclude that baclofen, acting at presynaptic terminals, demonstrates differential laminar effects in immature rat visual cortex.

486.9

DECREASED NEURONAL INHIBITION IN *IN VITRO* HIPPOCAMPUS AFTER 1 WEEK FLURAZEPAM (FZP) TREATMENT: AN INTRACELLULAR STUDY. X-H. Xie* and E. I. Tietz. Department of Pharmacology, Medical College of Ohio, Toledo, OH 43699

Chronic FZP treatment significantly reduces GABA-mediated hippocampal paired-pulse inhibition (Xie and Tietz, 1991). Orthodromic EPSP-IPSP sequences and action potential (AP) burst frequency and duration, elicited by a 1 sec depolarizing current (0.6 nA) injection, was examined in *in vitro* superfused slices 48 hr after 1 week FZP treatment. Male rats (175-200 gm) were offered FZP in the drinking water (100 mg/kg X 3 dy;150 mg/kg X 4 dy). Selected neurons from treated (8 cells/5 rats) and control (7 cells/4 rats) slices had stable resting membrane potentials (RMP: -64.5±0.8 vs. -63.3±1.3 mV), input resistances > 30 MΩ (78.3±10.6 vs 93.9±10.6) and antidromic APs > 70 mV (82.5±3.9 vs 83.5±1.5 mV). RMP was held at -60 mV throughout the experiment. EPSP-IPSPs elicited by just subthreshold Schaffer-collateral stimulation were recorded (3 M KAc filled glass pipette, 100-170 MΩ) from CA1 pyramidal cells (24°C). There was a significant ($p<.01$) reduction in the amplitude of the *f*IPSP (1.4±0.6 vs 6.3±0.7 mV) and *s*IPSP (3.5±0.4 vs 6.5±0.7 mV) and an increase in EPSP amplitude (10.6±1.3 vs 5.4±0.8 mV). The small increase in burst duration and frequency (265.4±60.4 ms, 34.0±2.4 Hz vs 242.1±50.7 ms, 25.2±4.2) was not significant ($p>.10$). These data provide additional evidence regarding the nature of the impairment in GABA-mediated hippocampal function in chronic benzodiazepine treated rats. Supported by NIDA grant RO1-DA04075.

486.10

FEEDFORWARD INHIBITION IS REDUCED IN HIPPOCAMPUS AFTER CHRONIC BENZODIAZEPINE TREATMENT. X. Zeng and E.I. Tietz* Dept. of Pharmacology., Med. Coll. Ohio, Toledo, OH 43699

Recurrent inhibition was reduced in CA1 region of *in vitro* hippocampal slices of benzodiazepine tolerant rats indicated by a reduction in both orthodromic-orthodromic and antidromic-orthodromic paired-pulse responses (Xie and Tietz, 1991). In this study the magnitude of feedforward inhibition in slices from 1 week chronic flurazepam (FZP) treated rats was evaluated according to Lupica and Dunwiddie (1992). Slices (400 μM) were cut, 48 hr after treatment, from male rats (175-200 gm) offered FZP (100 mg/kg X 3 dy;150 mg/kg X 4 dy) in the drinking water. Control rats received saccharin water. Control and test population spikes evoked by stratum radiatum stimulation (S2) on the subicular side of an extracellular recording electrode (2 M NaCl filled glass pipette, 2-5 Mohm) were compared. The test pulse followed an orthodromic conditioning pulse (S1), subthreshold for a population spike, with interpulse intervals (IPI) of 10-100 ms. A knife cut was made through the alveus between S1 and the recording electrode to block recurrent inhibition. Feedforward inhibition in control slices ranged from 100-55%. Treated slices showed a significant (20-40%, $p<.01$) reduction in feedforward inhibition at all IPI. These data suggest that all levels of GABA-mediated inhibition are impaired in CA1 region of hippocampus of chronic benzodiazepine treated rats. Supported by NIDA grant RO1-DA04075.

486.11

$GABA_A$ RECEPTOR ACTIVATION INDUCES GABA AND GLUTAMATE RELEASE FROM PREOPTIC AREA. A. Fleischmann*[1], M.H. Makman[2], A.M. Etgen[1,3] Depts. of Psychiatry[1], Biochemistry[2], Molecular Pharmacology[2] and Neuroscience[3], Albert Einstein College of Medicine, Bronx, NY 10461.

The effect of γ-aminobutyric acid (GABA) receptor agonists on release *in vitro* of radiolabeled GABA and glutamate was studied using a crude preparation of isolated nerve terminals (neurosomes). GABA agonists were incubated (2 min., 37°C) with neurosomes prepared from hypothalamus (HYP), preoptic area (POA) and cortex (COR) tissues. GABA and the $GABA_A$ receptor agonist muscimol, but not the $GABA_B$ receptor agonist (-)-baclofen, stimulated ^{3}H-GABA and ^{3}H-glutamate release from POA but not HYP or COR neurosomes of male rats. These effects were partially inhibited by the $GABA_A$ receptor antagonists picrotoxin and bicuculline. Muscimol-induced release of ^{3}H-glutamate and ^{3}H-GABA was dependent on extracellular Ca^{2+}. Muscimol failed to release ^{3}H-GABA and ^{3}H-glutamate from POA neurosomes of ovariectomized female rats. However, administration of estradiol and progesterone to ovariectomized females caused the appearance of muscimol induced-release of amino acids comparable to that obtained in male rats. Therefore, $GABA_A$ receptor-induced release of amino acids is brain region-specific and modified by hormonal status.

486.12

EFFECTS OF GABA ON AXONAL CONDUCTION AND EXTRACELLULAR POTASSIUM ACTIVITY IN THE NEONATAL RAT OPTIC NERVE. A. Z.Hassan*[1], K. Sakatani[2] and M. Chesler[1]. [1]Dept. of Neurosurgery, New York University Medical Center, 550 First Avenue, New York, NY 10016 and [2]Dept. of Neurosurgery, Sapporo medical college, South-1st West-16th Chuoku, Sapporo Japan 060.

GABA depolarizes rat optic nerve axons and modulates axonal conduction through the activation of GABA-A receptors. In a number of preparations, application of GABA also causes a rise in $[K^+]_e$. To address whether an increase of $[K^+]_e$ plays a major role in GABA actions on the rat optic nerve, we studied the effects of GABA on axonal conduction and $[K^+]_e$ in the neonatal rat optic nerve *in vitro*. Double-barrelled K^+-sensitive microelectrodes were used to record $[K^+]_e$. The extracellular compound action potential evoked by supramaximal stimuli was recorded from reference barrels of the K^+-sensitive microelectrodes. GABA (10^{-4}-10^{-3} M) increased $[K^+]_e$ in the neonatal optic nerve in a concentration-dependent manner. During prolonged application, the $[K^+]_e$ slowly recovered. Upon washout, a $[K^+]_e$ undershoot was observed, followed by a slow return to baseline. The increase in $[K^+]_e$ induced by GABA was markedly reduced by the GABA-A receptor blocker, bicuculline (10^{-4} M). Isoguvacine (10^{-4} M), a GABA-A agonist, mimicked the effect of GABA but produced larger responses at the same concentration. In contrast, baclofen (10^{-4} M), a GABA-B agonist, had no effect on $[K^+]_e$. The changes in the compound action potential induced by GABA correlated only partially with the $[K^+]_e$ changes; the compound action potential was still depressed when $[K^+]_e$ returned to baseline before the undershoot. Furthermore, the changes in the compound action potential induced by elevation of $[K^+]_e$ were far less than those induced by GABA. These results demonstrate that the GABA-evoked accumulation of $[K^+]_e$ plays a secondary role in GABA actions on the neonatal rat optic nerve. Supported by NINDS Grant NS 1064.

486.13

GABA MODULATES CALCIUM AND MEMBRANE POTENTIAL OSCILLATIONS IN IMMORTALIZED HYPOTHALAMIC NEURONS. A.C. Charles* and T.G. Hales. Depts. of Neurology and Anesthesiology, UCLA School of Medicine, Los Angeles CA 90024

Clonal GnRH-secreting hypothalamic neurons (GT1-7) in culture showed spontaneous oscillations in $[Ca^{2+}]_i$ as measured with fluorescence videomicroscopy. Ca^{2+} oscillations had a periodicity of 1-60 seconds and the peak $[Ca^{2+}]_i$ of each oscillation ranged from 100-500 nM. Ca^{2+} oscillations of individual cells were generally asynchronous, although some groups of cells showed synchronous oscillations. Spontaneous Ca^{2+} oscillations were reversibly abolished by 1μM TTX. Bath application of 1 μM GABA evoked an increase in $[Ca^{2+}]_i$ in most cells (100-200 nM), and increased the frequency of Ca^{2+} oscillations; 10 μM GABA evoked a greater increase in $[Ca^{2+}]_i$ (100-500 nM), in some cells further increasing the frequency of Ca^{2+} oscillations. These responses to GABA were inhibited by 10 μM bicuculline. Using the patch-clamp technique under current-clamp conditions, GT1-7 cells showed oscillations of membrane potential between -60 and -40 mV, with a periodicity of 0.5 -30 seconds. Each depolarizing oscillation was preceded by an action potential. Under voltage-clamp conditions, GABA activated Cl^- currents in all cells tested. Depolarizing test pulses activated inward Ca^{2+} currents with high and low threshold components, as well as Na^+ currents which were sensitive to TTX (0.5 μM). Cell-attached patch recordings revealed spontaneous action potentials. Transient application of GABA under these conditions evoked multiple action potentials. These results suggest that GABA has an excitatory action on GT1-7 cells which may be involved in the stimulation of GnRH secretion.

486.14

Immortalized Hypothalamic (GT1-7) Neurons Express Functional $GABA_A$ Receptors. R.W. Olsen, H. Kim, B. Longoni, A.J. Tobin and T.G. Hales*. Dept. Pharmacology, Dept. Biology and Dept. Anesthesiology, UCLA, LA, CA 90024.

Neuronal cell-lines provide a source of pure populations of neurons and allow the properties of many neurotransmitter receptors to be studied. None of these cells have been reported to express functional $GABA_A$ receptors. Indeed there have been no reports of cell-lines expressing functional amino acid receptors. Using biochemical and electrophysiological techniques, we have identified a neuronal cell-line expressing functional $GABA_A$ receptors. Membranes from immortalized hypothalamic (GT1-7) neurons bound [^{3}H]muscimol (213 ± 16 fmol/mg protein, 10 nM), but no significant clonazepam-displaceable [^{3}H]flunitrazepam binding was detected. In addition, diazepam (1 - 100 μM) was unable to enhance specific [^{3}H]muscimol binding. Under voltage-clamp conditions, GABA-activated chloride currents, recorded from GT1-7 cells, were blocked by bicuculline (1 μM) and Zn^{2+} (IC_{50} = 2.1 μM), but were insensitive to diazepam (1 - 10 μM). These results suggest that $GABA_A$ receptors on GT1-7 cells lack γ subunits. The neurosteroid 5α-pregnan-3α-ol-20-one (100 nM) and pentobarbital (100 μM) both potentiated GABA responses recorded from GT1-7 cells to 413 ± 89% and 450 ± 61% of control amplitude, respectively. GABA responses were also potentiated by propofol (2 μM). Interestingly neither the anesthetic steroid (1 μM) nor propofol (100 μM) directly activated $GABA_A$ receptors of GT1-7 cells. PCR analysis of the cells revealed the presence of mRNAs encoding α1, β1 and β3 polypeptides but not α2, α4, α6, β2, γ2 or δ. GT1-7 cells may provide a useful model system for studying the regulation of $GABA_A$ receptor polypeptide expression.

486.15

PERIPHERAL BENZODIAZEPINE RECEPTOR BINDING IN HYPOTHYROID RAT VENTRICLES

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Previously we reported the inhibition of triiodothyronine (T3) uptake into tissue cultured cells, by compounds from the benzodiazepine (BZ) class, but no interaction was seen in competition binding assays for the peripheral BZ receptor in liver preparations (Kragie & Doyle 1992 Endocrinology **130**:1211). Others have demonstrated upregulation of central and peripheral receptors after brief treatment with D-thyroxine (Gavish et al 1986 J Neurochem **47**:1106). We looked at binding of the peripheral receptor ligand, [^{3}H]R05 4864 in cardiac ventricular homogenates from thryroidectomized (TX) and sham-operated Holtzman adult male rats. Thirty days post-operative, TX animals were clinically hypothyroid, as demonstrated by low serum thyroxines and reduced heart / body weight ratios relative to sham controls. Hearts were harvested and flash frozen. Homogenates, 75-150 ug protein in PBS, were incubated with 0.25 to 12 nM [^{3}H]Ro5 4864 at 4°C for 1 hr. Nonspecific binding was determined using 1 uM cold Ro5 4864. Data were analyzed with LIGAND.

	SHAM	significance	TX
Bmax pmol/mg	11.01 +/- 1.16 (8)	p<0.002	6.17 +/- 0.75 (10)
HILL	0.96 +/- 0.02	NS	1.00 +/- 0.02
log Kd	-8.143 +/- 0.07	p<0.001	-8.453 +/- 0.04

The data show increased affinity and decreased receptor density in the TX hearts. Supported by NIH grant to LK: K11 DK01456

486.16

ANXIOLYTIC COMPOUNDS HAVE DIFFERENTIAL EFFECTS ON RAT EEG.

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Although currently available antianxiety agents are sedative, it is unlikely that sedation is a necessary property of this class of compound. Here, different anxiolytics were examined with a view to quantifying their effects on central arousal: as an example of a sedating compound, a barbiturate, Sodium Pentobarbital, was compared with a recently developed Type I sedative, Zolpidem, a non-selective benzodiazepine, Diazepam, and a serotonin ($5HT_{1A}$) ligand, Ipsapirone, which has been shown to exhibit little sedation. The electroencephalogram (EEG) was adopted as a sensitive measure of arousal. Rats were chronically implanted with cortical electrodes, and test compounds were administered via a cannulated tail vein. Vigilance was maintained by using a randomized disturbing noise source. The EEG, spectrally analyzed, demonstrated that all compounds except Ipsapirone significantly increased power in the freqency band above 8 Hz. Ipsapirone, in contrast, decreased power at these frequencies. The lowest freqency range (1-4 Hz) was also affected differentially: the barbiturate and Zolpidem increased, but Ipsapirone decreased, power at these frequencies; Diazepam had no effect. These data show that all compounds affected central arousal but there was no common action which might reflect an antianxiety effect. The EEG change produced by the two most sedating drugs indicated that there was increased recruitment of thalamocortical circuits. Such recruitment occurs with reduced vigilance. Diazepam acted similarly but Ipsapirone, under circumstances in which the compound reduces anxiety, increased vigilance. In conclusion these results indicate, firstly, that there is no preclinical EEG signature for anxiolytics because the EEG is a measure of the effect of these compounds on central arousal. And, secondly, that sedation is apparently not required for an anxiolytic action.

486.17

[^{123}I]IOMAZENIL SPECT IMAGING DEMONSTRATES SIGNIFICANT BENZODIAZEPINE (BZ) RECEPTOR RESERVE IN HUMAN AND NONHUMAN PRIMATE BRAIN.

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[^{123}I]Iomazenil has been used as a SPECT (single photon emission computed tomography) probe of the BZ receptor to measure receptor occupancy by agonists and antagonists. The present study involved parallel experiments in humans and monkeys to examine receptor occupancy by a clinically relevant dose of lorazepam.

A total of 36 [^{123}I]iomazenil SPECT scanning studies were performed in 12 humans and 2 monkeys. The effects of lorazepam (0.03 mg/kg i.v.) was assessed by the ratio of the brain washout rate of [^{123}I]iomazenil in the 45 min period after its injection relative to that in the 45 min period prior to injection, and this ratio was compared to that following placebo injection. Although this dose of lorazepam caused moderate sedation of the human subjects, the imaging results in both humans and monkeys could not demonstrate significant enhancement of washout of brain activity. The effects of higher cumulative doses of lorazepam (0.13, 0.25, and 0.5 mg/kg) were examined with a stepwise displacement paradigm and showed an ED50 of 0.17 ± 0.01 mg/kg (mean ± SEM, n=12) to displace specifically bound radioligand in brain. Log-probit plots of these data suggest that the clinically relevant dose of lorazepam (0.03 mg/kg i.v.) is associated with 3.1 ± 0.4% receptor occupancy. To examine whether endogenous GABA might modulates this *in vivo* potency measurement of lorazepam (i.e., ED50 to displace the radioligand), we examined the stepwise displacement with or without the concurrent administration of tiagabine, an inhibitor of the GABA transporter. In a series of 4 experiments, we could not demonstrate a significant *in vivo* GABA shift of lorazepam's potency.

These studies show that clinically relevant doses of lorazepam have low BZ receptor occupancy (< 4%) and suggest the BZ system has significant receptor reserve.

GABA RECEPTORS: FUNCTION V

487.1

PROGESTERONE-INDUCED ELEVATION IN BRAIN 3α-HYDROXY-5α-PREGNANE-20-ONE IS ASSOCIATED WITH ANXIOLYTIC BEHAVIOR AND AN INCREASE IN $GABA_A$ RECEPTOR FUNCTION.

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We have previously reported that intracerebroventricular administration of the progesterone metabolite, 3α-hydroxy-5α-pregnan-20-one (allopregnanolone), elicited significant anxiolytic behavior in a dose-dependent and stereospecific manner (Brain Res, 561: 157, 1991). We have also found that the anxiolytic efficacy of diazepam was enhanced in proestrous female rats, relative to ovariectomized (Ovx) rats, as was the efficacy of GABA-stimulated chloride (Cl-) influx in cortical synaptoneurosomes (Behav Neurosci, 105: 653, 1991). These results are consistent with the well-documented in vitro effects of 3α-hydroxy pregnane steroids as potent positive modulators of the $GABA_A$ receptor.

In the following experiments, the effect of a subcutaneous injection of progesterone (P: 0, 1, or 4 mg) in Ovx rats on exploration of an elevated plusmaze was examined. Blood serum and cortical concentrations of allopregnanolone were assessed. GABA-stimulated $^{36}Cl^-$ influx was determined in cortical synaptoneurosomes from a subgroup of Ovx females treated with vehicle or 4 mg of P. Significant anxiolytic activity was detected 4 hours after the administration of 1 or 4 mg of P; behavioral measures of anxiolytic efficacy were significantly correlated to blood and cortical levels of allopregnanolone. P treatment also increased the sensitivity of cortical synaptoneurosomes to GABA (i.e., decreased the EC_{50}) and increased the maximal efficacy with which GABA stimulated Cl- uptake. Together, these data support the hypothesis that the psychotropic effects of P are mediated by the bioconversion to allopregnanolone, and that allopregnanolone subsequently augments $GABA_A$ receptor-mediated function.

487.2

DEHYDROEPIANDROSTERONE (DHEA) IMPROVES MEMORY IN NORMAL MICE AND MICE WITH AGE-INDUCED MEMORY DEFICITS.

C.L. Melchior*, A. Glasky and R.F. Ritzmann. Olive View Medical Center, Sylmar, CA 91342.

DHEA is a steroid formed in the brain where it is active at the $GABA_A$ receptor complex. DHEA has been shown to enhance memory in avoidance paradigms. The purpose of these studies was to assess the effect of DHEA on working memory in the win-shift foraging paradigm. Briefly, a food deprived mouse is placed in a T-maze with a milk reward in both goal boxes. The mouse traverses the maze and is allowed to consume the milk in one of the goal boxes. On the next trial, if the mouse remembers which way it went on the previous trial, it will go the opposite way. By increasing the delay between trials the length of time a mouse can remember can be determined. Young adult C57BL/6 mice can remember at delays of 120 seconds. DHEA (0.005 mg/kg I.P.) increased the delay at which they could remember to 180 seconds. Young adult Swiss Webster mice are able to perform with delays up to 60 seconds. In 12 month old Swiss Webster mice, 70% had a memory deficit such that they could only remember at delays of 10 seconds. Physostigmine improved performance in these mice to a moderate extent, that is, they could remember with up to a 30 second delay. DHEA not only allowed the animals to perform at the 30 second delay but also, for 50% of these animals, extended the delay at which they could remember to 60 and 90 seconds. Thus, DHEA can improve working memory in young adult mice and improve the memory of mice with age-induced memory impairment to the level of young adults.

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487.3

PHYSIOLOGICAL VARIATIONS OF GONADAL STEROIDS MAY REGULATE $GABA_b$ SYNAPTIC TRANSMISSION IN THE CEREBRAL CORTEX. M.I. Al-Dahan, M.H. Jalilian-Tehrani and R.H. Thalmann*, Baylor College of Medicine, Houston, TX 77030

These results show that $GABA_b$ agonist binding in the cerebral cortex is consistently affected during normal variations in gonadal steroids that occur during the estrus cycle of the rat. Well-washed synaptic plasma membranes were prepared for filtration assays of the binding of the $GABA_b$ agonist (^{3}H)-baclofen. In addition, we examined the binding of the $GABA_a$ agonist (^{3}H)-muscimol and (^{3}H)-8-OH-DPAT, a 5ht1a agonist that depends upon the same G-proteins as do $GABA_b$ receptors. (^{3}H)-baclofen binding was lowest during estrus (72±10 fmol/mg protein) and then increased over metestrus (145±12) to reach a plateau by diestrus (215±29) and proestrus (237±31). In contrast, (^{3}H)-muscimol binding was at or near its maximum during estrus, and (^{3}H)-8-OH-DPAT binding declined slightly from proestrus to estrus, but then remained constant until the next proestrus. Saturation binding experiments showed that $GABA_b$ receptor density (Bmax) approximately tracked effects upon total specific binding in that Bmax was lowest during estrus. Intracellular recording methods will be used to assess the effects of this variation in $GABA_b$ binding upon receptor-channel coupling in slices harvested from animals in different stages of the estrus cycle. Supported by NIH grant NS-21713.

487.4

COMPLEX INTERACTIONS BETWEEN THE STEROID DERIVATIVE RU 5135 AND THE $GABA_A$-RECEPTOR COMPLEX. C Cadoni, LD McCauley and KW Gee*. Dept of Pharmacology, College of Medicine, University of California, Irvine, CA 92717.

The diverse pharmacological actions observed *in vivo* following the administration of RU 5135 range from sedation to convulsions. *In vitro* evidence has suggested that these effects may be mediated in part via the $GABA_A$ receptor complex (GRC). The modulation of [^{35}S]t-butylbicyclophosphorothionate (TBPS) binding was used to evaluate the actions of the steroid derivative RU 5135 at the GRC. The inhibition of [^{35}S]TBPS binding by GABA in the presence of various concentrations of RU 5135 was consistent with the hypothesis that RU 5135 is a competitive antagonist at the $GABA_A$ receptor. Schild analysis of 5α-pregnane-3α-ol-20-one (3α,5α-P) modulation of [^{35}S]TBPS binding in the presence of different concentrations of RU 5135 suggested that the action of RU 5135 was not competitive at the putative steroid site on the GRC despite common structural features with the neuroactive steroids (i.e., 3α-OH'ated, 5β-reduced A ring). On the other hand, the reduced potency of 3α,5α-P as an inhibitor of [^{35}S]TBPS binding in the presence of RU 5135, as well as blockade of 5α-pregnane-3α,20α-diol (5α-pregnanediol) inhibition of [^{35}S]TBPS binding by RU 5135 provide further support for the GABA antagonist properties of RU 5135. Moreover, this amidine steroid was able to partially inhibit [^{35}S]TBPS binding independent of GABA with nanomolar potency; yet the mechanism by which this occurs remains to be determined. (Supported by NIH grants NS25986 and NS24645).

487.5

CHRONIC GABA TREATMENT DOWNREGULATES $GABA_A$ RECEPTOR COMPLEX AND α mRNA SUBUNITS IN MAMMALIAN CORTICAL NEURONS. M.K. Ticku, M.C. Mhatre* and A.K. Mehta. Univ. TX Hlth. Sci. Ctr., Dept. of Pharmacology, San Antonio TX 78284-7764

Chronic exposure of GABA was investigated on the binding of ligands to GABA receptor complex, GABA-induced ^{36}Cl-influx and α subunit mRNA levels in cerebral cortical cultured neurons. Chronic GABA (500 μM, 5 days) decreased the specific binding of [^{3}H]flunitrazepam, [^{3}H]Ro15-1788, [^{3}H]Ro15-4513, [^{3}H]GABA and [^{35}S]TBPS by 35-45%. Chronic GABA treatment also decreased the GABA-induced ^{36}Cl-influx by 43% and GABA enhancement of [^{3}H]flunitrazepam binding by 31%. All these effects were blocked by concomitant exposure of the neurons to $GABA_A$ receptor antagonist, R 5135 (1μM). Chronic GABA exposure of cerebral cortical neurons (500 μM for 5 days) also produced 40-50% reduction in the level of $GABA_A$ receptor α_2 subunit mRNAs (6Kb = 54% reduction, 3 Kb = 43% reduction), an effect which was also blocked by R 5135. Chronic GABA treatment did not alter the level of poly A+ RNA. The GABA-induced reduction in $GABA_A$ α_2 subunit mRNA may underlie alteration in $GABA_A$ receptor function, down-regulation and/or uncoupling, observed following chronic GABA exposure of these neurons. Finally, GABA-induced downregulation, uncoupling and a decrease in α_2 mRNA subunit are a $GABA_A$-receptor mediated event. Supported by NINDS grant #NS15339.

487.6

$GABA_A$ RECEPTORS IN MOUSE CORTICAL TISSUE ARE PHOSPHORYLATED IN SITU BY ENDOGENOUS PROTEIN KINASE A. M.H. Jalilian Tehrani* and E.M. Barnes, Jr., Dept. of Biochemistry, Baylor Col. of Med., Houston, TX 77030.

In concordance with the recognition motif for protein kinase A (PKA) identified on all $GABA_A$ receptor (GaR) β subunits, it has been demonstrated that purified bovine GaRs can be phosphorylated by the PKA catalytic subunit. In order to study GaR phosphorylation under more physiological conditions, we have utilized crude membranes from mouse cortex or cortical homogenates eluted from Sephadex G-50. These preparations were incubated with [γ-^{32}P]ATP and 10 μM 8-Br-cAMP or Cl-phenylthio-cAMP. Extracts from these incubations were immunoprecipitated with polyclonal antibodies against affinity-purified GaR and then subjected to SDS-gel electrophoresis and autoradiography. In the absence of cAMP, there was little incorporation of ^{32}P into immunoprecipitates. Addition of cAMP analogs induced phosphorylation of a 57-kD peptide, and to a lesser extent, a 48-kD peptide. Similar results were obtained using purified mouse GaRs which were phosphorylated by the PKA catalytic subunit. The endogenous phosphorylation of native GaR peptides in the ATP-depleted cortical homogenates was markedly higher than that found with crude membranes, presumably due to a higher level of PKA in the former preparation. The in situ phosphorylation of both GaR peptides was time dependent and reached a maximum level in about 30 sec. Addition of PKA inhibitors (Walsh inhibitor peptide or H-89) abolished the cAMP-dependent phosphorylation. The 57-kD peptide is tentatively identified as a β subunit, since 47-kD and 51-kD peptides in cortical membranes were photolabeled using [^{3}H]flunitrazepam.

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487.7

Effect of Phosphorylation-dependent Rundown on $GABA_A$ Receptor Pharmacology. M. Gyenes, T. T. Gibbs*, & D. H. Farb, Dept. of Pharmacology & Experimental Therapeutics, Boston Univ. School of Medicine, Boston, MA 02118.

Repeated application of 30 μM GABA to cultured chick spinal cord neurons in the whole-cell voltage-clamp configuration resulted in a progressive decline, or "rundown" of currents induced by 30 μM GABA. In contrast, repeated application of 3 μM GABA did not elicit rundown, and responses to 3 μM GABA did not decrease even after induction of rundown by repeated application of 30 μM GABA. Although 3 μM GABA did not normally elicit run-down, run-down of the 3 μM GABA response was observed when the response was potentiated with 5α-pregnan-3α-ol-20-one (5α3α), or when alkaline phosphatase was added to the intracellular buffer. This result is consistent with a model in which $GABA_AR$ activation induces rundown by promoting $GABA_A$ receptor ($GABA_AR$) dephosphorylation. After rundown, there was a decrease in the maximum response to GABA, coupled with a leftward shift of the GABA EC_{50} from 16.7 μM to 6.4 μM. When ATPγS was present in the intracellular solution the decline in the maximum GABA-induced current was prevented and the shift in the EC_{50} was decreased (12.5 μM). Rundown was also associated with a decrease in the maximum percentage enhancement of the GABA response by positive modulators such 5α3α, progesterone, pentopbarbital, and chlordiazepoxide suggesting a decrease in the allosteric coupling of GABA and steroid recognition sites of the $GABA_AR$. In contrast, there was no change in the effects of negative modulators such as pregnenolone sulfate and Zn.

487.8

Protein Kinase C Inhibits Rundown of $GABA_A$ Receptor Currents in Mouse Cortical Neurons and Transfected L_{929} Cells. Y-F. Lin[1], L.J. Greenfield Jr.[2], M.D. Browning[3], and R.L. Macdonald[1,2], Depts of Physiology[1] and Neurology[2], U. of Michigan, Ann Arbor, and Pharmacology[3], U. of Colorado, Denver.

The $GABA_A$ receptor/chloride channel is an oligomeric polypeptide composed of homologous subunits (α,β,γ,δ), each containing four transmembrane segments (M1-M4) and a cytoplasmic loop between M3 and M4. The bovine γ_2 long (γ_{2L}) subunit includes in this loop a consensus sequence for phosphorylation by protein kinase C (PKC) and is phosphorylated by PKC *in vitro*. Phosphorylation by PKC may regulate $GABA_A$ receptor desensitization. During "whole cell patch" intracellular recording, GABA-induced currents gradually decrease in amplitude. This "rundown" is slowed by ATP and enhanced by alkaline phosphatase in the pipette medium, and may result from $GABA_A$ receptor dephosphorylation (Chen et al., J. Physiol. 420:207). We now report that intracellular PKC slows rundown of GABA currents in cultured mouse cortical neurons and L_{929} cells transfected with cDNAs encoding the bovine α_1, β_1 and γ_{2L} subunits. In cortical neurons, peak responses decreased 68% over 20 minutes in control cells, from 853 ± 151nA (mean ± SEM, n=10) to 275 ± 54nA, but only 21% in neurons treated with PKC (940 ± 225nA to 745 ± 209 nA, n=10). For transfected L_{929} cells, the normalized peak current in controls decreased to 56 ± 13% (n=7) at 12 minutes, while cells with PKC showed little decrement (97 ± 23%, n=5). These results suggest that the rate of $GABA_A$ current "rundown" is regulated by PKC, presumably by phosphorylation of the γ_{2L} subunit.

487.9

RAPID DESENSITIZATION OF THE $GABA_A$ RECEPTOR IN OUTSIDE-OUT PATCHES. J.J. Celentano*, and R.K.S. Wong. Dept. of Pharmacology, SUNY Health Science Center, Brooklyn, NY 11203.

Rapid desensitization of the $GABA_A$ receptor has been observed in $^{36}Cl^-$ flux studies ($t_{1/2}$=32 and 533 ms, Cash and Subbarao. Biochem 26:7556, 1987), but not in electrophysiological studies. We examined the effects of rapid GABA application on outside-out patches and found desensitization on the millisecond time scale. Patches were excised from acutely isolated pyramidal neurons of guinea pig hippocampus (Kay and Wong. J Neurosci Meth 16:227, 1986) using standard patch pipets (8-13 MΩ,). Extracellular buffer was (in mM): NaCl 145, $CaCl_2$ 1, $MgCl_2$ 1, glucose 25, Hepes 10, pH 7.4 NaOH, and intracellular buffer was: Tris HCl/ Tris base 130, NaCl 3, $MgCl_2$ 4, Na_2ATP 4, EGTA 10, HEPES 10, pH 7.3 methane sulfonic acid (final Cl^- 11 or 45 mM). Various GABA concentrations (in μM) were applied by means of a 7-barrel flow-tube (200 μm/barrel, 45 cm H_2O) capable of displacing the solution surrounding the patch pipet within 1.5 ms (determined by studying electrode junction potential changes). 1000 GABA typically induced outward currents of 50-150 pA (0 mV) which desensitized with biphasic kinetics (τ=44±5 ms, and 840±130 ms, n=20). 41% of the maximum response desensitized with rapid kinetics, and the total extent of desensitization (%D) was 91%. Over the concentration range of 3-3000 GABA, τ for the slow phase was about 1 sec, while %D increased with concentration from 50 to 95%. The fast phase was not observed at ≤50 GABA, and contributed to 35% of the response to 100 GABA (τ=64±11 ms, n=11). Responses to 300 and 3000 GABA desensitized with kinetics similar to that of 1000 GABA. These results indicate that the $GABA_A$ receptor is capable undergoing rapid desensitization which can result in an underestimation of the maximum GABA response thereby dramatically affecting EC_{50} and Hill slopes determinations. Experiments are currently underway to clarify these issues and to examine the effects of modulators on desensitization parameters.

487.10

$GABA_A$ RECEPTOR DESENSITIZATION: LACK OF MODULATION BY SPECIFIC PROTEIN KINASES AND PHOSPHATASES. D. J. Oh* and M. A. Dichter. Dept. of Neurology, University of Pennsylvania and Graduate Hospital, Philadelphia, PA 19104.

The $GABA_A$ receptor consists of multiple subunits which have consensus sequences for phosphorylation by various kinases, including PKA, PKC, and tyrosine kinase. Based on the structural homology between nACh and $GABA_A$ receptors, phosphorylation may modulate $GABA_A$ receptor desensitization. We studied various activators and inhibitors of protein kinases and phosphatases (listed below) with patch clamp techniques, after intracellular perfusion or extracellular application (DOG and OAG).

	Activators	Inhibitors
PKA (specific)	cAMP+IBMX, PKA	KT5720, PKI 5-24
PKC (specific)	DOG, OAG	Calphostin, PKC19-36
Non Specific Kinases		H7
Tyrosine Kinases		Genistein, Lavendustin A
Phosphatases	Alkaline phosphatase	Okadaic Acid Calyculin A
Tyrosine Phosphatase	EDTA, Spermine Citrate	Heparin Vanadate

These agents had no significant effects on $GABA_A$ receptor desensitization in cultured hippocampal neurons. Our data suggest that either phosphorylation does not regulate desensitization or a $GABA_A$ receptor-specific kinase may exist, analogous to β adrenergic receptor kinase.

PEPTIDES: RECEPTORS IV

488.1

ANGIOTENSIN II (AII) TYPE 2 (AT_2) RECEPTOR-MEDIATED EFFECTS ON POTASSIUM CURRENTS IN CULTURED NEURONS. J. Kang, C. Sumners* and P. Posner. Dept. of Physiology, Univ. of Florida Coll. of Med., Gainesville, FL 32610.

We have shown that AII increases net outward ionic current (I_{no}) in neurons from neonate rat hypothalamus and brainstem. This effect of AII is concentration-dependent, reversible, and mediated by AT_2 receptors. This study analyzed the stimulatory effect of AII on the K^+ component currents of I_{no}. In order to isolate K^+ current from I_{no}, neuronal cultures were superfused with a solution containing TTX (3 μM) and $CdCl_2$ (0.3 mM). The current recorded under this condition consists of I_K and I_A (I_{KTOT}). I_{Na}, $I_{Ca,K}$, and I_{Ca} are blocked. When the external K^+ concentration was changed, the amplitude of I_{KTOT} was changed. A linear relationship between the currents and log $[K^+]_o$ was fitted by the equation $I = g(V_m + 60\log([K^+]_{in}/[K^+]_o))$. ($V_m$ = +10 mV, $[K^+]_{in}$ = 135 mM; g does not change when V_m does not change.) The reversal potential of I_{KTOT} was approximately -90 mV when $[K^+]_o$ = 5.4 mM. The data generated by using pulse protocols to isolate I_K and I_A demonstrate that AII (10 nM) caused an increase in I_A and I_K in cultured neurons. To determine if this stimulatory effect of AII on I_{KTOT} was receptor-mediated, the selective nonpeptide AT_1 receptor blocker Losartan (DuP753) and the selective AT_2 receptor blocker PD123177 were tested. The increase in I_{KTOT} elicited by AII (10 nM) was antagonized by PD123177 (100 nM), but not by DuP753 (100 nM). Further studies of AII effects on I_{Ca} and $I_{Ca,K}$ showed no effects of AII on these currents. Thus, the enhancement of I_{no} by AII is mediated by the AT_2 receptor subtype through enhancement of I_K and I_A. (Supported by NIH Grant NS-19441).

488.2

REGULATION OF TYROSINE PHOSPHORYLATION BY ANGIOTENSIN II IN DIFFERENTIATED NG108-15 CELLS. M. D. Carrithers, M. N. Ghoneim, W. Schelman, and J. A. Weyhenmeyer*. Dept. of Cell and Structural Biology, University of Illinois College of Medicine, Urbana, IL 61801.

The neuroblastoma x glioma hybrid cell line, NG108-15, is used as a model system to study neuronal-glial type angiotensin receptors. When differentiated with 1.5% DMSO and low serum, these cells express both AT-1 and AT-2 receptors. Since both receptor types have been hypothesized to regulate cell growth, the present study was performed to determine the effects of angiotensin II (ANG II) on tyrosine phosphorylation in differentiated NG108-15 cells.

To determine regulation of tyrosine phosphorylation, cells were differentiated for four to five days and then incubated in the presence and absence of 10 nM ANG II for five minutes at 37°C. Cells were lysed and immunoprecipitated with a phosphotyrosine Ab (Oncogene Science). Samples were analyzed by SDS-PAGE followed by Western blotting. Blots revealed that ANG II stimulated phosphorylation of tyrosine residues in proteins of $M_r \cong$ 140, 30, and 27 kD. These responses were attenuated by pretreatment of the cells with 1 μM PD 123319, an AT-2 antagonist.

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488.3

GONADAL STEROIDS ALTER BRAIN ANGIOTENSIN II RECEPTORS IN OVARIECTOMIZED (ovx) RATS. K.L. Grove*, R.C. Speth, P.W. Sylvester, K.P. Briski, Dept. VCAPP, Washington State University, Pullman, WA 99164-6520.

The AT_1 receptor is the subtype expressed in most perihypothalamic nuclei (Rowe *et al., Eur. J. Pharm.* (1990) 186: 339). Previous studies of the effects of gonadal steroids on brain angiotensin II (AII) receptors are limited by the presence of sulfhydryl reducing agents (SHRA), which inhibits the AT_1 receptor subtype (Speth *et al., Brain Res.* (1991) 548:1).

Ovx rats, housed on a 12:12 light:dark cycle were treated with 20 μg of estradiol benzoate (EB) at 0900 h. on Day 1 and Day 4 of steroid treatment and injected with 2.0 mg of progesterone (P) on Day 5 at 1100 h. Animals were sacrificed at 1200, 1400 and 1500 hrs on Day 5. Using *in vitro* receptor autoradiography in the absence of SHRA with 500 pM ^{125}I-sar^1,ile^8 AII (± 3 μM AII) AII receptor expression in several hypothalamic nuclei was quantified. Plasma luteinizing hormone was measured by RIA. AII receptor binding in several perihypothalamic nuclei in the EB+P treated ovx female rat increased over time as follows:

Time(hr)	BSTV	SCh	Arc	ME
1200(n=6)	647±100*	2882±211*	405±43*	745±104
1400(n=6)	1014±72	3668±198	552±43	1084±164
1500(n=4)	1257±107	4184±574	589±89	878±130

Values are fmol/mg tissue ± S.E.M. BSTV-ventral portion of the bed n. of the stria terminalis; SCh-suprachiasmatic n.; Arc-arcuate n.; ME-median eminence. *Significantly different from 1400 and 1500 ($P < 0.05$).

This data suggests that AII receptors in brain areas regulating LHRH release increase prior to the gonadal steroid induced LH surge.

488.4

INCORPORATION OF CYTOSOLIC AT_2 ANGII RECEPTORS INTO MEMBRANES OF DIFFERENTIATED N1E-115 NEUROBLASTOMA CELLS. I.R. Siemens*, R. Mir, R.L. Soffer and S.J. Fluharty. Depts. of Animal Biology and Pharmacology, and Institute of Neurological Sciences, University of Pennsylvania, Phila., PA 19104 and Cornell University Medical Center New York, NY.

The murine neuroblastoma N1E-115 cell line possesses two subtypes of membranous receptors (AT_1 and AT_2) for the neuroactive peptide angiotensin II (AngII). Upon neuronal differentiation of these cells the density of the AT_2 receptor subtype is substantially increased while the density of the AT_1 receptors remains unchanged. Moreover, only a portion of this increase is prevented by transcriptional blockade suggesting the possible involvement of translational and post-translational processes. In this regard N1E-115 cells also possess a large pool of soluble cytosolic AT_2 binding proteins which require PCMS for binding activity. In the present study we have investigated the possibility that cytosolic AngII binding sites translocate to the membrane and contribute to the large increase of AT_2 receptors observed during cell differentiation. Binding experiments revealed that the increase in AT_2 receptors in differentiated membranes was proportional to the observed decrease in cytosolic binding. Antibodies (Ab_C) directed against cytosolic AngII binding sites immunoprecipitated 80% of cytosolic AngII binding proteins. The same antibody precipitated a small population of AT_2 receptors in crude CHAPS solubilized membranes. Partial purification of AngII receptors from solubilized membranes using heparin sepharose chromatography (HSC) resulted in elution of two major [^{125}I]-AngII binding peaks. The first peak eluted in the absence of any cations and contained approximately 80% of initial [^{125}I]-AngII binding activity. The second binding peak required 1.5 M NaCl for elution and contained 20% of initial [^{125}I]-AngII binding activity. Immunoprecipitation experiments showed that 90% of [^{125}I]-AngII binding activity in peak II could be precipitated with Ab_C, however, no immunoprecipitation was observed in peak I. Despite the high degree of immunoreactivity binding to peaks II was completely independent of PCMS. Collectively these data strongly suggest that cytosolic AngII binding sites can translocate into the cell membrane of differentiated N1E-115 cells by a posttranslational modification that may involve high affinity for HS and altered disulfide bridges. Supported by NS23986, HD25857 and MH43787.

488.5

REGULATION OF cGMP AND NITRIC OXIDE PRODUCTION BY ANGII AND CALCIUM IN NEURONAL CELLS. L.P. Reagan*, E.D. Zarahn and S.J. Fluharty. Depts. of Animal Biology and Pharmacology, and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

Angiotensin II (Ang II) elicits a rapid and dose-dependent increase in cGMP levels in murine neuroblastoma N1E-115 cells. We have recently demonstrated that this rise in cGMP levels is mediated by AT_1 and AT_2 AngII receptor subtypes. Moreover, the stimulatory effects of Ang II on cGMP formation were attenuated by the nitric oxide synthase (NOS) inhibitor *N*-monomethyl-L-arginine (NMMA). In the present report we have demonstrated that Ang II (100nM) elicits a 2-3 fold increase in the conversion of the NOS substrate [^{3}H]-arginine to [^{3}H]-citrulline, which indirectly demonstrates the production of NO in these cells. This Ang II-induced conversion was blocked by NMMA and also by the Ang II receptor antagonist Sarc^1-Ile^8 (SARILE). Moreover, the calcium ionophore ionomycin (10μM) caused a 10 fold increase in cGMP levels which was blockable by NMMA. On the other hand, sodium nitroprusside (1mM), which spontaneously releases NO and directly stimulates guanylate cyclase, increased cGMP levels, but this response was not blocked by NMMA. Finally, Ang II-induced cGMP production was attenuated by brief exposure to 5 mM EDTA, suggesting a possible role for extracellular Ca^{++} in this response. Collectively these results further demonstrate that the Ang II-induced increase in cGMP levels is mediated by NO, and that this response may require Ca^{++} from both intracellular and extracellular sources. Supported by NS23986 and MH43787.

488.6

INTERACTIONS BETWEEN AT_1 AND AT_2 ANGII RECEPTOR SUBTYPES DURING AGONIST-INDUCED DOWNREGULATION IN N1E-115 CELLS. A.K. Butler and S.J. Fluharty*. Departments of Animal Biology and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

In many of its target cells, angiotensin II (AngII) is known to regulate the density of its receptor *in vivo*. Recently, we have demonstrated that AngII produces a rapid decrease in AT_1 receptors and a more delayed decline in the AT_2 subtype in murine N1E-115 cells. In the present series of experiments we have examined the relative contribution of each AngII receptor subtype during agonist-induced downregulation. When AngII cells were exposed to AngII (10 nM for 20 min) their was a significant decrease in the density of both AT_1 and AT_2 receptors. Similarly, when cells were concurrently treated with AngII and CGP42112A (0.5 μM), a selective AT_2 receptor antagonist, comparable downregulation of both receptor subtypes was observed. In market contrast, when N1E-115 cells were treated with AngII in the presence of the AT_1 receptor antagonist, DuP753 (2 μM), there was no decline in either receptor subtype. In fact, both AT_1 and AT_2 receptors significantly increased after this combination of agonist and selective antagonist exposure. These results demonstrate that AngII interaction with AT_1 receptors is necessary for downregulation of both subtypes, and that selective stimulation of AT_2 receptors appears to result in an upregulation of AngII receptors. As much, these data suggest that reciprocal interactions between AT_1 and AT_2 receptor subtypes may occur during agonist-induced downregulation in neuron-like cells. Supported by NS23986 and MH43787.

488.7

BIOCHEMICAL CHARACTERIZATION OF THE AFFINITY PURIFIED AT_2 ANGIOTENSIN II RECEPTOR FROM MURINE NEUROBLASTOMA N1E-115 CELLS. P. He, I.R. Siemens, M.K. Raizada, M.M. White* and S.J. Fluharty. Depts. of Animal Biology and Pharmacology, and Institute of Neurological Sciences, University of Pennsylvania, Phila., PA 19104 and Dept. of Physiology Univ. of Florida, Gainesville, FL.

Differentiated murine neuroblastoma N1E-115 cells posses both subtypes (AT_1 and AT_2) of membrane associated angiotensin II (AngII) receptors, which are similar in specificity, affinity, and molecular weight to those in rat brain. Functional solubilization of N1E-115 cell membranes with the detergent CHAPS exclusively solubilizes AT_2 receptors while AT_1 receptors remain in the 105,000 x g pellet. Covalent cross-linking of [^{125}I]-AngII to solubilized AngII receptors with the homobifunctional cross-linker DSS specifically labeled two proteins, one minor protein of 102 kDa and one major protein of 69 kDa molecular weight. Affinity purification of solubilized AngII receptors with AngII affinity columns resulted in elution of one high molecular weight band (110 kDa) and one low molecular weight band (66 kDa) when excess amounts of agonist were used to elute the proteins from the column. In contrast, when proteins were eluted with an excess amount of an antagonist only one protein band eluted from the column with a molecular weight of 66 kDa. Covalent cross-linking of [^{125}I]-AngII to affinity purified proteins derived from both sets of experiments resulted in specific labeling of one high (102 kDa) and one low molecular weight band (66 kDa) in the presence of an agonist, but only one low molecular weight band (66 kDa) was labeled when an antagonist was used. The single 66 kDa protein was not immunoreactive with antibodies directed against AT_1 receptors. Moreover, proteolytic digestion of the affinity purified protein resulted in peptide fragments whose amino acid sequences were not homologous with cloned AT1 receptors. Supported by NS23986 and MH43787.

488.8

CORTICOSTEROID REGULATION OF PLC-α IN RAT BRAIN AND CULTURED NEURONAL CELLS. R. Maki*, P. He, D.M. Zhang, J.R. Williamson and S.J. Fluharty. Depts. of Animal Biology and Biochemistry, and Institute of Neurological Sciences, University of Pennsylvania, Phila., PA 19104.

Many of the cellular actions of angiotensin II are mediated by phosphoinositide (PI) hydrolysis and the attendant mobilization of intracellular Ca^{2+}. Recently, we have reported that AngII receptors are coupled to a 60 kDa PI-specific phospholipase C, PLC-α in cultured neuronal cells. In the present study, we have used two-dimensional (2-D) gel electrophoresis to the examine the distribution of immunoreactive PLC-α in rat brain, as well as its regulation by corticosteroids. The PLC-α antibody was raised against a 60 kDa protein purified from rat liver. The N-terminal amino acid sequence of this protein corresponded to the sequence of PLC-α purified from guinea pig uterus. Rats were injected either with aldosterone (ALDO - 250 ug/day) or corticosterone (CORT - 50 ug/day). Control animals were injected with a propylene glycol vehicle. After 3 days, cerebellum, cerebral cortex, hippocampus, amygdala, anteroventral third ventricle (AV3V), and pituitary were dissected and proteins were analyzed by 2-D gel electrophoresis followed by immunoblotting with PLC-α antisera. The results indicated that PLC-α was present in each of the brain regions examined. Moreover, in brain the antisera detected two proteins of 60 and 70 kDa with different pI values, whereas in N1E-115 cells only the 60 kDa species was detected by the antisera. In AV3V, medial amygdala or pituitary, but not other brain regions, ALDO or CORT altered the pI value of both the 60 and 70 kDa protein, and the relative amount of immunoreactivity appeared to change in cultured cells as well. The physiological relevance of steroid regulation of neuronal PLC-α remains to be determined. Supported by NS23986, HD25857 and MH43787.

488.9

SOME NEUROPHARMACOLOGICAL PROPERTIES OF PD 123319, A NON-PEPTIDE ANGIOTENSIN II AT_2 RECEPTOR LIGAND. T.A. Pugsley*, S. Whetzel and T. Heffner. Parke-Davis Pharmaceutical Research Div., Warner-Lambert Co., Ann Arbor, MI 48105.

Angiotensin (Ang II) is a peptide hormone that regulates many physiological functions including blood pressure, fluid and electrolyte homeostasis and neuronal activities of various target cells. Two types of receptors for Ang II have been described, AT_1 and AT_2. While both subtypes bind Ang II and its analogs with comparable affinity, AT_1-1 receptors have high affinity for DuP 753, and AT_2 receptors have high affinity for PD 123177, PD 123319 (PD) and CGP 42112A. Ang II has been reported to interact with cholinergic and dopaminergic neurotransmitter systems. We investigated the AT_2 ligand PD for its effects on cholinergic and related indicies in rat brain. PD (1-60 mg/kg, i.p.) given 30 min prior to sacrifice caused a dose dependent decrease of acetylcholine (Ach) content in rat striatum. This effect lasted for up to 6 h after a 30 mg/kg dose of PD. In contrast the AT_1 receptor ligand DuP 753 was inactive in altering Ach levels at 30 mg/kg, in rat striatum. The effect was not selective to striatum as a significant decrease of Ach was observed in hippocampus at 30 mg/kg of the agent. PD in vitro did not alter high affinity choline uptake; thus it is unlikely to be causing the decrease in Ach levels by blocking choline uptake, suggesting that it may be releasing Ach. Basal cerebellar cGMP levels [a well characterized second messenger response modulated by the N-methyl D-aspartate (NMDA) receptor complex] were decreased by PD at 30, but not at 3 and 10 mg/kg. These results suggest that in vivo PD may alter cholingeric function and NMDA receptor complex mediated responses.

488.10

INHIBITION OF SODIUM APPETITE BY OXYTOCIN IN FEMALE BUT NOT IN MALE RATS. C. Polidori*, P. Pompei, M. Massi, A.N. Epstein and R.R. Sakai. Department of Biology, University of Pennsylvania, Philadelphia, PA 19104 and Lab. of Neuroendocrinology, The Rockefeller University, New York, NY 10021.

Sodium intake is sexually dimorphic in rats. Female rats drink more than male rats when they are sodium depleted (sodium appetite) and when they are sodium replete (need-free sodium intake)(Chow, et al. 1992). Oxytocin (OT), a peptide hormone secreted by the paraventricular and supraoptic nucleus of the hypothalamus, has been correlated with sodium intake in rats. We first tested OT's effects on furosemide induced sodium depletion (10mg/rat/sc) both in male and female rats and secondly on need-free sodium intake. After sodium depletion a pulse intracerebroventricular (pICV) injection of OT (1μg/μl) reduced the sodium appetite in female rats by 65% and by 20% in male rats. Similarly, female rats reduced their need-free sodium intake by 75% after a pICV injection of OT (1μg/μl). This inhibition of need-free intake was blocked by prior injection of the selective OT antagonist OTA (1μg/μl). In summary, these results suggest that central OT is more effective in female than in male rats and that OT may play a role in the expression of need-free and need-induced sodium intake in female rats. Supported by National Inst. of Neurological Disorders and Stroke NS-03469 and MH 43787.

488.11

CHARACTERIZATION OF GUANINE NUCLEOTIDE EFFECTS ON ^{125}I-ENDOTHELIN BINDING IN RAT BRAIN AND HEART. L. Yu, A. Wu, N. Davis, L.C. Wince* and R.A. Colvin. Biological Sci. Dept., Ohio University College of Osteopathic Medicine, Athens, OH 45701

We characterized the binding of ^{125}I-Endothelin (ET-1) to purified plasma membranes from whole rat brain and heart. Binding reactions were done in 5 ml of 137 mM choline Cl, 10 mM HEPES (pH 7.4), 0.1% BSA, and the following protease inhibitors: soybean trypsin inhibitor (50 μg/ml), leupeptin (0.5 μg/ml) and pepstatin (0.7 μg/ml). To distinguish ET receptor subtypes we performed displacement curves using 0.2 nM ^{125}I-ET-1 and increasing concentrations of either ET-1 or ET-3. Rat heart plasma membranes showed high affinity binding for ^{125}I-ET-1 that was only displaced by ET-1 (IC_{50} = 0.15 nM). In contrast, ^{125}I-ET-1 binding in rat brain was equally displaced by either ET-1 or ET-3 (IC_{50} = 0.2 nM). Alamethicin (50 μg/tube) was shown to increase ^{3}H-ouabain binding to rat brain (40%), but had no effect on ^{125}I-ET-1 binding. Alamethicin was included in assays to characterize the effects of added $MgCl_2$ and guanine nucleotides. Scatchard analysis showed no effect of 10 mM $MgCl_2$ on ^{125}I-ET-1 binding Kd and Bmax (0.17 nM, 0.14 nM; 0.19 pmol/mg, 0.18 pmol/mg; with or without $MgCl_2$ respectively). Increasing concentrations of either GTPγS or GDPβS had no effect on ^{125}I-ET-1 binding to rat brain. Similarly, GDPβS (100 μM) had no effect on ^{125}I-ET-1 Kd or Bmax when assayed in either rat brain or rat heart. In conclusion, both ET-A (heart) and ET-B (brain) receptors exist in a high affinity state in purified plasma membranes, which is not affected by the presence of $MgCl_2$ or guanine nucleotides such as GTPγS and GDPβS.

488.12

USE OF SELECTIVE ANTAGONISTS TO SUBSTANTIATE THE EXISTENCE OF A B_2-KININ RECEPTOR SUBTYPE IN SPINAL CARDIOVASCULAR CONTROL. P. Lopes[1], D. Regoli[2], M. Thakur[1]* and R. Couture[1]. [1]Dept. Physiology, Faculty of Medicine, Université de Montréal, Montréal, Canada H3C 3J7 and [2]Dept. Pharmacology, Faculty of Medicine, Sherbrooke University, Sherbrooke, Canada J1H 5N4.

A role for kinins has been suggested in spinal cardiovascular regulation and autoradiographic studies have demonstrated the presence of B_2-kinin receptors in the rat spinal cord. In this study, selective antagonists were used to further characterize the receptor subtype involved in the intrathecal (i.t.) action of bradykinin (BK) on mean arterial pressure (MAP) and heart rate (HR) of the conscious rat. The i.t. injection of BK (81 pmol at T-9) elicited a transient increase of MAP and a longer lasting decrease of HR. The cardiovascular response to BK was significantly and dose-dependently inhibited by the prior i.t. injection (717-800 pmol, 3-5 min earlier) of three B_{2B} receptor antagonists (D-Arg[Hyp^3,D-Phe^7,Leu^8]-BK, D-Arg[Tyr^3,D-Phe^7,Leu^8]-BK, Tyr,D-Arg[Hyp^3,D-Phe^7,Leu^8]-BK) but remained unaffected by pretreatment with similar doses of antagonists for the B_{2A} receptor (D-Arg-[Hyp^3,Leu^8]-BK, D-Arg-[Hyp^3,Gly^6,Leu^8]-BK) or the B_1 receptor ([Leu^8]desArg^9-BK). Similarly, D-Arg[Hyp^3,Gly^6,D-Phe^7,Leu^8]-BK, D-Arg[Hyp^2,Thi^{5,8}, D-Phe^7]-BK, two non selective antagonists for B_{2A} and B_{2B} receptors, failed to antagonize the cardiovascular responses to BK and displayed agonistic activities at higher doses. Doses 10-fold higher (7.7 nmol) of Hoe 140, a potent antagonist for peripheral B_2 receptors, were required to inhibit the response to BK. These results suggest that BK may affect the cardiovascular system by acting on a B_{2B} receptor subtype in the rat spinal cord. [Supported by the MRC of Canada].

488.13

CHARACTERIZATION OF NON-OPIOID [^{3}H]-DYN A-(1-13) BINDING SITES IN RAT HEART MEMBRANE PREPARATIONS. M. Dumont* and S. Lemaire. Department of Pharmacology, University of Ottawa, Ottawa, Ontario Canada K1H 8M5.

In the brain, Dynorphin A (DYN A) interacts with both opioid and non-opioid receptors, the stimulation of the non-opioid receptor resulting in a loss of tail flick reflex and hindlimb paralysis. In the heart, DYN A also induces non-opioid effects such as a positive inotropic effect and cardiac arrhythmia. Using membrane binding techniques, we have characterized the cardiac non-opioid DYN A receptor using [^{3}H]-DYN A-(1-13). Binding assays were performed with two ml-aliquots of membrane (0.8 mg protein) in 5 mM Tris-HCl (pH 7.4); 0.2% BSA at 4°C for 120 min followed by filtration through polyethylenimine treated glass microfiber filters. [^{3}H]-DYN A-(1-13) binding sites were sensible to trypsin and Scatchard analysis yielded linear plots with a K_d of 56.5 nM and a B_{max} of 1.91 pmol/mg protein. In competition experiments, [^{3}H]-DYN A-(1-13) binding was displaced by DYN A-(1-13), DYN A and DYN A-(2-13) but not by DYN A-(1-8) and levallorphan. [^{3}H]-DYN A-(1-13) binding was insensitive to σ (DTG, (+)-3 PPP, (+)-SKF-10047) and PCP (TCP, MK-801) ligands. These results suggest that [^{3}H]-DYN A-(1-13) labels a non-opioid DYN A receptor in the heart which upon stimulation may lead to cellular damage as seen in myocardial ischemia. Supported by HSFO.

488.14

PRESENCE OF FUNCTIONAL CORTICOTROPIN-RELEASING HORMONE RECEPTORS IN HUMAN Y-79 RETINOBLASTOMA CELLS. Maria C. Olianas* and Pierluigi Onali, Department of Neurosciences, University of Cagliari, Cagliari, Italy.

In human Y-79 retinoblastoma cells corticotropin-releasing hormone (CRH) produces a marked (60-fold) and rapid increase of adenylyl cyclase activity. The concentrations of the peptide producing half-maximal (EC50) and maximal stimulations are 60 nM and 1-5 µM, respectively. The effect of CRH is GTP - dependent, being minimal in the absence of added nucleotide and maximal at 10 µM GTP. The specific CRH receptor antagonist α-helical CRH 9-41 competitively counteracts the CRH stimulation with a Ki value of 80 nM. Sauvagine and urotensin I, two peptides displaying sequence homology with CRH and high affinity for CRH receptors, mimick the effect of CRH with EC50 values of 10 and 11 nM, respectively. These results demonstrate the presence of functional CRH receptors in human Y-79 retinoblastoma cells and suggest that this cell line may be a suitable model in which to study the action of CRH on human retinal cell function.

488.15

CORTICOTROPIN RELEASING HORMONE (CRH) RECEPTORS INVOLVED IN MEDIATING FEAR IN THE RHESUS MONKEY. N.H. Kalin,[#] D.E. Grigoriadis,[2] E.B. DeSouza,[2] J.E. Turner,[1] G.W. Dent,[2] S.E. Shelton,[1] and H. Uno.[3] [1]Dept. of Psychiatry, Univ. of Wisconsin-Madison, WI; [2]Dupont-Merck, Wilmington, DE; [3]Wis. Regional Primate Center, Madison, WI.

CRH systems are involved in integrating various aspects of the stress response. CRH receptors in the pituitary mediate stress-induced ACTH release, whereas receptors in other brain regions likely mediate fear-related behavioral and autonomic responses. The distribution and density of CRH receptors has been studied extensively in rats, but little work has been done in primates. We examined the regional distribution of CRH receptors in adult rhesus monkeys (Macaca mulatta). Pituitary had the highest receptor density (302 fmol/mg protein). In brain, amygdala and cerebellum had significantly more CRH receptors than cortex, caudate, hippocampus, and hypothalamus. Scatchard analyses revealed similar binding affinities in pituitary, amygdala, and cortex (0.169-0.347 nM). The high density of receptors in pituitary and amygdala is interesting because these sites are major mediators of endocrine and behavioral changes associated with fear. Previously we showed that the pituitary-adrenal response in rhesus monkeys is not mature until animals are 12 weeks old. Quantitative autoradiographic studies were performed in pituitary and discrete brain regions from 2- and 12-week-old monkeys. In pituitary, no significant age-related differences were found. CRH receptors were evident in anterior and intermediate but not posterior lobes, and their density in the intermediate lobe was greater than in the anterior lobe. Binding sites in the anterior lobe had a "cluster-like" distribution similar to that of corticotropes. CRH receptors in the intermediate lobe were more uniformly distributed, reflecting the distribution of opiomelanocortin-producing cells in this region. Data from other brain regions will be presented.

488.16

HYPOTHALAMIC LOCALIZATION OF PROLACTIN AND OPIOID BINDING SITES IN A MALE MIGRATORY SONGBIRD. P. Deviche* and J.D. Buntin. Inst. Arctic Biology, Univ. Alaska Fairbanks, Fairbanks, AK 99775 and Dept. Biolog. Sciences, Univ. Wisconsin, Milwaukee, WI 53201.

Studies on several avian species have demonstrated that prolactin (PRL) and opioids (OP) regulate feeding, but neither the mechanisms nor the sites involved are identified. To address this question, we measured the density of specific ^{125}I-ovine PRL and ^{3}H-DPDPE (a delta receptor ligand) binding sites in brain regions of adult male dark-eyed juncos (*Junco hyemalis*) by *in vitro* autoradiography. We found that specific PRL and OP binding sites are present in several hypothalamic regions (PRL: infundibulum (INF) > ventromedial hypothalamus (VMH) > paraventricular nucleus (PVN) > lateral hypothalamus (LHy); OP: PVN > INF > VMH > LHy). Some regions (VMH; LHy) of the avian brain that contain a high density of PRL and OP binding sites control feeding behavior. Thus, these regions may constitute sites of action on this behavior of PRL or of a PRL-like molecule, and also of endogenous OP.

488.17

IMMUNOHISTOCHEMICAL LOCALIZATION OF SP RECEPTOR (SPR) IN RAT BRAIN. C.L. Veenman*, L. Medina, A. Reiner, C. Crankshaw and J.E. Krause. Dept. Anat. & Neurobiol., Univ. TN, Memphis, TN 38163 and Dept. Neurobiol. & Anat., Wash. Univ., St. Louis, MO 63110.

Five antisera against segments of rat SPR (88-T4 against the third cytoplasmic loop; 9323 against the second extracellular loop; and A2P5, 81-7, 80-X against third extracellular loop regions) were used to examine the distribution of SPR in rat brain. The specificity of all antisera was confirmed by solution phase binding, ELISA and/or immunoprecipitation experiments. All antisera extensively labeled the perikaryal and proximal dendritic membranes of a population of neurons in layer 5 of isocortex and in the subiculum. Double-labeling showed that some of these neurons contained parvalbumin, but none contained calbindin or somatostatin. A2P5 and 88-T4 also showed granular labeling on or within neurons in deep cortical layers, pallidum, basal forebrain and substantia nigra pars reticulata (SNr). A2P5 also showed fine granular labeling of structures the apparent size of terminals, particularly in the neuropil of superficial cortical layers, in medial striatum and patches throughout striatum, in globus pallidus, entopeduncular nucleus, thalamic reticular nucleus, superficial layers of the superior colliculus and in SNr. Labeling with A2P5 and 88-T4 could be blocked with synthetic antigen. These results are largely consistent with previous descriptions of SPR distribution based on ligand binding, with the major exception that they do indicate the presence of SPR in SNr. Supported by NS-28721 and NS-19620 (AR), the Spanish Ministry of Education (LM) and NS-21937 (JEK).

PEPTIDES: PHYSIOLOGICAL EFFECTS IV

489.1

MODULATORY ACTIONS OF SEVERAL NEWLY IDENTIFIED LOCUST NEUROPEPTIDES ON LOCUST NEUROMUSCULAR PREPARATION. M. Schiebe* and L. Schoofs. Abt.Angew. Physiol., U of Ulm, D-7900 Ulm, Germany and KU Leuven, B-3000 Leuven, Belgium.

Several modulatory actions of the invertebrate FMRF-NH2 and related peptides on tension generated in the extensor tibiae muscle of the locust hindleg by stimulation of the slow excitatory motor neurone (SETi) are now well established. Here we demonstrate effects of four newly sequenced neuropeptides isolated from the locust which are unrelated to FMRF-NH2. The following parameters were examined: Tension, contraction rate, relaxation rate, E_m and ejp amplitude. The preparations were constantly perfused at 1.4ml/min and kept at 20° C. Peptides were applied as 100 µl aliquots into the superfusion line were they were diluted to a final concentration of $5*10^{-7}$ M. All peptides were isolated from extracts of brain complexes of Locusta migratoria. The following observations were made: 1) Locustatachykinin II (APLSGFYGVR-NH2), increased tension, the relaxation rate, ejp amplitude and could induce a contracture at a stimulation frequency of 0.3 Hz. Effects outlasted the presence of the peptide in the superfusate by several minutes. 2) Locustatachykinin III (APQAGFYGVR-NH2) had identical effects. 3) Locustamyotropin III, a peptide belonging to a separate family of locust peptides which is supposed to stimulate locust oviducts in vitro, had short-lasting, but dramatic inhibiting effects on tension in this preparation, accompanied by a reduction in ejp amplitude and a small depolarisation. 4) Locustamyoinhibiting hormone (AWQDLNAGW-NH2) which suppresses contractions of the hindgut and oviduct of locusts also had inhibiting effects on muscle, similar to Locustamyotropin III but at a higher dose (10^{-6}M). The results of these experiments suggest, that the hindleg of the locust may be subject to complex modulatory actions of different families of peptides. It is as such and as a experimental model particularly useful.

489.2

PEPTIDE IMMUNOREACTIVITY IN THE GENITAL GANGLION OF *APLYSIA*.
S. B. Moffett* Dept. of Zoology, Washington State University, Pullman, WA 99164.

The roles of bag cells and some other CNS neurons in *Aplysia californica* reproduction are well established, but neurons of the genital ganglion, located at the juncture of the genital nerve with the sperm-oviduct, may also play a role. Indirect immunofluorescence in wholemounts of the ganglia of young animals (30-50g) revealed intense immunoreactivity to *Mytilus* inhibitory peptide (MIP) in a quarter of the cells and SCPb-like immunoreactivity in a non-overlapping population. MIP varicosities are densely distributed on the sperm-oviduct and ovotestis as well as in baskets of endings surrounding some non-immunoreactive cells. Antibodies generated against both catch relaxing peptide (CARP) and buccalin reveal several axons which enter the ganglion and project toward the ovotestis. The strategic position of the genital ganglion neurons and the inhibitory role that some of the peptides associated with the ganglion play in muscle contraction in other systems (Kobayashi and Muneoka, 1990, Zool. Sci. 7:801; Kiss, 1991, Comp. Biochem. Physiol. 95C:207) suggest that genital ganglion neurons and bag cells may interact in coordinating egg-laying behavior.

489.3

CONANTOKIN GV, THE "SLEEPER" PEPTIDE, PRODUCES HYPERACTIVITY IN ADULT RATS. K.A. Serpa* and L.T. Meltzer. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105

Conantokin GV (C-GV) is a neuropeptide isolated from the marine snail Conus geographus. C-GV was originally isolated on the basis of its ability to induce a sleep-like state in young mice following ICV injection (Olivera et al., Toxicon 23: 277, 1985). In older mice, C-GV produced hyperactivity or "sleepy climber" activity, in which the hyperactivity alternated with periods of behavioral inactivity. We examined the effects of ICV injection of C-GV in adult rats implanted with EEG and EMG electrodes. Animals were placed in an isolation chamber lit with a red light and connected to a swivel to allow free movement. Behavioral effects were visually assessed. C-GV 0.11 nmol/rat had no effect on sleep or behavior. C-GV 1.1 nmol/rat induced hyperactivity as well as ataxia. The EEG during this time appeared to be of greater amplitude than normal awake EEG, with overriding high amplitude waves, and could not be scored for sleep-awake stages. 5 of the 8 rats exhibited seizure-like EEG. The behavioral effects of the high dose appear similar to those seen in adult mice.

489.4

HELODERMIN EVOKED RELEASE OF ENDOGENOUS ACETYLCHOLINE FROM GUINEA PIG LONGITUDINAL MUSCLE-MYENTERIC PLEXUS (LM-MP) PREPARATIONS. P.K. CHIANG*, R.R. GRAY, C.B. REAVES, and R.K. GORDON. WALTER REED ARMY INSTITUTE OF RESEARCH, DIVISION OF BIOCHEMISTRY, WASHINGTON DC 20307-5100.

Vasoactive intestinal peptide (VIP) and related peptides are distributed in the CNS and peripheral nervous systems, and are found in high concentrations in the intestines. We previously showed (Arch. Int. Pharmacodyn. 305:14, 1990) that two VIP analogs induced a concentration-dependent contraction of guinea pig ileum, and that these analogs also induced a concentration-dependent secretion of endogenous acetylcholine (ACh). We now demonstrate that one peptide component of the venom of the Gila monster lizard *Heloderma suspectum*, which has a high homology to VIP, induced the release of [^{3}H]ACh from a LM-MP preparation of guinea pig ileum. The helodermin toxin was approximately equal in potency to the most effective VIP, and the maximum evoked release of [^{3}H]ACh was at 10 µM of each peptide. The evoked [^{3}H]-ACh released was ≈ 0.3% of the total content of tritium in the tissue, which contrasts with 20 µM nicotine inducing ≈ 0.4%, and 25 mM KCl inducing ≈ 5%. These results show that helodermin toxin resembles VIP in specificity and ability to bind to receptors in LM-MP preparations which cause the secretion of endogenous ACh.

489.5

ATRIAL NATRIURETIC FACTOR INJECTED INTO RAT SUBFORNICAL ORGAN BLUNTS VASOPRESSIN RELEASE INDUCED BY ANGIOTENSIN II. L. Steardo*, M. Iovino§, J. Perez, N. Brunello and G. Racagni. Center of Neuropharmacology, Institute of Pharmacological Sciences, University of Milan, Via Balzaretti 9, 20133 Milan and §Department of Neurology, 2nd Medical School, University of Naples, Via Pansini 5, 80131 Naples, Italy.

The recently discovered atrial peptides are thought to be importantly involved in controlling body fluid homeostasis, both in animals and in humans. The site and mechanism by which these peptides, collectively indicated as Atrial Natriuretic Factor (ANF), regulate salt–water balance have been the subjects of intensive research over the last few years. ANF has been recognized in the brain. Its receptor sites have been identified in close vicinity to those of angiotensin II in circumventricular organs, such as subfornical organs (SFO) and the organum vasculosum laminae terminalis (OVLT). In the light of this close anatomical association, it has been possible to speculate that both peptides may interact with each other to regulate salt–water balance. Therefore to test this hypothesis, the effect of ANF given into SFO on Ang II induced vasopressin release in rats has been investigated. ANF in dose dependent manner has been able to attenuate the enhancing effect of peripherally injected Ang II (192 μg/Kg/min) on plasma vasopressin levels. These findings support the hypothesis that ANF influences the Ang II effecton fluid balance and they indicate SFO as one of the main sites at which this interaction occurs.

489.6

ANGIOTENSIN II_1 RECEPTOR ANTAGONISTS BLOCK ANGIOTENSIN-INDUCED EXCITATION OF HYPOTHALAMIC PARAVENTRICULAR AND SUBFORNICAL ORGAN NEURONS IN RAT BRAIN SLICE. Z. Li and A.V. Ferguson*, *Dept. of Physiology, Queen's University, Kingston, Ont. K7L 3N6*

High densities of angiotensin (ANG) II receptors have been revealed within the hypothalamic paraventricular nucleus (PVN) and the subfornical organ (SFO) of the rat. We have recently shown that intravenous losartan (Dup-753), a nonpeptide antagonist of ANG II_1 (AT_1) receptors attenuates systemic ANG-induced increases in neuronal activity in PVN, and blocks the elevation in blood pressure elicited by electrical stimulation of SFO.

Extracellular single unit recordings were made from 79 PVN and 54 SFO neurons in brain slices (350 μm) of adult male Sprague-Dawley rats (140-220 g). Spontaneous activity of these groups of neurons was 1.72±0.14 Hz (MEAN±SEM) and 3.31± 0.25 Hz, respectively. Bath application of ANG II ($7x10^{-9}$-$7x10^{-8}$ M) for 2-5 min increased firing in 40 (50.6%) PVN neurons, and 29 (53.7%) SFO neurons tested. The mean activity of PVN neurons was increased by 105.2%, while that of SFO neurons was increased by 242.3%. The effects of losartan on ANG II responsiveness of 12 PVN and 14 SFO cells was tested. Bath administration of losartan (10^{-6}-10^{-4}M, 4-5 min) abolished the excitatory effects of ANG II on all PVN (95.6±7.4% reduction in response), and 92.9% (92.9±12.2% reduction in response) of SFO, neurons. Partial recovery of excitatory neuronal responses to ANG II was observed when low concentrations of this antagonist were applied. The ability of PD-123319, an AT_2 antagonist, to block neuronal responses to ANG II was also evaluated and excitatory effects of ANG II were blocked by this drug in one of 5 cells tested. These results demonstrate that losartan can directly block ANG II-induced neuronal excitation on the PVN and the SFO and suggest the predominant existence of AT_1 receptors within these nuclei.

489.7

NEUROENDOCRINE, BEHAVIOURAL AND CARDIOVASCULAR RESPONSES TO CORTICOTROPIN-RELEASING HORMONE INTO THE CENTRAL AMYGDALA IN STRESS-FREE AND STRESS SITUATIONS. A. Wiersma, A.D. Baauw, S.F. de Boer*, B. Bohus and J.M. Koolhaas. University of Groningen, Dept. of Animal Physiology, P.O.BOX 14, 9750 AA Haren, The Netherlands.

The central nucleus of the amygdala (CEA) is known to be involved in the regulation of the parasympathetic and passive coping response to conditioned and acute stressors. Neuroanatomical studies revealed that the majority of the corticotropin-releasing hormone (CRH) containing neurons in the CEA have direct connections with autonomic regulatory nuclei in the brainstem. A 7-min infusion of 30 ng CRH (in 1 μl CSF) into the CEA of freely moving male Wistar rats under stress-free conditions, led to an increase in heart rate, without changes in plasma noradrenaline (NA), adrenaline (A), and corticosterone. The CRH-induced tachycardia was effectively blocked by pretreatment with 1 μg α-hCRH, a receptor antagonist of CRH. Both CRH infusion and pretreatment with α-hCRH caused a slight behavioural activation. However, administration of α-hCRH alone did not induce a behavioural activation. The shock-probe defensive burying test was used to examine the conditioned stress response. The results show that CRH infusion in the CEA failed to affect heart rate in a conditioned stress test. The behavioural activity showed a remarkable response towards a more active (sympathetic) behavioural response after CRH infusion, which was blocked by pretreatment of α-hCRH.

The present results suggest that CRH induces a reduction of the parasympathetic output systems and passive coping strategies. As CRH is given at the level of the cell body of the CRH neurons in the CEA, the results can be explained as an autoreceptor mediated inhibition of CRH connections from the CEA with parasympathetic brainstem nuclei.

489.8

BOMBESIN EXCITES NEURONS IN HYPOTHALAMIC ARCUATE AND SUPRACHIASMATIC NUCLEI IN BRAIN SLICES. J.T. Pan*, J.Y. Lin and K.C. Teng. Inst. of Physiology, Natl. Yang-Ming Med. Coll., Taipei, Taiwan 11221, R.O.C.

Both bombesin, a putative peptide neurotransmitter, and its receptors have a wide and extensive distribution in the hypothalamic areas, including the arcuate (ARC) and the suprachiasmatic (SCN) nuclei. A total of 70 ARC and 174 SCN neurons were recorded and tested for bombesin in brain slices. Over 75% of ARC neurons were excited by application of 0.5 nmol of bombesin into the slice chamber. The effects of bombesin on SCN neurons, however, can be differentiated between neurons with different firing patterns, viz. it excited 75% of irregular firing neurons (n=113), while it only excited 17% and inhibited 34% of regular firing neurons (n=61). A dose-dependent effect of bombesin (from 0.005 to 5 nmol) on SCN neurons was also demonstrated. In 25 SCN neurons tested for both bombesin and gastrin-releasing peptide, 24 showed similar responses (67% were excited by both peptides). Pretreatment with Leu^{13}-(CH_2NH)Leu^{14}]-bombesin, a bombesin receptor antagonist, blocked 67% of the bombesin effect in SCN neurons. The data implicate that bombesin may play a significant role in the regulation of selective SCN and ARC neurons.

489.9

CRF STIMULATES CATECHOLAMINE RELEASE IN RAT MEDIAL PREFRONTAL CORTEX AND MEDIAL HYPOTHALAMUS, ASSESSED BY MICRODIALYSIS. Jan Lavicky, R. Don Brown*, and Adrian J. Dunn. Department of Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130-3932.

In vivo microdialysis was used to measure changes of extracellular concentrations of catecholamines in freely moving rats in response to administration of corticotropin-releasing factor (CRF). With dialysis probes in the medial hypothalamus, intracerebroventricular (icv) administration of CRF (17 or 330 pmoles) dose-dependently increased dialysate concentrations of norepinephrine (NE), dopamine (DA), and all their measurable catabolites except normetanephrine (NM). Dialysate concentrations of serotonin could not be measured reliably, but those of its catabolite, 5-hydroxyindoleacetic acid (5-HIAA) were also elevated. DA and NE increased within the first two 20 min collection periods, and returned to baseline within 3 h. Similar data were obtained with dialysis probes in the medial prefrontal cortex after 17 or 167 pmoles of CRF icv. IP administration of CRF (1 nmole) similarly elevated dialysate concentrations of NE, DA, 5-HIAA, and all catecholamine catabolites except NM in both the medial hypothalamus and the medial prefrontal cortex. These results support earlier neurochemical data suggesting that CRF administered either centrally or peripherally stimulates the release of both DA and NE in the brain.

Supported by a grant from NINDS (NS 27283)

489.10

EFFECT OF GALANIN ON TUBEROINFUNDIBULAR DOPAMINERGIC NEURONAL ACTIVITY AND PROLACTIN SECRETION IN MALE AND FEMALE RATS **C. GOPALAN*, Y. TIAN, K.E. MOORE and K.J. LOOKINGLAND. Dept. of Pharm/Tox., Michigan State Univ., E.Lansing, MI 48824.**

Tuberoinfundibular dopaminergic (TIDA) neurons terminating in the median eminence tonically inhibit the secretion of prolactin from the anterior pituitary. Prolactin, in turn, stimulates the release of dopamine from TIDA neurons and thereby regulates its own secretion. Central administration of the neuropeptide galanin is reported to increase prolactin secretion, but it is not clear if this effect is mediated by changes in the activity of TIDA neurons. In the present study, the effects of galanin on the basal and prolactin-stimulated activity of TIDA neurons were examined by measuring the ratio of 3,4-dihydroxyphenylacetic acid (DOPAC) to dopamine in the median eminence of both male and female rats. Intracerebroventricular (i.c.v.) administration of galanin (2 μg/rat) produced a rapid (by 15 min) increase in plasma prolactin concentrations, but failed to alter the ratio of DOPAC to dopamine in the median eminence of either male or female rats. These results indicate that galanin-induced activation of prolactin release is not mediated by changes in the activity of TIDA neurons. On the other hand, galanin decreased the ratio of DOPAC to dopamine in the median eminence of both male and female rats whose TIDA neuronal activity was stimulated following experimental procedures that increase circulating prolactin concentrations (i.e. administration of the dopamine antagonist haloperidol [1 mg/kg; s.c.; 12 h]. Taken together, these results indicate that the inhibitory effects of galanin on the activity of TIDA neurons is dependent upon the level of activity of these neurons in both male and female rats (supported by ADAMHA grant MH 42802).

489.11

VASOPRESSIN RELEASE FROM AMYGDALA IN VITRO. J. Raber, E. Merlo Pich, G.F. Koob and F.E. Bloom*. Department of Neuropharmacology, Scripps Research Institute, 1006 N. Torey Pines Rd., La Jolla, CA 92037.

Arginine Vasopressin (AVP) containing neurons have been shown to occur in limbic structures, including the hippocampus and amygdala, as well as in the classic magnocellular hypothalamic nuclei. We have investigated the release mechanism of AVP in vitro from dissected rat brain regions. Minced amygdala, hypothalamus and somatosensory cortex were each incubated in balanced Earle's salt solution for a total time of 180 minutes at 37o C. After three 20 minute periods for collecting basal release, depolarization with KCl (60 mM) caused a marked stimulation of AVP release (amygdala; basal level 58 +/- 18 pg/ml, stimulated 90 +/- 23 pg/ml, hypothalamus; basal level 392 +/- 36 pg/ml, stimulated 831 +/- 91 pg/ml). No detectable (stimulated) release was found with slices from the somatosensory cortex. This stimulation effect is calcium dependent and was blocked in the presence of EGTA (10 mM). These results indicate that depolarizing agents induce the release of AVP-like immunoreactivity not only from the hypothalamus but also from limbic structures, such as the amygdala. This system may be suitable for evaluating the effects of other regulatory signals, such as the cytokines, on neuronal neuropeptide release.

489.12

MELATONIN EFFECTS ON TWO ANXIOLYTIC TEST IN RATS. Naranjo-Rodríguez E.B., Vázquez A.M. and C. Reyes-Vázquez*. Sec. Farmacología, Depto. Farmacia, Facultad Química and Depto. Farmacología, Facultad Medicina, UNAM.

Anticonvulsive, hypnotic and sedative effects had been described after melatonin (MEL) systemic administration. The mechanism of action used by MEL to produce such effects are unknown. However, MEL enhances GABA "in vitro" and elicits some biochemical effects resembling those produced by diazepam (DIAZ), chlorodiazepoxide (CDP) and buspirone (BUS). These data suggest that MEL could exert other characteristic effects of these drugs, like anxiolysis. Male Wistar rats (120-230 g) were used in two experiments. Some rats were deprived of food and water for 48 h prior to test on a conflict procedure. Then, after 20 licks from a water bottle with a metal drinking tube, the animal received an electric shock (0.5 mA/2 sec). Also, some rats were tested in a force swimming procedure, in which total immobility during a 5 min period was recorded. The effects of three classical anxiolytic drugs, DIAZ (2 and 5 mg/kg), CDP (5 and 10 mg/kg) and BUS (5 and 10 mg/kg) were compared with those produced by MEL (1 and 2 mg/kg). All drugs were applied 20 min before both tests. Anxiolytic drugs as well as MEL produced a dose-dependent increase in the number of shocks received and in the total immobility time. These results suggest an anxiolytic action of MEL.

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489.13

CHRONIC PEPTIDE T ADMINISTRATION PREVENTS NEOCORTICAL ATROPHY RESULTING FROM NUCLEUS BASALIS LESIONS IN AGED RATS. D.J. Socci[1], J.M. Hill[2], C.B. Pert[3], M.R. Ruff[3] and G.W. Arendash.*[1] Dept. of Biology[1], Univ. of S Florida, Tampa, FL 33620; NICHD[2], NIH, Bethesda, MD 20892; Center for Molecular & Behav. Neurosci.[3], Rutgers Univ., Newark, NJ 07102.

Vasoactive intestinal peptide (VIP) is co-localized in cholinergic terminals within neocortex. Since VIP has neurotrophic actions *in vitro*, it may be involved with, and/or be capable of preventing, degenerative changes in neocortex resulting long-term after cortical cholinergic denervation induced by nucleus basalis (NB) lesioning (*Science* 238:952, 1987). The two-fold purpose of the present study was: 1) to determine the long-term degenerative effects of NB lesions in the neocortex of aged rats, and 2) to test the ability of "peptide T" (PT), which has a pentapeptide sequence homologous with VIP (7-11), to prevent any observed degenerative changes. Aged (20-21 month old) male Sprague-Dawley rats received NB infusions of ibotenic acid (5 µg/1 ul PBS) bilaterally. Immediately after lesioning, animals began receiving daily i.p. injections of either PT (1 mg) or vehicle solution. After sacrifice 5 months later, brain sections were stained with thionine for analysis of neocortical thickness (Layers II-VI) at the mid-parietal level. Compared to measurements from unoperated controls, NB lesioned animals given vehicle alone exhibited a significant 17.4% decrease in overall cortical thickness ($p<.002$); there was a 13.2% decrease in Layer II ($p<.02$), a 28.6% decrease in Layers III-V ($p<.0001$), and a 15.1% decrease in Layer VI ($p<.02$). Chronic PT treatment completely eliminated the lesion-induced decrease in overall cortical thickness, as well as that within Layers II and VI. Also, the lesion-induced decrease in Layers III-V was significantly attenuated by PT ($p<.03$). PT's action in preventing NB lesion-induced neocortical atrophy may involve a direct or indirect (glial-mediated) neurotrophic mechanism, presumably through VIP receptor activation.

489.14

BLOCKADE OF THE CARDIOVASCULAR AND TOXIC EFFECTS INDUCED BY INTRATHECAL INJECTION OF ENDOTHELIN-1 IN THE CONSCIOUS RAT. P. Poulat[1], P. D'Orléans-Juste[2], M. Yano[3], J. de Champlain[1*] and R. Couture[1]. [1]Dept. Physiology, University of Montreal, Montreal, Qué., Canada H3C 3J7, [2]Dept. Pharmacology, Sherbrooke University, Sherbrooke, Qué., Canada and [3]Banyu Pharmaceuticals Co., Tsukuba, Japan.

Evidence suggests that endothelin-1 (ET-1) acts as a neuropeptide within the CNS by interacting with specific receptors. In the rat spinal cord, the presence of ET_A receptors was demonstrated in binding assay. This study was to determine the intrathecal (i.t.) action of ET-1 and BQ-123 (antagonist of ET_A receptors) on mean arterial pressure (MAP) and heart rate (HR) of the conscious rat. The i.t. injection of ET-1 (6.5 - 650 pmol at T-9) produced dose-dependent increases in MAP and decreases in HR. During the dose-response curve, 40-50% of the rats died while 650 pmol ET-1 caused death of 100% of the animals within 3-5 min after an initial increase of blood pressure. Both the cardiovascular changes and the toxic action of 650 pmol ET-1 were blocked by the prior i.t. injection of 65 nmol BQ-123 (25 min earlier) but were unaffected when the same dose of antagonist was administered i.v. BQ-123 was devoid of intrinsic activity when given either i.t. or i.v. The pressor response to 65 pmol ET-1 was significantly inhibited after i.v. injection of phentolamine while the bradycardia was abolished by either atropine or pentolinium. The latter three treatments failed, however, to prevent the toxic action of i.t. ET-1. These results suggest that the cardiovascular and toxic effects induced by i.t. ET-1 are mediated by ET_A receptors in the spinal cord. The pressor response appears to be due to the activation of the sympathetic nervous system while the bradycardia is ascribed to a vagal reflex. [Supported by the MRC of Canada].

OPIOID RECEPTORS: COUPLING AND BIOCHEMISTRY

490.1

SELECTIVITY PROFILES AND REGULATION BY GUANYL NUCLEOTIDES OF LIGAND BINDING TO μ, δ AND κ OPIOID RECEPTORS IN BRAIN MEMBRANES. M. Williamson and J.M. Herz*. Biochemical Pharmacology Group, Panlabs, Inc., Bothell, WA 98011

We have used specific radioligand binding assays for the μ, δ and κ opioid receptors in whole brain membranes from guinea pig to establish selectivity profiles for various opioids and investigate effects of guanyl nucleotide regulation of agonist binding. [^{3}H]Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (DAMGO), [^{3}H](D-Pen^2,D-Pen^5)-enkephalin (DPDPE), and [^{3}H]U69,593 were used as specific radioligands for the μ, δ and κ receptors, respectively. Assays were carried out in modified Krebs-Hepes buffer containing protease inhibitors. Saturation isotherms revealed a single class of high affinity sites for [^{3}H]DAMGO. Inhibition of [^{3}H]DAMGO binding yielded the following K_is: fentanyl (1.2 nM), naloxone (0.76 nM), DPDPE (370 nM), naltrindole (2.5 nM), and norbinaltorphimine (26 nM). Indirect Hill coefficients for competing ligands were near 1.0. High selectivity of DAMGO for μ-receptors was observed since the ratio of the K_is for δ/μ and κ/μ were 926 and 3,700, respectively. GTP-γ-S completely inhibited [^{3}H]DAMGO binding with an IC_{50} of 0.89 μM. The K_d determined from saturation isotherms for binding of [^{3}H]U-69,593 was found to be 2.2 nM for a single class of sites. U50,488 was found to be highly selective for κ receptors since μ/κ=211 and δ/κ=11,430. Norbinaltorphimine exhibited a K_i of 0.35 nM and a n_H of 0.39, indicating heterogeneity in labeled sites. [^{3}H]DPDPE was found to be highly selective for δ-receptors, with μ/δ=168 and κ/δ=500. Saturating concentrations of GTPγS or GDPβS reduced binding of [^{3}H]U69,593 to 34% and 40% of control with IC_{50}s of 0.70 and 2.0 μM, and reduced the binding of [^{3}H]DPDPE to 12% and 1% of control with IC_{50}s of 0.15 and 0.92 μM. These results establish that guanyl nucleotides act with similar affinity to convert μ, δ and κ receptors to states of reduced affinity for agonists in guinea pig brain membranes

490.2

OPIOID RECEPTOR REGULATED SECOND MESSENGER SYSTEMS IN PHEOCHROMOCYTOMA CELLS M.E. Abood* and J.S. Eubanks. Dept. of Pharm., Med. Coll. of Virginia, Virginia Commonwealth Univ. Richmond, VA 23298.

PC12 rat pheochromocytoma cells are useful as a model system for neuronal development. In one subclone of PC12 cells, PC12h, low levels of δ-type opioid receptors markedly increase in response to nerve growth factor (NGF) (Inoue, N. and Hatanaka, H. *J. Biol. Chem.* 257: 9238 1981). We have been investigating the consequences of the appearance of opioid receptors in PC12h cells and examining concurrent changes in the expression of opioid-regulated genes. After 10 days of treatment with NGF, the number of opioid receptors (as measured by ^{3}H-diprenorphine binding), increases from a B_{max} of 40 to 220 fmols/mg protein. We have previously demonstrated that the opioid receptors in this cell line can be down-regulated by etorphine, indicating that they are capable of responding to opioid agonists. In the current study, the question of whether opioid receptors in PC12h cells couple to inhibition of adenylyl cyclase was addressed. Etorphine caused a dose-dependent inhibition of cAMP accumulation. This effect was reversed by naloxone. Furthermore, etorphine inhibition of cAMP accumulation was found in non-NGF as well as NGF-treated PC12h cells. These data indicate that the opioid receptors on PC12h cells are coupled to adenylyl cyclase, similar to δ receptors in other cell lines. (Supported by DA-06867)

490.3

EVIDENCE FOR LIGAND BINDING TO TRANSMEMBRANE SEGMENTS OF OPIOID RECEPTORS. J.C. Schaeffer[1], J.A. Bell[2]*, D.K. Arquette[1] and E.T. Consorti[1]. [1]Dept. of Chem. Cal State U., Northridge, CA 91330 and [2]Addiction Research Center, NIDA, Baltimore, MD 21224.

Considerable evidence suggests that opioid receptors are members of the G-protein linked receptor family. For several G-protein coupled receptors, biochemical and genetic analyses have implicated transmembrane segments of the receptor as the sites of ligand binding. The relative potencies of 7α-aminomethyl-6,14,-endo-ethenotetrahydrooripavine(I) (close structural relative of etorphine) and a series of N-acyl derivatives were assessed using a ^{3}H-naloxone/rat brain membrane binding assay. N-acetylation(II) had no effect on potency whereas the introduction of a hydrophobic N-phenylacetyl(III), N-phenylpropionyl (IV), or N-phenylbutyryl(V) substituent increased binding potency 40-fold, and made these compounds as potent as etorphine (IC_{50} = 0.3 nm). The effects of these compounds on an electrically evoked potential from isolated neonatal rat spinal cord showed them to be potent agonists, with III as potent as etorphine and I, II, IV and V being less effective. The electrophysiological effects were reversed by naloxone, but upon naloxone wash-out, effects of the more hydrophobic compounds (III, IV, V) persistently returned. These results suggest that partitioning of these compounds into the hydrophobic core of the neuronal plasma membrane may play a vital role in their duration of action because the μ opioid binding site is located in the transmembrane region of opioid receptors. However, because III is a more potent agonist than IV or V, small structural changes must affect the ligand/receptor complex conformation that transmits binding information to the G-protein involved in the transmembrane signaling process.

490.4

MECHANISMS OF LOW pH PRETREATMENT-MODIFIED G PROTEIN-ADENYLYL CYCLASE INTERACTION IN NG108-15 CELL MEMBRANES. D. E. Selley* and S.R. Childers. Dept. Physiol./Pharmacol., Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, NC 27103.

Pretreatment of rat brain or NG108-15 cell membranes at pH 4.5 prior to assay at pH 7.4 produces the following modifications in signal transduction pathways that regulate adenylyl cyclase (AC): 1) decreases stimulation of AC by G_s, 2) increases receptor-mediated inhibition of AC, and 3) increases inhibition by Na^+ and GTP of agonist binding to opioid receptors, with no effect on binding in the absence of Na^+ and GTP. In NG108-15 membranes, low pH pretreatment did not increase opioid stimulation of low K_m GTPase activity, or Gpp(NH)p inhibition of AC, in the presence of 120 mM NaCl. However, studies of the effect of Na^+ concentration on AC and GTPase activity in NG108-15 membranes have revealed that low pH pretreatment: 1) decreased basal AC activity in a manner that was inversely related to Na^+, 2) increased opioid inhibition of AC at Na^+ concentrations below that required to support opioid inhibition of the enzyme in control membranes, and 3) decreased basal low K_m GTPase activity and increased opioid agonist stimulation of low K_m GTPase in a manner that was inversely related to Na^+. The common effect of low pH pretreatment on both AC and GTPase was to increase agonist effects at low Na^+ concentrations. These data suggest that a complex interaction between Na^+, G_s, G_i, and inhibitory receptors is altered by low pH pretreatment.

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490.5

OPIATE RECEPTOR AGONISTS REGULATE PHOSPHORYLATION OF SYNAPSIN I IN SPINAL CORD-DORSAL ROOT GANGLION COCULTURES. Z. Vogel*, D. Saya, S-Y. Nah. LMB, NINDS, NIH, Bethesda, MD 20892; Dept. Neurobiol., Weizmann Inst. Science, Rehovot, 76100 Israel.

κ-Opiate receptor agonists were shown to inhibit adenylate cyclase activity as well as the voltage-dependent Ca^{2+} channels in spinal cord-dorsal root ganglion (SC-DRG) cocultured neurons (J. Neurochem. *52*, 360, 1989; J. Biol. Chem. *264*,347, 1989). We have, therefore, investigated the effect of κ receptor agonists on the phosphorylation of synapsin I, a synaptic vesicle-associated protein whose phosphorylation was shown to be regulated by cAMP, depolarization, and intracellular Ca^{2+} concentration. Depolarization of SC-DRG cocultures (by high K^+ or veratridine) as well as the addition of forskolin (which activates adenylate cyclase) leads to increased phosphorylation of synapsin I. The addition of κ opiate agonists (such as U50488 and EKC) attenuated both the K^+ depolarization- and forskolin-induced phosphorylation of synapsin I. The EC_{50} obtained for U50488 was ~ 5 and 1 μM, respectively. This attenuation by κ agonists was blocked by the opiate antagonist naloxone. μ and δ opiate receptor agonists had a much weaker effect compared with κ. Similarly, κ opiate agonists attenuated (by 30-50%) the high K^+ or veratridine-induced phosphorylation of synapsin I in synaptosomes prepared from spinal cord. These results show that opiate ligands modulate synapsin I phosphorylation. Moreover, the data could explain the alterations in synaptic efficacy and reduction in neurotransmitter release observed following opiate treatment. *(Supported by the Minerva and Schilling Foundations, the National Institute of Drug Abuse, and the German-Israeli Foundation for Scientific Research and Development.)*

490.6

NOVEL OPIOID BINDING SITES ASSOCIATED WITH THE NUCLEI OF NG108-15 NEUROHYBRID CELLS. M.M.Belcheva#, J. Rowinski#, J.Barg#, W.Gregg Clark#, C.Gloeckner#, X.-M.Gao†, D.-M.Chuang† and C.J.Coscia*#.#Dept. of Biochem. and Mol. Biol., St. Louis Univ. Sch. Med. St. Louis, MO 63104 and †Biological Psychiatry Branch, NIMH, Bethesda, MD 20892

Nuclear opioid binding sites have been discovered in NG108-15 cells. Marker enzyme analyses, electron and fluorescence light microscopy studies attested to the purity of nuclear preparations. Immunohistochemical staining of cryostat sections of NG108-15 cells with an anti-opioid receptor antibody corroborated a nuclear localization. ^{3}H-DPDPE, ^{3}H-DADLE and ^{3}H-diprenorphine homologous binding (K_d and B_{max}), ^{3}H-DSLET heterologous competition curves, stereospecificity and kinetic data, satisfied criteria for the presence of δ opioid sites in purified nuclear preparations; neither μ- nor κ- specific binding were detectable. Agonists, ^{3}H-DADLE and ^{3}H-DPDPE, bind with high affinity to nuclear membranes and with lower affinity to chromatin. In contrast, partial agonist ^{3}H-diprenorphine high affinity binding sites were in chromatin, while low affinity binding was found in nuclear membranes. Gpp(NH)p sensitivity of ^{3}H-DADLE binding was detected in nuclear membranes but not chromatin. Opioid binding to nuclear membranes and chromatin was abolished upon cycloheximide treatment of cells. The results suggest that NG108-15 cells contain newly synthesized G protein-coupled δ receptors in nuclear membranes and uncoupled, internalized opioid sites in chromatin.

490.7

DOWNREGULATION OF KAPPA OPIOID RECEPTORS IN A MURINE LYMPHOMA CELL LINE. D.B. Joseph* and J.M. Bidlack. Dept. of Pharmacology, Univ. of Rochester Sch. of Med. and Dent., Rochester, NY 14642

Previous studies from this laboratory have demonstrated the presence of κ opioid receptors on membranes from the mouse thymoma cell line, R1.1. Chronic exposure of these cells to the κ agonist U50,488 produced changes in the receptor population, as indicated in ligand binding assays using the κ-selective agonist [^{3}H]U69,593. The binding of 1 nM [^{3}H]U69,593 to membranes from U50,488-treated cells was reduced by as much as 55% in comparison to control cells, which were treated with an equivalent concentration of U50,488 for 15 min. The effect of U50,488 was both concentration- (EC_{50} = 30 nM) and time-dependent, with the maximum decrease in binding observed after 24 hr of exposure. The decrease in [^{3}H]U69,593 binding was due to changes in both receptor density and affinity. Twenty-four hr exposure to 100 nM U50,488, a maximally effective concentration, produced a 50% decrease in the B_{max} value and a 2-3 fold increase in the K_d value. The effects of chronic exposure of the cells to 100 nM U50,488 were completely blocked by 1 μM naloxone. Treatment of R1.1 cells with 10 μM morphine or 100 nM [D-Ala^2, D-Leu^5] enkephalin for 24 hr produced no change in [^{3}H]U69,593 binding to membranes prepared from these cells, suggesting that μ and δ opioids did not produce a change in the number or affinity of κ opioid receptors. The R1.1 cell line will provide a model for characterizing the mechanisms of drug dependence associated with κ opioid receptors. The results from the present studies are the first evidence for the occurrence of opioid receptor downregulation in cells of the immune system. (Supported by USPHS DA04355 and DA07232.)

490.8

THE PRESENCE OF δ OPIOID RECEPTORS ON COS-7 CELL MEMBRANES. T. Zalewska, E. Malatynska, H.I. Yamamura*. Department of Pharmacology, College of Medicine, The University of Arizona, Tucson AZ, 85704.

COS-7 cells are very often used in transient gene expression. They might be considered as part of an expression system for opioid receptor cloning. We report the presence on COS-7 cell membranes of high affinity binding sites for [^{3}H]naltrindole (an antagonist of δ opioid receptor). Equilibrium binding studies show that [^{3}H] naltrindole labels a homogenous population of binding sites with a dissociation constant (K_d) of 72.6 pM and Hill value not different from unity. The measured receptor density (B_{max}) is 17 fmol / mg protein. Naltrindole and a ligand selective for δ opioid receptor, p-Cl-DPDPE, displaced [^{3}H]naltrindole from its binding site with high affinity. The K_i value for naltrindole is 200 pM, and for p-Cl-DPDPE is 315 pM. The ligands selective for μ (PL-17) and κ (U69,593) opioid receptors only inhibited [^{3}H]naltrindole binding at micromolar concentrations. This study demonstrates the presence of δ opioid receptor sites on COS-7 cell membranes. It is concluded that these cells should be avoided for the expression of putative δ opioid receptor genes. Supported in part by NIDA grants.

490.9

KAPPA-OPIATE RECEPTORS ON ASTROCYTES STIMULATE L-TYPE Ca^{2+} CHANNELS. Peter S. Eriksson[1], Michael Nilsson[1], Maria Wågberg[1], Elisabeth Hansson[1] and Lars Rönnbäck*[1,2] Institute of Neurobiology[1] and Department of Neurology[2] University of Göteborg, Göteborg, Sweden. Cultured astrocytes from the cerebral cortex respond to κ-receptor stimulation with a substantial elevation of the cytoplasmic free calcium, visualized through the use of the fluorescent calcium indicator fura-2. The stimulation of κ-receptors using the agonist U-50,488H increase the level of calcium through a stimulatory effect on the transmembrane calcium influx. The transmembrane influx was dose dependent. Furthermore, it was completely blocked by the selective κ-receptor antagonist *nor*-Binaltorphimine. The presence of L-type channels was verified by the use of Bay K8644. The effect of Bay K8644 was completely blocked by nifedipine, indicating the involvement of L-type channels. L-type channel coupled κ-receptors on astrocytes might represent a novel mechanism contributing to the depressant action of opioids on synaptic transmission via decreasing the availability of extracellular calcium necessary for presynaptic transmitter release.

490.10

CHARACTERIZATION OF A PUTATIVE OPIOID RECEPTOR IN THE PROTOZOAN *STENTOR*. M.J. Marino*, T.G. Sherman, and D.C. Wood. *Department of Cellular and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260*

An understanding of the biochemical events underlying opioid mediated processes has been hindered by the complexity inherent in mammalian systems. Therefore, a simple systems approach may prove beneficial in understanding the biochemistry of opioid-mediated events. The protozoan *Stentor* may express an opioid receptor and this simple organism could provide a useful system for the biochemical study of such receptors. We have previously shown that β-endorphin (β-end) modulates a mechano-sensory conductance in *Stentor* with an EC_{50} of 3.0 μM. The result of this modulation is behaviorally apparent as a marked decrement in the probability that a cell will contract when mechanically stimulated. The effect of β-end appears to be receptor mediated for several reasons. First, the β-end effect is mimicked by morphine and DAGO, but not by dynorphin or the enkephalins indicating a putative μ-like receptor. Second, the actions of β-end, DAGO, and morphine are sensitive to equimolar concentrations of the opiate antagonist naloxone. Third, the effect of β-end is pertussis toxin (PTX) sensitive. *Stentor* incubated for 24 hours in 200 μg/ml of PTX do not exhibit a depression in mechanical stimulus response probability when exposed to 1 μM β-end or 20 μM DAGO. This PTX treatment has no effect on control cells' response probability and does not effect the cells' electrical threshold. This suggests that these opioids act through a G-protein linked opioid receptor. Finally, *Stentor* exhibit tolerance with the continued exposure of β-end. Current work is aimed at identifying any endogenous opioid ligands as well as characterizing this receptor utilizing pharmacological and molecular techniques.

490.11

GENETIC CORRELATION BETWEEN THE NUMBER OF MU AND DELTA BINDING SITES IN WHOLE BRAIN PREPARATIONS FROM 8 INBRED MOUSE STRAINS. K. Shimosato*, N. Goodman & R. Marley. NIDA-Addiction Res. Ctr., Box 5180, Baltimore, MD 21224.

To identify animal models differing in opioid receptor binding parameters for use in further examination of opioid-mediated behaviors, we have examined the number (B_{max}) and affinity (K_d) of μ and δ receptor in whole brain preparations from 8 inbred mouse strains. ^{3}H-DAMGO (0.5 - 21 nM) and ^{3}H-pCl-DPDPE (0.04 - 4 nM) were used to characterize μ and δ receptors, respectively. Scatchard analyses revealed that the B_{max} values for μ receptors ranged from 75 to 116 fmol/mg, with a rank order among the strains of BALB, C3H, C57/6J, CBA < C57/6ByJ, DBA, SJL < AKR. No differences in μ receptor affinity were observed among the strains (K_d = 3 - 4 nM). The number of δ receptors among the strains ranged from 55 - 83 fmol/mg, with a rank order (BALB < C3H, C57/6J, C57/6ByJ, CBA < DBA < SJL < AKR) very similar to that observed for μ receptors. There were no differences among the strains in δ receptor affinity (K_d = 0.4 - 0.5 nM). Statistical analyses revealed a significant genetic correlation between the number of μ and δ receptors in whole brain preparations from the 8 inbred strains ($r = 0.88$, $p < 0.01$). The strong genetic correlation between the number of μ and δ opioid receptors suggests that there may be common mechanisms associated with the regulation of the expression of these receptor subtypes.

490.12

INTERACTIONS OF OPIOID PEPTIDES AND OTHER DRUGS WITH MULTIPLE DELTA BINDING SITES IN RAT AND MOUSE BRAIN: FURTHER EVIDENCE FOR SUBTYPES OF THE δ_{ncx} BINDING SITE. H. Xu*[1], J.S. Partilla[2], A.E. Jacobson[1], K.C. Rice[1] and R.B. Rothman[2]. [1]LMC, NIDDK, NIH, Bethesda, MD 20892. [2]NIDA Addiction Research Center, PO Box 5180, Baltimore MD 21224.

These studies tested the hypothesis that there exist subtypes of delta receptors. Rat and mouse brain membranes were depleted of μ binding sites using the irreversible ligand, BIT. δ_{ncx} binding sites were labeled with [^{3}H]DADL (4 - 6 hr at 25° C, in 10 mM TRIS-HCl, pH 7.4, 100 mM choline chloride, 3 mM $MnCl_2$). Initial experiments with rat and mouse brain membranes demonstrated that DPDPE or DPLPE inhibition curves were characterized by low slope factors, both in the absence and presence of 50 μM GppNHp. Binding surface analysis of the interaction of DADL, DPDPE, deltorphin-I and oxymorphindole with [^{3}H]DADL binding sites readily resolved two binding sites in both rat brain membranes. Similar studies with mouse brain membranes also readily resolved two sites, which had a different ligand selectivity profile from the two sites observed using rat brain. Sodium chloride (100 mM) decreased [^{3}H]DADL binding, and increased the Ki values of DPDPE, deltorphin-II and naltrindole for the two sites. These data support the hypothesis of subtypes of the δ_{ncx} binding site, and suggest that there may also be significant difference among species. Additional studies will be needed to test this hypothesis further.

490.13

AUTORADIOGRAPHIC LOCALIZATION OF THE μ AND δ RECEPTORS IN THE BRAIN OF MICE SELECTED FOR THEIR DIFFERENCES IN VOLUNTARY ETHANOL CONSUMPTION. J.-P. DE WAELE* and C. GIANOULAKIS Douglas Hospital Research Center and McGill University, Montréal, Québec, Canada

The implication of the endogenous opioid system in the control of voluntary ethanol consumption is suggested by the observation that the administration of both specific (ICI-174864) or non-specifc (naltrexone) opiate antagonists induces a decrease in voluntary ethanol consumption in rats. Using specific μ (FK-33824) and δ (DPDPE) ligands, we studied the distribution of μ and δ binding sites in the brain of the C57BL/6 (ethanol-preferring) and DBA/2 (ethanol-aversive) mice. It was demonstrated that there is higher release of hypothalamic ß-endorphin and more mRNA coding for POMC in the hypothalamus of the C57BL/6 mice than of the DBA/2 mice. There is a predominance of μ over δ receptors in both strains of mice, with μ receptors present in limbic and thalamic areas, while δ receptors density is low in regions such as the septum or the thalamus. Strain-related differences in μ receptor density may be observed in certain nuclei of the limbic system (n. accumbens, septum, VTA) and of the thalamus. Differences in the opioid receptors distribution between the C57BL/6 and DBA/2 mice may explain in part the differences in their ethanol-drinking behavior.

490.14

EXPRESSION OF OPIOID RECEPTORS IN *XENOPUS* OOCYTES INJECTED WITH RAT BRAIN MESSENGER RNA. S. Kaneko, J. Yuasa, H. Takahashi and M. Satoh*. Dept. Pharmacol., Facl. Pharm. Sci., Kyoto Univ., Kyoto 606-01, Japan.

Voltage-clamp recording was used to detect functional expression of opioid receptors in the *Xenopus* oocyte translation system. By injecting poly(A)$^+$RNA isolated from 3 week-old rat striatum or whole brain, the oocytes often acquired intracellular Ca^{2+}-related oscillatory responsiveness to DAMGO (μ agonist), DADLE (μ+δ agonist), DPDPE (δ agonist) and U-50488H (κ agonist) at a concentration of 1 μM. These responses were very transiently expressed after injection of mRNA, however, water-injected oocytes never responded any of the opioid agonists. After sucrose-density fractionation, RNA size class of about 3 kbase encoded these opioid receptors. In the oocytes injected with striatal mRNA, DADLE and DPDPE evoked the fluctuating current with higher probability and in larger amplitude than other agonists, whereas whole brain mRNA produced DAMGO and U-50488H responses predominantly. The DPDPE response of striatal mRNA-injected oocytes was antagonized by naloxone as well as the δ-specific antagonists ICI 154129 and ICI 174864. DAMGO and U-50488H responses have not been characterized yet because of strong desensitizing property. These observations suggest that putative μ, δ and κ subtypes of opioid receptors mobilizing intracellular Ca^{2+} are expressed in *Xenopus* oocytes by rat brain mRNA.

490.15

IN VIVO IMAGING OF OPIOID RECEPTORS WITH [^{125}I]-IOXY, A NEW IODINATED NALTREXONE DERIVATIVE.

M.J. Iadarola*[1], L.S. Brady[2], M.V. Green[3], K.F. Berman[4], B.R. de Costa[5] and K.C. Rice[5]. [1]NAB, NIDR; [2]CNE, NIMH; [3]DNM, CC; [4]CBDB, NIMH and [5]LMC, NIDDK, NIH, Bethesda, MD 20892.

We have synthesized and radiolabeled Ioxy (de Costa et al., J. Med. Chem. in press) for use in single photon emission computed tomography (SPECT) imaging of endogenous opioid receptors in humans. Ioxy, an iodo-derivative of naltrexone, functions as an antagonist and is an analog of cycloFoxy, a [^{18}F]-labeled compound used in positron emission tomography (PET). Intravenous injection of 20 uCi to rats yielded highest levels of binding in striatum and thalamus, lesser binding in other areas, and the fewest counts in cerebellum. Counts were displaced to levels in cerebellum by pretreatment with (-) but not (+) naloxone. Autoradiography of brain sections after i.v. administration showed dense binding to striatal patches, laminae I-II of spinal cord, medial habenula and interpeduncular nucleus. We used an experimental gamma camera to obtain a brain scan of the distribution of [125I]-Ioxy in a living rat. A planar scan in the vertex projection revealed dense accumulation of radioactivity in the thalamus, midbrain and bilaterally in the caudate nucleus. These studies introduce a new method for imaging receptors in small animals in vivo and suggest that Ioxy will be an effective agent for use as a ligand in SPECT studies of human opioid receptors.

490.16

GLUCOSE METABOLISM AND OPIATE RECEPTOR BINDING IN THE DEAFFERENTED CORTEX IN MONKEY BY PET. D.J. Doudet*, R.E. Carson, M.A. Channing, R. Saunders, M. Der and R.M. Cohen. Clinical Brain Imaging/ NIMH; Nuclear Medicine Dept, NIH, Bethesda, MD.

To examine the role of the cortical opiate system, we studied glucose metabolism and F-18 cyclofoxy (CF) binding in the visual system of rhesus monkeys (N=4). The forebrain commissures and one optic tract were sectioned. CF distribution volume (DV) and glucose metabolic rates (CMRg) were determined for left and right hemispheres. The deafferented occipital and parietal and temporal areas had a significantly increased DV of CF (respectively 33, 18 and 9 %) and a significant decrease in CMRg (12, 8 and 6%) compared to the contralateral side ($p<0.05$). Compared to normal rhesus, CF binding was significantly increased (25%) in the contralateral occipital, parietal and temporal areas and the ipsi and contralateral central and frontal areas, but glucose metabolism was normal. CF binding and CMRg were normal in subcortical areas.

This increase in DV of CF, likely due to an increase in unoccupied opiate receptors, in areas with decreased functional activity suggests that sensory inputs play a role in the modulation of opiate function and that cortico-cortical connections are important in its regulation.

CATECHOLAMINES: RECEPTORS III

491.1

ACTIVATION OF POSTSYNAPTIC BUT NOT PRESYNAPTIC DOPAMINE RECEPTORS BY DIHYDREXIDINE, A POTENT D1 AND D2 RECEPTOR LIGAND. N.F.Nichols,* P.J.K.D.Schreur, M.W.Smith, W.E.Hoffmann, D.E.Nichols and M.F.Piercey, The Upjohn Co., Kalamazoo, MI 49001, Purdue U., W. Lafayette, IN 47906.

Mottola et al. (Neurosci. Abs. 17:818) found that the D1 agonist dihydrexidine (DX) weakly bound to D2 receptors, using radiolabeled spiperone, a D2 antagonist, as a D2 receptor marker. Like D2 agonists, DX depressed tyrosine hydroxylase, but this was not altered by D2 antagonists. In binding tests, we find that DX had higher affinity for D2 receptors (Ki=3.3 nM vs ^{3}H-U-86170, a dopamine [DA] agonist) than for D1 sites (Ki=38.5 nM vs ^{3}H-SCH 23390) and α-2 sites (Ki-62.5 nM vs ^{3}H-clonidine). In reserpinized mice, doses ≥1.0 mg/kg i.p. stimulated locomotor activity similar to other D2 agonists. This effect was blocked by the D2 antagonist raclopride (1 mg/kg s.c.). However, unlike other D2 agonists, DX, 0.1, 1.0, and 10.0 mg/kg i.p. did not block amphetamine-induced locomotor activity in non-reserpinized mice. It did not alter firing rates of DA neurons in substantia nigra pars compacta (SNPC) with doses up to 3 mg/kg i.v. (apomorphine ED50=.009 mg/kg i.v.), nor did it antagonize DA agonists effects in SNPC. In summary, DX behaves like a D2 agonist with some affinity also for D1 receptors, similar to apomorphine. However, it does not stimulate or block the DA autoreceptor, a D2 binding site.

491.2

EFFECTS OF DEPRENYL ON DOPAMINERGIC NEUROTRANSMISSION IN THE RAT MESOSTRIATAL SYSTEM. [1]M. Savasta*, [1]M. Chritin, [2]P. Assouly, [2]H.C. Neuckirch, [2]F. Bougeard, [1]E. Beurriand, [1]M. Manier, [1]P. Pollak and [1]C. Feuerstein. 1. INSERM-LAPSEN U.318, Pavillon de Neurologie, CHU de Grenoble, BP 217, 38043 Grenoble cedex 9, France. 2. SCHERING PLOUGH, 92 Rue Baudin, 92307 Levallois Perret cedex, Fance.

Deprenyl, a monoamine oxidase B inhibitor, has been reported to delay the initiation of levodopa therapy in patients with Parkinson's disease (PD), although the slowing of the progress of PD caused by the drug remains controversial. The present experiments were undertaken in order to examine the influence of this drug on mesostriatal dopaminergic neurotransmission in control and 6-OHDA-denervated rats.

We studied the effect of chronic treatment by deprenyl (0.25mg/Kg, s.c./day) for 15 and 30 days on striatal DA, DOPAC and TH contents, on D_1 and D_2 DA receptor striatal densities and on D_2 mRNA levels in two groups of rats (G_{15} and G_{30}) according to the delay of treatment. Each group was composed of shams (n=8), shams + deprenyl (n=10), 6-OHDA lesioned (n=6) and 6-OHDA lesioned + Deprenyl (n=10). Striatal levels of : DA and the metabolite DOPAC were determined by HPLC-EC ; DA D_1 and D_2 receptors were measured by autoradiography, using [^{3}H]SCH 23390 and [^{3}H]Raclopride respectively as ligands ; D_2 mRNAs were examined by in situ hybridization using a specific oligonucleotide ; TH was studied by radioimmuno-histochemistry. The results obtained showed that :

- striatal DA levels were significantly increased (+70%) in shams+deprenyl animals of G_{30} group. In lesioned animals where more than 90% of DA neurons were destroyed, no consistant effect could be defined ;
- the effects of deprenyl on D_2 receptor expression are clear only after 15 days of treatment. These effects concerned, firstly, shams+deprenyl animals where striatal D_2 receptor densities were significantly increased (18 to 25%, $p<0.001$) as compared to shams ; secondly, lesioned+deprenyl animals where D_2 mRNAs levels were increased by 12 to 17 % ($p<0.01$) as compared to lesioned rats. No effect could be observed for either striatal D_1 receptors and TH levels.

491.3

D_2 DOPAMINE RECEPTOR TURNOVER AND mRNA LEVELS IN THE NEUROLEPTIC-RESPONSIVE (NR) AND NEUROLEPTIC NON-RESPONSIVE (NNR) LINES OF MICE. **Y. Qian*, B. Hitzemann, G. Yount, J. White and R. Hitzemann . Dept.'s of Psychiatry and Neurobiology, Div. of Endocrinology, SUNY at Stony Brook, NY 11794.**

The NR and NNR lines of mice differ >10 fold in their sensitivity (ED_{50s}) to catalepsy induced by neuroleptics with a high D_2/D_1 dopamine receptor activity profile. Recovery of pre- and postsynaptic D_2 receptors was assessed by quantitative receptor autoradiography following *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) blockade. Significant differences in receptor turnover were found between the two lines in the nucleus accumbens (NA) and the caudate putamen (CPu), but not in the substantia nigra zona compacta (SNc) and the ventral tegmental area (VTA). For both lines, receptor production rates in the NA and CPu are higher than those in the SNc and VTA. For the NA and CPu, receptor production rates of the NNR line were lower than those of the NR line. Preliminary nuclease protection data show that such receptor turnover differences are associated with parallel differences in the D_2 receptor mRNA level.

491.4

USE OF BXD RECOMBINANT INBRED MICE TO DETECT GENOMIC MARKERS FOR NEUROLEPTIC SENSITIVITY. S.J. Kanes*, B. Hitzemann, T. Phillips, J. Belknap, J. Crabbe and R. Hitzemann, Departments of Psychiatry and Pharmacology, SUNY at Stony Brook, NY 11794-8101 and VAMC, Portland, OR 97201.

Our laboratory has used selective breeding and standard inbred strains to investigate the biochemical and genetic factors associated with response and non-response to neuroleptic induced catalepsy (see e.g. Kanes et al., Soc. Neurosci. Abst.# 539.6, 1991). The study of recombinant inbred mouse strains provides a mechanism for detecting linkage between the phenotype of interest and specific Quantitative Trait Loci (QTL). The strain distribution pattern (SDP) for the haloperidol ED_{50} in 18 strains of the BXD recombinant inbred series (RI) has been determined. ED_{50}'s in the RI strains are normally distributed confirming that sensitivity to neuroleptic induced catalepsy is a polygenic quantitative trait. This SDP was correlated with the SDP of 360 genomic markers mapped in the BXD's. This analysis indicates 14 markers significantly associated with the catalepsy SDP. Eight markers were found on chromosome 4, six of which were centered on FV-1, which lies 76 cM from the centromere. Correlated responses to neuroleptic-catalepsy include the density of pre and post-synaptic D_2 receptors, the density of midbrain DA neurons and the density of striatal cholinergic neurons. The SDPs for these responses and the associated QTL analysis will be presented.

491.5

CHOLINERGIC AND DOPAMINERGIC REGULATION OF NEUROLEPTIC RESPONSE IN SELECTED AND INBRED STRAINS OF MICE. B. Hitzemann, S. Kanes, Y. Qian and R. Hitzemann* Departments of Psychiatry, Pharmacology and Neurobiology SUNY at Stony Brook, NY 11794-8101 and VAMC, Northport, NY 11768.

The successful selection of the neuroleptic responsive (NR) and neuroleptic non-responsive (NNR) lines of mice (Hitzemann et al. 1991) and the marked variation in neuroleptic sensitivity among inbred strains of mice (Kanes et al. 1991) argues strongly that genetic mechanisms contribute significantly to the variance in neuroleptic-induced catalepsy. The precise nature or extent of these mechanisms remains unclear. Given the well established role of the striatal cholinergic system in regulating extrapyramidal neuroleptic responses, we have investigated striatal cholinergic cell number [choline acetyltransferase (ChAT) positive neurons] in the NR and NNR lines and 8 inbred strains. In comparison to the NNR line, cholinergic cell number is significantly increased 40 to 60% in the rostral but not caudal striatum. Similarly, we have found that among the inbred strains there is a significant correlation ($r = -0.75$ or better, $p < 0.05$) between the ED_{50} for haloperidol-induced catalepsy and cholinergic cell number in both the rostral and caudal striatum. Focusing on the rostral striatum, there is no difference between the NR and NNR lines in D_2 dopamine receptor density; however, for the inbred strains, D_2 receptor density is inversely correlated ($r = -0.72$, $p < 0.05$) to cholinergic cell number. This difference between the selected and inbred lines argues that D_2 receptor and cholinergic cell number can have independent genetic regulation within the striatum.

491.6

PHARMACOLOGICAL CHARACTERIZATION OF HUMAN DOPAMINE D_{2A}, D_{2B} AND D_3 RECEPTORS. Å.H. Malmberg[1], D.M. Jackson*[1], U. Hacksell[1,2], A.M. Johansson[2], A-M. Eriksson[1], N.A. Mohell[1] Astra Arcus AB, S-151 85 SÖDERTÄLJE, Sweden[1] and Department of Organic Pharmaceutical Chemistry, Uppsala Biomedical Center, Uppsala University, S-751 23 UPPSALA, Sweden[2].

The genes coding for six subtypes of dopamine (DA) receptors have recently been characterized and expressed in various cell lines. In this study we have compared the pharmacological properties of the human D_3 receptor with the two alternatively spliced forms of the human D_2 receptor, D_{2A} (long) and D_{2B} (short). The D_3 receptor was expressed in hamster ovary cells and the D_2 receptors in mouse fibroblasts. In vitro radioligand binding studies demonstrated that the benzamide radioligands [^{3}H]raclopride and [^{125}I]NCQ298 bound with high affinity, saturability and low non-specific binding to the three DA receptor subtypes. The K_d (dissociation constant) values of [^{3}H]raclopride for D_{2A}, D_{2B} and D_3 were 1.41, 0.77 and 1.58 nM, and the K_d values of [^{125}I]NCQ298 for D_{2A} and D_3 were 31 and 17 pM, respectively. As expected the binding of the benzamide radioligands was regulated by sodium ions. In competition studies most of the antagonists tested showed about equal affinities for the three DA receptor subtypes. However, remoxipride and spiperone had ten fold lower affinity, and some 2-aminotetralin derivates twenty fold higher affinity for D_3 than D_2 receptors. Among agonists DA, quinpirole and several 2-aminotetralin derivates displayed up to fifty fold higher potencies at D_3 receptors. Two affinity states for DA and quinpirole were observed in all three subtypes. It is concluded that in spite of the high level of homology in transmembrane (ligang binding) domains it is possible to obtain D_2/D_3 selective drugs that migth prove useful for development of antipsychotics.

491.7

EFFECTS OF D2 AND D3 RECEPTOR ACTIVATION MEASURED BY MICROPHYSIOMETRY. M.P. Rosser,*M.R. Kozlowski, R.L. Neve, and K.A. Neve. Screening and Biochemical Research, Bristol-Myers Squibb, Wallingford, CT 06492; Molecular Neurogenetics Laboratory, McLean Hospital, Belmont, MA 02178; Research Service, VA Medical Center, Portland, OR 97207.

The dopamine (DA) D3 receptor is a novel binding site for DA, first identified by cloning and expression of a novel cDNA from rat brain. The D3 receptor has been classified as a subtype of the D2 receptor based on its D2-like pharmacological profile and its sequence homology to D2 receptors. C_6 glioma cells transfected with the rat cDNA for the D3 receptor expressed, at a density of 200-300fmol/mg protein, a binding site with high affinity for ^{125}I-epidepride and low affinity for D2 antagonists. These characteristics are consistent with the expression of a D3 receptor. A silicon microphysiometer (Molecular Devices) was used to compare second messenger responses to stimulation of D2 and D3 receptors. The DA agonist, quinpirole, causes an increase in the rate of acid extrusion in cell lines expressing recombinant D2 receptor. The response is inhibited by amiloride and amiloride analogs selective for the Na^+/H^+ antiporter, but not affected by pertussis toxin. D2 receptor stimulation, therefore, appears to accelerate Na^+/H^+ exchange via a pathway that does not involve inhibition of adenylyl cyclase or pertussis toxin sensitive G proteins. In the presence of quinpirole, C_6 cells expressing recombinant D3 receptors also increased their rate of acid extrusion. As with D2-expressing cells, this increase was inhibited by amiloride and its analogs, suggesting that D3 receptor stimulation also activates a Na^+/H^+ antiporter. This is the first report of a functional response to stimulation of D3 receptors.

491.8

INTERNAL ACCEPTOR SITE DIRECTS ALTERNATIVE SPLICING IN THE MOUSE D_3 DOPAMINE RECEPTOR. Fishburn C.S., David C., Carmon S. and Fuchs S*. Dept. of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Dopamine receptors have been implicated in several neuropathological conditions. A number of receptor subtypes are recognised, and their respective roles in normal brain function and in neurological disorders may be advanced by the availability of cloned receptor cDNA forms. We have cloned the mouse D3 dopamine receptor from olfactory tubercle cDNA using PCR, and have found it to exist in two alternatively spliced forms. These two mRNA isoforms differ by the presence of 63bp, which encode 21 amino acids in the putative third cytoplasmic loop of the receptor. The longer form bears sequence homology of 94% to the rat D_3 dopamine receptor. We have also found alternative splicing to occur in the rat D_3 receptor. Using PCR analysis on different mouse brain regions, we have shown the long and short D_3 receptors to be present in the same tissues, the longer form invariably being the predominant one. Analysis of the gene for the mouse D3 dopamine receptor revealed an acceptor site at the 3' end of the 63bp stretch. This suggests that the alternatively spliced shorter D_3 mRNA isoform may be the result of splicing at this alternative acceptor site. It therefore appears that the D 3 receptor undergoes alternative splicing in the third cytoplasmic loop, in a region similar to the location of the splicing reported to occur for the D_2 dopamine receptor.

491.9

CHARACTERIZATION OF THE HUMAN D3 DOPAMINE RECEPTOR EXPRESSED IN TRANSFECTED MAMMALIAN CELL LINES. **R.G. MacKenzie*, D. VanLeeuwen, T.A. Pugsley, P. Boxer, L. Tang++, R.D. Todd+ and K.L. O'Malley++, Dept. Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48106, +Dept. Psychiatry, ++Dept. Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.**

CHO-K1, human neuroblastoma SK-N-MC and SK-N-Be(2), mouse fibroblast CCL1.3 and rat glioma/mouse hybrid NG108-15 cells were transfected with a pcDNAI/neo vector containing the cDNA of the human D3 dopamine receptor. Transfection of the CHO-K1 and SK-N-MC cells resulted in high expression of the D3 receptor (>1 pmol receptor/mg membrane protein) as evidenced by radioligand binding assays. In the CHO-K1-D3 cells, a small guanine nucleotide shift was noted for dopamine binding but all other tests for D3 function were negative, including inhibition of forskolin-stimulated cAMP generation, [3H]thymidine uptake as a measure of mitogenesis and Ca2+ flux in single cells containing the Ca2+-sensitive dye, FURA-2. Stable transfectants of CCL1.3 (≈500 fmoles/mg membrane protein) exhibited the expected D3 pharmacological profile. However, there was no guanine nucleotide-induced shift of dopamine binding, no effect of receptor activation on adenylyl cyclase activity and no inositol phosphate responses. In contrast, D2 receptors expressed in CCL1.3 cells inhibited adenylyl cyclase and increased inositol phosphate metabolism. Therefore, D3 receptors do not appear to couple to the same G-protein(s) as do D2 receptors. Experiments testing the function of the D3 receptor in other cell lines are in progress.

491.10

CHARACTERIZATION AND REGULATION OF DOPAMINE D3 RECEPTORS. B.A. Cox*, R.A. Henningsen, K. Duwe, R.L.Neve, and K.A. Neve, VA Medical Center and Oregon Health Sciences University, Portland, OR 97213, and McLean Hospital, Belmont, MA.

The dopamine (DA) D3 receptor is a novel D2-like receptor whose pharmacological profile differs from that of DA D2 receptors. We have stably expressed D3 receptors in C6 glioma cells (C6-D3) and have characterized their binding using the radioligand [^{125}I]epidepride, which has high affinity (60-100 pM) for D3 receptors. Drug potencies in rat basal forebrain minus neostriatum (BF) were compared to those using tissue from rat neostriatum (NS), and recombinant D3 (C6-D3) and D2 (C6-D2) receptors. Two site analysis of the data indicated that a small population (11-20%) of binding sites in rat BF a pharmacological profile similar to that of C6-D3 receptors. For neostriatum, however, curves are best fit to only one site.

	IC_{50}, in nM (n=3-4)				
Drug	C6-D3	BF-D3	BF-D2	NS	C6-D2
quinpirole	198	59	11700	10700	11000
spiperone	6	3.5	0.16	0.17	0.32
domperidone	117	125	2.5	3.4	3.4

Treating C6-D3 cells with agonists caused a concentration-dependent increase in the density of D3 receptors. Maximal increases observed, as determined by saturation analysis using [^{125}I]epidepride, were over 500% for DA and NPA and 373% for quinpirole. Increases were not prevented by treatment with pertussis toxin. (MH45372)

491.11

THE D_3 ANTAGONIST, AJ76, INCREASES ACCUMBENS DOPAMINE AND SEROTONIN RELEASE: SIMULTANEOUS DETECTION *ON LINE* WITH BEHAVIOR. F. Eng*, F.T. Phelan, R.T. Wechsler, M.F. Piercey[1] and P.A. Broderick. Dept. Pharmacol., CUNY Med. Sch., Convent Ave. & W. 138th St., NY, NY 10031, USA, CNS Res., The Upjohn Co.[1], Kalamazoo, MI 49007, USA.

AJ76 is a dopaminergic (DA-ergic), antagonist with a higher affinity for the D_3 than the D_2 receptor (Sokoloff *et al., Nature* 347:146; 1990). AJ76 is a weak stimulant due to DA autoreceptor antagonism (Svensson, *et al., J. Neural Transm.* 65:1; 1986), with preferential activity at the nerve terminal (Piercey and Lum, *Eur. J. Pharmacol.* 282: 219; 1990). AJ76 increases Ca^{++} dependent DA release (Waters *et al., Eur. J. Pharmacol.* 187:425; 1990). We now study the effects of AJ76 on concurrent accumbens (NAcc) release of DA and serotonin (5-HT) with *in vivo* electrochemistry. Simultaneously, activity patterns were studied by infrared photocell beam detection. Stearate working microelectrodes (Broderick, P.A., *Brain Res.* 495:115,1989) were implanted in NAcc, under Na pentobarbital anesthesia; 9-15 recovery days were allowed. The results show that AJ76 increased both DA and 5-HT release, while concomitantly increasing locomotor activity, rearing behavior, stereotypy and agoraphobic inhibitory behavior (central ambulations). While DA release remained above baseline in the second hour, 5-HT release in concert with behaviors, decreased to baseline values or below. Thus, AJ76 maintains an upregulated DA release while 5-HT and behavioral stimulant components subside, without sedation. Consistent with electrophysiological data (Piercey *et al., Neurosci. Abstr.* 15:1234; 1989), the data demonstrate that AJ76 may have an autoreceptor antagonist mechanism for 5-HT. [Supp: in part Upjohn Co. RF #7-76207].

491.12

MICRODIALYSIS STUDIES OF DOPAMINE (DA) SYNTHESIS IN VIVO: EFFECTS OF D_2/D_3 PREFERRING AGONISTS AND ANTAGONISTS

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To investigate the regulation of DA synthesis in vivo, we used microdialysis to measure striatal DOPA accumulation after perfusion with the aromatic amino acid decarboxylase inhibitor NSD-1015 (m-hydroxybenzylamine)[1]. D_2 and D_3 receptor preferring agonists and antagonists were perfused through concentric type microdialysis probes located in the anterior lateral striata of chloral hydrate anesthetized rats beginning 40 min. before perfusion of NSD. For each subject, probes were inserted bilaterally and levels of Dopa were compared with control responses from the contralateral striatum. Data are presented as the ratio of Dopa levels in drug-treated fractions over Dopa levels in the control striatum. Perfusion with NSD-1015 (100 μM) increased levels of Dopa to 24 ± 2.8 pg/μl in dialysate from control striata at 2 hr. (n = 12). Agonists and antagonists were chosen for their relative D_3 and D_2 receptor selectivity, based on K_i values reported by Sokaloff et al.[2] Perfusion with the DA agonist quinpirole (10 μM) (K_{iD2}/K_{iD3} = 113) resulted in a treated/untreated ratio of 0.63 ± 0.04. Perfusion with the non-selective DA agonist apomorphine (10 μM) (K_{iD2}/K_{iD3} = 1.2) resulted in a ratio of 0.70 ± 0.03. Co-perfusion of the D_2 preferring antagonist haloperidol (30 μM) (K_{iD2}/K_{iD3} = 0.046) with apomorphine resulted in a ratio of 1.04 ± 0.17. Co-perfusion of the D_3 preferring antagonist (+)UH232 (K_{iD2}/K_{iD3} = 4.4) with apomorphine resulted in a ratio of 1.21 ± 0.47. Thus, administration of either D_2 or D_3 preferring agonists decreases DA synthesis in a DA antagonist sensitive manner. These preliminary results (n = 3/group) suggest that microdialysis can be used to determine the relative influence of D_2 and D_3 receptor stimulation on both DA synthesis and release in vivo. [1]Westerink et al., J. Neurochem., (1990) 54:381; [2]Sokoloff et al., Nature, (1990) 347:146. (Supported by DA-04120, and the Mich. Dept. of Mental Health).

491.13

COMPARISON OF DOPAMINE- D2 AND D3 AGONISTS AND ANTAGONISTS [(+)UH-232] AT SYNTHESIS MODULATING DA AUTORECEPTORS in vitro. M.P. Galloway*, M.J. Keegan, and S. Benloucif, Cellular & Clinical Neurobiology, Wayne State Univ Sch Med, Detroit, MI 48207 USA

The recent discovery that mRNA levels for the dopamine (DA)-D3 receptor subtype are sensitive to 6-OHDA lesions suggests that expressed D3 receptors may function as DA autoreceptors. Since DA autoreceptors are defined functionally according to the function they regulate (i.e., DA synthesis, release, and cell firing), we have investigated the effects of D3-preferring agonists (7-OHDPAT) and antagonists [(+)UH232, (+)AJ76] at the DA synthesis modulating autoreceptor. Using the accumulation of DOPA in the presence of the decarboxylase inhibitor NSD1015 as a measure of tyrosine hydroxylation in K^+-stimulated (30mM) striatal slices, we found potent and efficacious agonist activity of monohydroxylated aminotetralins such as 7-OHDPAT and 5-OHDPAT. The DA agonist effects (0.3 μM) were fully reversed by (+)UH232, (+)AJ76, sulpiride, clozapine, haloperidol, and eticlopride. The calculated (Gaddum equation) equilibrium dissociation constant (K_B) for the antagonists at the DA autoreceptor were determined in the presence of 7-OHDPAT: HAL = 3nM, ETIC = 19nM, (-)SULP = 81nM, (+)UH232 = 112nM, clozapine = 427nM, and AJ76 = 1.8μM. In the presence of either 5 mM K^+ or forskolin (10μM), K_B values for (+)UH232 or SULP increased approximately 10 fold suggesting that anatagonist potency in vitro is enhanced by elevated levels of extracellular DA. Comparison of these data with kinetic parameters (K_i) derived from ligand binding studies may elucidate a functional role for D3-DA receptors. Supported by MI Dept of Mental Health and NIDA R01-04120 (MPG).

491.14

IODINATED 7-OH-DPAT: A POTENTIAL LIGAND FOR THE D3 DOPAMINE RECEPTORS. C. Foulon, M-P Kung, J. Billings, V.A. Boundy, P.B. Molinoff, and H.F. Kung*. Depts. of Radiology, Psychiatry and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104

Recent success in cloning the D3 dopamine receptors and defining its properties has generated significant interest in developing new ligands for *in vivo* and *in vitro* studies of this receptor subtype. D3 selective ligands could be useful as pharmacological tools and therapeutic agents. Quinpirole, AJ76, UH232 and 7-OH-DPAT have been reported to be D3 selective. Among them, 7-OH-DPAT appears to show highest affinity (Kd=0.7nM) and selectivity (ratio for Ki of D2/D3>100). 7-OH-DPAT was synthesized according to the published method, and iodination produced the corresponding 6-iodo-7-OH-DPAT(racemic). Spectra, HPLC and elemental analysis are consistent with the expected chemical structure. In *in vitro* binding assays using [^{125}I]NCQ 298 showed similar affinity for D2 (rat striatal membrane) and D3 (membranes are from Sf9 cells infected with a recombinant baculovirus containing the rat D3 gene) The Ki values for D3 receptors were 0.7±0.18 and 12±1.8 nM for 7-OH-DPAT and 6-I-7-OH-DPAT, respectively; while the Ki values for D2 were 156±17 and 264±33 nM, respectively. Adding the iodine atom clearly decreases the D3 affinity of 7-OH-DPAT by 17-fold. Nonetheless, the 6-I-7-OH-DPAT retained D3 selectivity. Radioiodination was accomplished by reacting sodium [^{125}I]iodide in the presence of peracetic acid as the oxidant. *In vivo* biodistribution studies in rats indicated that the 6-[^{125}I]I-7-OH-DPAT crosses the blood-brain barrier easily. Further studies are needed to validate the nature of brain localization. It appears that the 6-[^{125}I]I-7-OH-DPAT may be a useful agent and warrants further evaluation. Acknowledgement: Support from PHS (NS-24538 and NS-18591) and Region Centre (91298012), France.

491.15

CLONING OF THE HUMAN DOPAMINE D3 RECEPTOR AND ITS EXPRESSION AND PHARMACOLOGICAL CHARACTERIZATION IN A VARIETY OF MAMMALIAN CELL LINES. R.A. Horlick, D.S. Conklin, D.G. Grigoriadis, G.M. Cooke, K.D. Cornwell, S. Hutton, E.B. DeSouza and B.G. Sahagan*. CNS Research and Biotechnology Dept., The DuPont Merck Pharmaceuticals Co., Wilmington, DE. 19880-0400

Dopamine receptors are thought to play a key role in the clinical manifestations and treatment of psychoses including schizophrenia. The discovery of multiple dopamine receptors offers new tools for the development of receptor subtype-specific ligands that will effectively ameliorate psychotic symptoms without unwanted extrapyramidal or neuroendocrine side-effects. The dopamine D3 receptor, identified by molecular cloning (*Nature* **347**:146,1990), has an unique localization in the brain. Its presence in areas of the brain linked to the limbic regions and its absence from the pituitary make it a likely target for antipsychotic drug activity without the generation of undesirable side-effects. Here we describe the cloning of the human dopamine D3 receptor from human nucleus accumbens cDNA using PCR. The receptor was introduced into a panel of mammalian cell lines: CHO (Chinese Hamster ovary epithelial cells), 293 (Human embryonic kidney cells), Ltk- (murine fibroblast cells), and GH4C1 (rat pituitary lactotroph cell). The relative levels of expression of the D3 receptor in CHO, 293 and Ltk- cells are as follows: 12,500, 625, and 250 fmol/mg of protein. In all four backgrounds, the receptor exhibited [3H]spiperone binding which was saturable, membrane concentration dependent, temperature dependent, stereoselective and of high affinity. These four dopamine D3 receptor lines exhibit dopaminergic pharmacology with the appropriate rank-order profile for dopamine receptors.

491.16

WITHDRAWN

492.1

ACUTE AUTONOMIC EFFECTS OF D,L-HOMOCYSTEIC ACID LESIONS OF THE INSULAR CORTEX IN SPONTANEOUSLY HYPERTENSIVE AND WISTAR RATS. K.S. Butcher*, V.C. Hachinski and D.F. Cechetto. Robarts Research Institute, University of Western Ontario, London, Ontario, Canada N6A 5K8.

Middle cerebral artery occlusion (MCAO) results in an acute increase in sympathetic activity in Wistar rats, and a decrease in spontaneously hypertensive rats (SHR). There is evidence to suggest that these autonomic changes are due to damage of the insular cortex (IC). The IC was selectively lesioned, using D,L homocysteic acid (DLH; 1 M), in urethane-anesthetized Wistar and SHR rats. Arterial pressure (AP), heart rate (HR), renal sympathetic nerve discharge (SND), and ECG were measured in male SHR (12) and Wistar (12) rats, following a 500 nL injection of DLH or saline control into the IC. Initial AP and SND were not significantly different in SHR (85 ± 3 mmHg; 27 ± 9 $\mu v \cdot s$) and Wistar rats (78 ± 3 mmHg; 26 ± 9 $\mu v \cdot s$). Wistar HR (375 ± 11) was initially higher than that of SHR (319 ± 10; $p<0.05$). In the SHR, AP rose continuously in control animals, and was significantly greater than initial, as well as IC lesioned rats, by 3 hr after injection. SND did not change in SHR control animals, but declined significantly in IC lesioned rats, by 3 hr after DLH injection. HR increased in both groups of SHR. The pattern of changes was different in Wistar rats. IC lesion, in Wistar, resulted in a significant increase in AP by 4 hr after DLH injection. There was no change in the AP or SND of Wistar controls. HR increased in both Wistar groups. IC lesion appears to result in a fall in sympathetic activity in the SHR and a pressor response in the Wistar rat. This suggests that the IC is at least partially mediating the autonomic responses to MCAO. (Supported by HSFC and HSFO)

492.2

CARDIOVASCULAR RESPONSES TO NEURONAL ACTIVATION OF THE EXTENDED AMYGDALA IN ANESTHETIZED AND CONSCIOUS RATS. A.J. Gelsema*, N.E. Copeland, G. Drolet and H. Bachelard. Hypertension Unit, University of Ottawa Heart Institute, Ottawa, and Unité d'Hypertension du CHUL, Université Laval, Québec, CANADA.

The bed nucleus of the stria terminalis (BNST) and sublenticular substantia innominata (SLSI) are considered rostral extensions of the amygdaloid nuclei, but their involvement in cardiovascular control has not been studied. We explored these areas systematically in 27 spontaneously breathing, urethan-anesthetized Wistar rats for sites from where changes in arterial pressure (AP) and heart rate (HR) could be obtained by injection of 20 nL glutamate (Glu, 0.5M). Injections into 84 of the 130 histologically verified sites in the BNST and SLSI were followed after a 8.0 ± 0.7 s. latency by depressor responses ranging from -4 to -33 (mean -13.3 ± 0.8) mmHg, accompanied by variable changes in HR. Pressor responses (4.5 ± 1.3 mmHg) were found after stimulation of only three sites; 43 sites were not responsive. Depressor responses evoked by stimulation in 17 sites in 6 rats before and during paralysis and artificial ventilation were not significantly different ($p>.05$).

A different group of 10 rats was instrumented for bilateral Glu injections (200 nL, 0.1M) into the lateral BNST and for the recording of AP, HR and regional blood flow (using pulsed Doppler flow probes) in the conscious state. Decreases in AP (-10.2 ± 1.6 mmHg) were elicited exclusively, accompanied by small (9.8 ± 4.4 bpm) increases in HR and renal conductance ($11.1 \pm 2.2\%$) and larger ($32.2 \pm 11.3\%$) increases in hindquarter conductance, while mesenteric conductance decreased by $7.9 \pm 3.7\%$.

These results suggest that the BNST and SLSI may participate in the cardiovascular correlates of the defense reaction.
(Supported by the Heart and Stroke Foundations of Ontario and Quebec, and FRSQ)

492.3

CHANGES IN THE NEUROCHEMICAL ORGANIZATION OF FOREBRAIN AUTONOMIC SITES FOLLOWING FOCAL CEREBRAL ISCHEMIA. G.V. Allen*, M.A. DiCarlo and D.F. Cechetto. Robarts Research Institute, University of Western Ontario, London, Ontario, Canada, N6A 5K8.

Occlusion of the middle cerebral artery (MCAO) at the level of the rhinal fissure in anesthetized rats mimics the cardiovascular abnormalities that are observed in patients following focal cerebral ischemia. The stroke-induced autonomic symptoms may be due to pathophysiological changes that occur centrally following brain ischemia. MCAO or sham MCAO was done in pentobarbital-anesthetized male, Wistar rats. Five days after MCAO, the animals were perfused and reacted for the immunohistochemical demonstration of neuropeptide-Y (NPY), leu-enkephalin (leu-ENK) and neurotensin (NT) using the diaminobenzidine reaction. A computerized-microscopic imagine system was used to quantify differences in staining between the two sides of the MCAO brains and between the MCAO rats and sham animals. A three-fold increase in the density of NPY labeled fibers and terminals was observed in the insular cortex and basolateral amygdala ipsilateral to the MCAO compared to that of the contralateral side and sham-operated controls. In the central nucleus of the amygdala, two to three-fold increases in NT and leu-ENK labeled fibers and terminals were also observed ipsilateral to the MCAO. The marked changes in the neurochemical organization of the insular cortex and amygdala following MCAO may underly the autonomic changes that occur following MCAO in the rat and the similar effects observed clinically. (Supported by HSFC and MRC).

492.4

Fos IMMUNOREACTIVITY IN RAT BRAIN ELICITED BY ELECTRICAL STIMULATION OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS. K.H. Harris*, J.H. Jhamandas, and T.L. Krukoff. Depts. of Medicine (Neurology) and Anatomy & Cell Biology, Univ. of Alberta, Edmonton, Canada T6G 2E1.

To further study the role of the paraventricular nucleus (PVN) in autonomic regulation, Fos-immunoreactivity (Fos-ir) was examined as a marker of neuronal activiation after electrical stimulation of the PVN. In urethane-anesthetized rats, the PVN was unilaterally stimulated (25-40 V, 10 sec pulsed trains, 20 Hz, 100 μsec duration) for 1-2 h so that arterial pressure (AP) was elevated 10-25 mm Hg. In control rats, the electrode was placed in the PVN, current was passed briefly to verify placement with AP changes, and current then discontinued. PVN stimulation led to ipsilateral induction of Fos-ir in piriform cortex, insular cortex, medial amygdala, lateral septum, and several hypothalamic nuclei (medial preoptic area, anterior, arcuate, posterior, dorsomedial, ventromedial). In control rats, increases in Fos-ir were found in the ipsilateral piriform cortex and insular cortex. To assess whether increases in Fos-ir were due to ortho- or antidromic stimulation of neurons, PVN magnocellular neurosecretory cells (MNCs) were antidromically activated following pulsed electrical stimulation of their terminals within the neurohypophysis. Preliminary data indicate that there are no differences in Fos-ir within MNCs between stimulated and control animals.

Our results suggest that electrical stimulation of the PVN leads to increases in Fos-ir in many forebrain areas known to receive projections from the PVN. Our data from anesthetized rats also suggest that changes are due to orthodromic stimulation of target neurons and not to antidromic stimulation of inputs to PVN.
Supported by the Medical Research Council of Canada.

492.5

ELECTROPHYSIOLOGICAL AND ANATOMICAL CHARACTERIZATION OF A PROJECTION FROM THE PARABRACHIAL NUCLEUS TO THE DIAGONAL BAND OF BROCA IN THE RAT. J.C. Easaw*, T. Petrov, and J.H. Jhamandas. Depts. of Medicine (Neurology) and Anatomy and Cell Biology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2E1.

The brainstem parabrachial nucleus (PBN) is viewed as an important site for the transfer of cardiovascular-related information from peripheral receptors to the forebrain. Anatomical and electrophysiological methods were utilized in this study to characterize the projection from the PBN to the diagonal band of Broca (DB) a forebrain nucleus implicated in central autonomic regulation.

Rats received iontophoretic injections of the anterograde tracer phaseolus vulgaris leucoagglutinin (PHA-L) in the PBN region. After 14-17 days, the DB from perfused brains was immunocytochemically processed for detection of PHA-L fibers and choline acetyltransferase (ChAT), a synthesizing enzyme for cholinergic neurons. PHA-L fibers with axonal varicosities and boutons were observed to course over ChAT-positive neurons in both the horizontal and vertical limbs of the DB. Extracellular recordings from 348 DB cells revealed both excitatory and inhibitory responses following electrical stimulation in the PBN. 25% (93/348) of DB neurons displayed an increase in excitability (latency 38.81 ± -2.67 ms; duration 47.98 ± 2.97 ms), whereas, 6% (23/348) exhibited a depressant response (latency 28.39 ± 3.69 ms; duration 70.59 ± 13.59 ms) consequent to the PBN stimulus.

These data provide evidence for a predominantly excitatory projection from the PBN to the DB and a portion of this input appears directed towards cholinergic neurons.

Supported by MRC of Canada and AHFMR

492.6

PARABRACHIAL NUCLEUS PROJECTION TO THE AMYGDALA IN THE RAT: ELECTROPHYSIOLOGICAL AND ANATOMICAL OBSERVATIONS. J.H. Jhamandas*, K.H. Harris, T. Petrov, T. Vu and T.L. Krukoff. Depts. of Medicine (Neurology) and Anatomy & Cell Biology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2E1.

The pontine parabrachial nucleus (PBN) is a major relay for the transmission of autonomic inputs to the forebrain. This study describes the synaptic responses evoked within the amygdala (AMYG) following activation of PBN efferents and the chemical identity of AMYG target neurons that receive PBN projections.

Extracellular recordings from 152 AMYG neurons in anaesthetized rats revealed a set of complex synaptic responses following electrical stimulation in the PBN. 47 cells displayed a short duration (34.4 ± 2.0 ms) excitatory response while 30 cells demonstrated a long duration (158.1 ± 15.1 ms) excitation. Inhibitory responses were observed in 17 AMYG neurons. In 6 rats, iontophoretic injections of the anterograde tracer PHA-L were made in the PBN and after 14-18 days, the forebrains of perfused animals were processed immunocytochemically for visualization of PBN projections to AMYG neurons. PHA-L labelled fibers were found throughout the amygdaloid complex; within the lateral and medial subdivisions of the central nucleus of AMYG, fibers with axonal varicosities and boutons were observed coursing over galanin and neurotensin immunopositive neurons.

These results indicate that the PBN input to the AMYG is predominantly excitatory with a less frequently observed inhibitory component. Furthermore these projections are, in part, directed at identified peptidergic neurons.

Supported by the MRC of Canada and AHFMR

492.7

NEUROCHEMICAL CHARACTERIZATION OF PONTINE NEURONS WITH COLLATERALS TO THE CENTRAL NUCLEUS OF THE AMYGDALA (CNA) AND THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS (PVN) IN THE RAT. T. Petrov*, J.H. Jhamandas, and T.L. Krukoff. Depts. of Anatomy and Cell Biology and Medicine (Neurology), Univ. of Alberta, Edmonton, Alberta, Canada T6G 2H7.

It is becoming increasingly clear that brainstem neurons have the potential to simultaneously innervate distant forebrain areas via branching collaterals. Within the nucleus of the solitary tract and the ventrolateral medulla catecholamines have been implicated in such pathways; in the dorsal raphe nucleus,serotonin (5-HT) neurons provide collateral inputs to various targets.

In the present study we examined transmitter- and peptide-containing pontine neurons that innervate, via branching collaterals, the CNA and PVN. Rats were stereotaxically injected with fluorescent labelled retrogradely transported latex microspheres (LLM). Rhodamine-LLM (R-LLM) and fluorescein-LLM (F-LLM) were injected in the CNA and in the PVN respectively. After 4-5 days a third fluorophore (AMCA-conjugated to anti-rabbit IgG) was used to detect substance P (SP), galanin (GAL), choline acetyltransferase (ChAT), tyrosine hydroxylase (TH) or 5-HT immunoreactivity in 40 μm thick pontine coronal sections.

Triple-labelled neuronal profiles containing both R-LLM and F-LLM that were immunopositive for TH or GAL were located in the locus coeruleus; ChAT or SP- in the laterodorsal tegmental nucleus; and 5-HT- in the dorsal raphe nucleus. These results suggest that there exist distinct pathways, originating in the pons that utilize neurotransmitters and neuropeptides to innervate simultaneously the CNA and the PVN. Such chemically specific circuits may selectively transmit and modulate autonomic- and pain-related information within the neuraxis. Supported by MRC and AHFMR.

492.8

DIRECT INNERVATION OF CIRCUMVENTRICULAR ORGANS BY ARCUATE NUCLEUS NEURONS. M.P. Rosas-Arellano* and J. Ciriello. Dept. of Physiology, Univ. of Western Ontario. London, Canada, N6A 5C1.

The arcuate nucleus (ARC) has been suggested to be involved in the regulation of the neuroendocrine and cardiovascular systems, and in body fluid balance. This study was done to investigate the innervation of the circumventricular organs (CVO's) by neurons in ARC using the anterograde tract-tracer Phaseolus vulgaris leuccoagglutinin (PHA-L; 2.5%) in combination with the immunocytochemical localization of atrial natriuretic peptide (ANP) in the rat. PHA-L was iontophoresed into either the dorsomedial (dm), ventromedial (vm) or ventrolateral Arc. After 9-14 days the rats were perfused transcardially and brain sections were processed using the double labelling immunofluorescence technique for identification of PHA-L and ANP. Projections from the dmARC and vmARC were observed to the subfornical organ (SFO), the organum vasculosum lamina terminalis (OVLT), the median eminence (ME), and the area postrema (AP). As the CVO's are midline structures, PHA-L labelled fibers and their presumptive terminals were found throughout these areas. A moderate number of these PHA-L fibers were also labelled for ANP in SFO, OVLT and ME. These data suggest that ARC neurons may be involved in modulating the function of CVO's with regards to drinking behavior, sodium excretion and release of vasopressin to systemic changes in circulating levels of Angiotensin II and plasma osmolality. (*fellow from the Universidad Nacional Autónoma de México; supported by MCR and ICCS of Canada).

492.9

THE EFFECTS OF CARDIOVASCULAR PERTURBATIONS ON NEURONS OF THE BED NUCLEUS OF THE STRIA TERMINALIS (BST). M.F.Wilkinson and Q.J.Pittman. K.E.Cooper*. Dept. of Med. Physiology, Univ. of Calgary, Calgary, Alberta, CANADA T2N 4N1.

Control of febrile body temperature and blood pressure homeostasis are two autonomic functions thought to involve the BST. Via the ventral septal area (VSA), BST neurons affect body temperature during febrile episodes. This pathway may be responsible for the antipyretic effect observed following hemorrhage. We investigated, using in vivo electrophysiology, the effects of blood pressure changes on BST neurons with projections to the VSA. In addition, BST projections to other limbic areas, namely the paraventricular nucleus (PVN) and the lateral habenula (HAB) were also investigated following blood pressure (bp) alterations. Single unit extracellular voltage recordings were made in the BST of urethane-anesthetized (1.5g/kg, ip) S-D rats. Cannulation of the femoral artery and veins were performed for the monitoring arterial bp and iv administration (or hemorrhage, 3mL) of pressor (angiotensin II, 100ng or methoxamine, 40ng) or depressor drugs (nitroprusside, 0.2mg), respectively. Bipolar stimulating electrodes were placed into the VSA, PVN and HAB to evoke antidromic or orthodromic potentials in BST neurons. 50 BST units were antidromically invaded following stimulation of the VSA (n=33), HAB (n=12) or PVN (n=5). No PVN projecting BST neurons responded to changes in bp. However, of the VSA projecting neurons, activity was altered in 19% following increases in bp and 24% following decreases in bp. 25% of HAB projecting BST neurons changed their firing rate following increases in bp and 11% responded to decreases in bp. We also observed bp sensitive BST units with orthodromic connectivity (approx. 20%) in all areas examined. These studies show that neurons of the BST are sensitive to changes in bp and that BST to VSA pathways may play a role in hemorrhage-induced antipyresis. It is also apparent that bp-sensitive BST units are modulated by input from a variety of limbic sources.

492.10

ELECTROPHYSIOLIGICAL AND MORPHOLOGICAL PROPERTIES OF CAUDAL HYPOTHALAMIC HYPOXIC- AND HYPERCAPNIC-SENSITIVE NEURONS *IN VITRO*. G.H. Dillon* and T.G. Waldrop. Dept. of Physiology and Biophysics, Neuroscience Program, and College of Medicine, University of Illinois, Urbana, IL 61801

We have reported previously that hypoxia and hypercapnia stimulate separate populations of caudal hypothalamic neurons in a brain slice preparation. The purpose of the present study was to examine the electrophysiological and morphological properties of these groups of neurons, as well as those neurons unexcited by either stimulus. 400-500 µm coronal slices were taken from Sprague-Dawley rats. Slices were placed in an interface chamber and perfused with nutrient media equilibrated with 95% O_2/5% CO_2. Whole-cell patch recordings were obtained during hypoxic (10% O_2/5% CO_2/85% N_2 or 5% CO_2/95% N_2) and hypercapnic (7% CO_2/93% O_2 or 10% CO_2/90% N_2) bouts. The fluorescent dye lucifer yellow was included in the tips of several electrodes for subsequent morphological analysis. Neurons activated by hypoxia (77% of cells studied) had a resting membrane potential (V_m) of 52.6±1.6 mV; peak input resistance (R_n) was 429.0±72.6 MΩ. Hypoxia elicited a significant depolarization (8.3±1.7 mV) with an accompanying decrease in R_n (to 84.0±5.4% of control) in excited neurons. Hypercapnia-stimulated neurons (35% of cells studied) tended to have a lower resting V_m (48.4±1.7 mV) and a lower R_n (353.1±60.4 MΩ) than those stimulated by hypoxia. CO_2 elicited a depolarization of 2.9±0.6 mV in stimulated cells. Neurons unexcited by either stimulus were electrophysiologically similar to hypoxia-stimulated neurons (V_m = 51.9±2.9 mV, R_n = 442.5±61.9 MΩ). Post-inhibitory rebound and inward rectification were present in all cell types. Hypoxia-stimulated neurons had typically 3-4 main projections, many with subsequent branching, from the cell body. Projections toward the third ventricle and/or ventral surface were common. We suggest electrophysiological, and possibly morphological, properties may underlie the different responses to hypoxia and hypercapnia seen in these cell populations. (Supported by NIH 38726 and AHA).

492.11

AFFERENT PROJECTIONS TO THE DORSOMEDIAL HYPOTHALAMIC AREA MEDIATING STRESS-INDUCED CARDIOVASCULAR CHANGES. T.J. Cavanagh* and J.A. DiMicco. R&D Division, Boehringer Mannheim Corp., and Prog. in Med. Neurobiol., Ind. Univ. Sch. of Med., Indianapolis IN 46202.

Previous studies suggest that neurons in the dorsomedial hypothalamus mediate stress-induced cardiovascular changes in rats. Blockade of inhibitory $GABA_A$ or stimulation of excitatory amino acid receptors in this region elicits cardiovascular effects resembling those caused by experimental stress. Conversely, stimulation of $GABA_A$ or blockade of excitatory amino acid receptors at the same sites abolishes or attenuates the increases in heart rate and blood pressure seen in a paradigm for emotional stress. In order to identify afferents to this region, WGA-HRP (1 or 2%) was coinjected with kainate (5 pmol) in 5 nL. Retrograde transport was assessed after 48 hrs only in those experiments where heart rate increased by >80 beats/min with onset <20 sec. In all experiments where the cardiovascular response met these criteria, injection sites were clearly located in the hypothalamic dorsomedial nucleus (DMH). Most areas previously shown to project to the DMH were labelled, but no labelling was present in the hippocampus. Some of the previously described afferents were more precisely defined in light of new cytoarchitectonic descriptions. Also, labelling was noted in regions not previously described as projecting to the DMH, including the anterior parvicellular paraventricular nucleus (APVHp), and cells within the posterior intralaminar complex of the thalamus (subparafascicular and lateral parafascicular nuclei, retroethmoid nucleus, posterior intralaminar nucleus). Although neuropeptide content of APVHp has been characterized immunohistochemically, its afferent and efferent connections are largely unknown. The posterior intralaminar complex, noted for its dense distribution of CGRP-immunoreactive cells, is thought to relay visceral sensory information to insular cortex and to project to amygdala and medial preoptic nucleus, regions implicated in central cardiovascular control. These findings (1) support previous suggestions of a key role for the DMH in the integration of the cardiovascular response to emotional stress, and (2) provide novel evidence for afferent projections to the DMH from the APVHp and the posterior intralaminar complex of the thalamus. (Supported in part by USPHS Grant NS 19883)

492.12

SYMPATHOADRENAL AND VASOPRESSIN (AVP) INVOLVEMENT IN THE PRESSOR RESPONSE TO ADMINISTRATION OF CARBACHOL (CCh) INTO THE POSTERIOR HYPOTHALAMIC NUCLEUS (PHN). J. R. Martin*, Dept. of Pharmacol., KCOM, Kirksville, MO 63501.

Injection of CCh into the PHN of conscious rats evokes a dose-dependent rise in mean arterial pressure (MAP). To define the mechanisms involved in this increase, antagonists were administered intravenously to Sprague-Dawley rats instrumented for MAP measurement and injection of CCh (50 nl of 5.5 or 3.3 nmol) into the left PHN. Pretreatment with prazosin (PRAZ; 0.2 mg/kg) or yohimbine (YOH; 0.3 mg/kg) attenuated the CCh-induced increase in MAP. Pretreatment with an AVP V_1 receptor antagonist (AVPX; 20 µg/kg) attenuated the pressor response to 5.5 but not 3.3 nmol of CCh. Combining YOH or AVPX with PRAZ further attenuated the pressor response. Combining PRAZ, YOH and AVPX resulted in an initial decrease in MAP which was reversed by addition of propranolol (PRO; 1 mg/kg) thereby revealing an underlying increase in MAP. Pretreatment with PRO alone enhanced the increase in MAP evoked by 5.5 but not 3.3 nmol of CCh. Only the combination of pentolinium (10 mg/kg), methyl-atropine (2 mg/kg) and AVPX completely blocked the CCh-evoked increase in MAP. These results suggest that AVP, epinephrine, norepinephrine, and a fourth unidentified substance are involved in the pressor response evoked by injection of CCh into the PHN. (Supported by AHA MO Affiliate and HL-44531.)

492.13

INTERACTION BETWEEN THE LATERAL HYPOTHALAMUS AND THE MEDIAL SEPTAL AREA IN THE CONTROL OF CARDIOVASCULAR, FLUID AND ELECTROLYTIC RESPONSES INDUCED BY CENTRAL CHOLINERGIC ACTIVATION IN RATS. A.S. Haibara, L.A. De Luca Jr.*, W.A. Saad, L.A.A. Camargo, A. Renzi and J.V. Menani. Dept. of Physiology, School of Dentistry, UNESP, Araraquara, SP 14800, Brazil.

In the present study we investigated the effect of bilateral electrolytic lesions of the lateral hypothalamus (LH) on the pressor, dipsogenic, natriuretic and kaliuretic responses induced by cholinergic activation (carbachol injection) of the medial septal area (MSA). In addition, the effect of opiate agonist (FK 33824) injection into the LH on the same responses was also studied. Male Holtzman rats were used. The bilateral lesion of the LH (1 and 18 days) or the injection of FK 33824 (100 ng) into the LH impaired the pressor, dipsogenic, natriuretic and kaliuretic responses induced by the injection of carbachol (2 nmol) into the MSA. The results show that pathways dependent on the LH integrity are involved in the cardiovascular, fluid and electrolytic responses to a cholinergic activation of the MSA. They also suggest that opiate pathways of the LH have an inhibitory action on the effects produced by cholinergic activation of the MSA.

Research supported by FAPESP and CNPq.

492.14

CARDIOVASCULAR RESPONSES TO PARAVENTRICULAR (PVN) INJECTIONS OF OPIOID AGONISTS IN CONSCIOUS RATS. Hélène Bachelard* and Guy Drolet. Unité de Recherche sur l'Hypertension, Centre de Recherche du CHUL, Université Laval Québec (Qc), G1V 4G2.

The present study was designed to investigate the regional haemodynamic effects of some opioid agonists injected bilaterally into the PVN of conscious Wistar Kyoto rats. The rats were chronically instrumented with intracerebral cannulae, intravascular catheters and pulsed Doppler flow probes in a three step surgery. PVN microinjection of artificial CSF had no consistent effects whereas the μ-opioid agonist, DAGO (D-Ala^5, MePhe^4-Gly^5-ol-enkephalin) produced dose-related cardiovascular effects. DAGO (1.0 nmol) produced a significant ($P<0.05$, ANOVA followed by a Dunnett test) increase in mean arterial blood pressure (maximum, +17 $\pm$ 3 mm Hg, mean + s.e.m.), a fall in both renal (-27 $\pm$ 7%) and mesenteric (-45 $\pm$ 4%) vascular conductances and an increase in hindquarter (+79 $\pm$ 22%) vascular conductance. There was no significant change in heart rate. Moreover, PVN microinjections of increasing doses (0.01-5.0 nmol) of a δ-opioid agonist, DPDPE (D-Phe^{2,5}-enkephalin) and a κ-opioid agonist, U50488H, had no cardiovascular effects. Together these results suggest that PVN μ-opioid receptors might be important in cardiovascular regulation.

The work was supported by the FRSQ, Fondation des Maladies du Coeur du Québec, MRCC and Banting Research Foundation.

492.15

INTRACELLULAR RECORDINGS FROM RAT DIAGONAL BAND OF BROCA (DBB) NEURONS AND RESPONSES TO NOREPINEPHRINE (NE) IN BASAL FOREBRAIN-HYPOTHALAMIC EXPLANTS J.T. Cunningham*, C.R. Jarvis, R. Nissen, B. Hu & L.P. Renaud, Neuroscience Unit, Loeb Res. Inst., Ottawa Civic Hospital, Ottawa, Ontario K1Y 4E9.

Electrophysiological studies in the rat have demonstrated that the noradrenergic innervation of the DBB mediates the baroreceptor-induced inhibition of vasopressin-secreting (Vp) neurons in the hypothalamic supraoptic nucleus. Moreover, NE injections in the DBB selectively arrest the spontaneous firing of supraoptic Vp neurons. In the present study, we utilized intracellular recordings in basal forebrain explants to define the effects of NE on DBB neurons whose axons project towards the supraoptic region. Virtually all DBB neurons antidromically activated from the supraoptic region have action potentials ranging 0.9 to 1.45 ms and afterhyperpolarizations greater than 100 ms. Similar features have been ascribed to cholinergic neurons in the DBB. Both antidromic and non-antidromic DBB neurons displaying these features were consistently depolarized by bath application of NE (100-120 uM) from membrane potentials of -60 to -70 mV. The results indicate that putative-cholinergic neurons in the DBB are sensitive to NE and may participate in the baroreceptor-induced inhibition of supraoptic Vp neurons. (Supported by Heart & Stroke Foundation of Ontario and the MRC).

492.16

INFLUENCE OF INTRAVENTRICULAR PRETREATMENT WITH α-AGONISTS AND THAT OF HYPOTHALAMIC STIMULATION ON THE PRESSOR ACTION INDUCED BY VASOPRESSIN IN RATS. N. Ono*, T. Furukawa and H. Kamiya, Dept. of Pharmacol., Fac. of Pharmaceut. Sci., Fukuoka Univ., Fukuoka, 814-01, Japan.

Influences of α-agonists on the central regulation of blood pressure induced by vasopressin were investigated using urethane-anesthetized rats. Vasopressin (1-10 nmol) administered intracerebroventricularly (i.c.v.) elicited dose-relatedly the pressor and positive chronotropic effects restored about 1-2 hr after administration. The pressor action of vasopressin were inhibited by the intravenous pretreatment with pentolinium and phentolamine, the i.c.v. pretreatment with α-agonists, norepinephrine, phenylephrine, methoxamine and V1-antagonist, d(CH2)5OMe(Tyr)-AVP, but not the i.c.v. pretreatment with clonidine and β-blocker. While the pressor action was augmented by the i.c.v. pretreatment with prazosin. In the experiment of electric stimulation to the hypothalamic nucli, the pressor action of vasoressin was supressed significantly by the continuous stimulation (for 15 min, 200 μA, 2 msec) of anterior hypothalamic area. The supression was frequence-dependently (10 and 12.5 Hz). These results suggest that vasopressin may be affected by α-1 subsystem of the CNS in cardiovascular regulation through the activation of sympathetic nervous system, all involving in the CNS.

492.17

ROLE OF CORTICOTROPIN-RELEASING FACTOR IN MEDIATING CARDIOVASCULAR RESPONSES TO SEROTONIN AND A SEROTONIN$_{1A}$ RECEPTOR AGONIST. A. Dedeoğlu and L.A. Fisher*. Department of Pharmacology, College of Medicine, University of Arizona Health Sciences Center, Tucson, AZ 85724.

Corticotropin-releasing factor (CRF), by virtue of its central nervous system (CNS) distribution and actions, is hypothesized to be a neurotransmitter in brain pathways mediating the endocrine, autonomic and cardiovascular responses to stressful stimuli. Hypophysiotropic CRF neurons (i.e., those regulating pituitary ACTH secretion) are stimulated by serotonin (5-HT) and several 5-HT receptor subtype-selective agonists. The purpose of the present study was to test the hypothesis that 5-HT and related agonists likewise produce excitatory effects on CRF-containing neurons governing cardiovascular function. This hypothesis was based on previous studies demonstrating that low doses (< 10 nmol) of 5-HT and the 5-HT$_{1A}$ receptor agonist, 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), act within the CNS to elevate arterial pressure (AP) and heart rate (HR), effects similar to those produced by CRF administration. All experiments were performed in conscious, unrestrained male Sprague-Dawley rats (220-250 g) previously instrumented with lateral cerebroventricular (icv) guide cannulas and iliac arterial catheters. Icv administration of CRF (0.15 nmol), 5-HT (1 nmol), and 8-OH-DPAT (3 nmol) produced concurrent increases in AP (12-15 mm Hg) and HR (45-75 beats/min). Icv administration of the CRF receptor antagonist, α-helical CRF$_{9-41}$ (9 nmol), did not alter AP or HR when given alone. Co-administration of α-helical CRF$_{9-41}$ significantly attenuated (by 50-100%) the pressor and tachycardic responses to injections of CRF, 5-HT, and 8-OH-DPAT. It is concluded that the cardiovascular actions of low icv doses of 5-HT and 8-OH-DPAT are in part mediated by the release of CRF within the CNS.

492.18

SELECTIVE ELIMINATION OF HYPOTHALAMIC NEURONS BY GRAFTED HYPERTENSION-INDUCING NEURAL TISSUE R.Eilam, R. Malach, L. Sklair*, M. Segal, Dept. Neurobiology, Brain Research, The Weizmann Institute, Rehovot 76100, Israel.

Embryonic hypothalamic tissue originating from spontaneously hypertensive rats (SHR) was implanted in young normotensive (Wistar Kyoto; WKY) rats in an attempt to localize brain regions directly responsible for the induction of hypertension. A 30% elevation in host systolic blood pressure was noted 3 months after implantation in animals grafted with rostral hypothalamic tissue (R-SHR), while that of the group receiving caudal tissue did not change. The hypertension in the R-SHR group was accompanied by hypertrophy of the heart and kidneys. A 77% reduction of vasopressin immunoreactive (VP+) of parvocellular cells was noted in the paraventricular nucleus (PVN) of the R-SHR group. In the C-SHR animals, on the other hand, the parvocellular VP+ remained unaltered but the magnocellular VP+ was reduced by 53%. TH immunoreactivity did not show difference in PVN cells number between experimental and control groups, suggests a possible specificity of disappearance of VP+ cells. While we do not know why these cells degenerate, the disappearance of VP+ PVN parvocellular cells in the R-SHR's indicates that they may contribute to the development of hypertension.

492.19

C-FOS EXPRESSION IN HYPOTHALAMIC NEURONS OF HEMORRHAGIC AND HYPOTENSIVE RATS. E. Shen*, S. L. Dun, T. H. Chiu and N. J. Dun. Dept. of Anatomy & Dept. of Pharmacol., Medical College of Ohio, Toledo, OH 43699

One hour after lowering the arterial blood pressure of adult Sprague Dawley rats to 60-70 mm Hg by either withdrawing 5-6 ml of blood from the femoral artery or infusion of nitroprusside (2 mg/ml,total volume injected 60-120 μl/hr), Fos-immunoreactivity (Fos-IR) was detected in neurons of the supraoptic (SON) and paraventricular (PVN) nuclei. Double staining with antibodies to vasopressin (AVP) and oxytocin (OXY) showed that 70% and 20% of Fos-IR neurons in the SON and PVN were AVP-positive; whereas, 5% and < 1% Fos-IR cells in SON and PVN were OXY-positive. Sectioning of the carotid sinus nerve reduced the number of Fos-IR neurons in the epsilateral hypothalamus. An increase of Fos-IR neurons could be detected as early as 15 min and reached the plateau one hour after hemorrhage or hypotension; the number remained elevated in 3 hr. Northern blot analysis of c-fos mRNA from the hypothalami showed a consistent increase, reaching the peak between 30-60 min. The results show that lowering of blood pressure effectively induces c-fos mRNA and Fos-IR in hypothalamic neurons known to be associated with cardiovascular regulation. (Supported by NS 18710 & NS24226).

492.20

PROJECTIONS FROM THE PARAVENTRICULAR NUCLEUS TO THE CAUDAL VENTROLATERAL MEDULLA. S.L. Cravo, L.R.G. Britto*, L.V.Bonaldi. Dept. of Physiology, São Paulo Medical Sch.; Dept. of Physiol. Biophysics, Univ. of São Paulo, Brazil.

The paraventricular nucleus of hypothalamus (PVH) plays a major role in the regulation of body fluid homeostasis. Besides acting through the secretion of arginine-vasopressin (AVP), the PVH can also modulate the activity of other brain autonomic nuclei. We sought to determine the relationship between PVH and the vasomotor centers of the ventrolateral medulla (VLM). In anesthetized rats, the anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L) was iontophoresed into PVH. After 7-14 days, animals were reanesthetized, perfused and tissue was processed immunocytochemically for PHA-L. Our results showed that: i) PHA-L-immunostained fibers were found in the ipsilateral n. of tractus solitarius (NTS), and rostral (RVL) and caudal (CVL) nuclei of VLM; ii) RVL fibers were distributed over the region of C1 adrenergic neurons; iii) PVH projections to VLM were particularly dense at the CVL level. A dense network of fibers and terminals was observed extending from the parambigual region to the ventral surface between the lateral reticular and the V nerve spinal nuclei. Double label experiments combining retrograde transport of fluorescein- labeled microspheres with AVP immunocytochemistry demonstrated double-labeled cells in the PVH after injections into CVL. We conclude: a) PVH projects to both vasomotor center of ventrolateral medulla, particularly to CVL; b) PVH vasopressinergic cells are part of this projection. (Supported by FAPESP, Brazil).

CARDIOVASCULAR REGULATION: UPPER BRAINSTEM MECHANISMS

493.1

CHARACTERISTICS OF CENTRAL SEROTONIN (5-HT) UPTAKE SITES IN HYPERTENSIVE RATS: INTERACTION WITH CLONIDINE AND CENTHAQUIN Anil Gulati, Ramesh C. Arora and John W. Crayton*, Dept. of Pharmacodynamics, Univ. of Illinois, Chicago, and Section Biol. Psychiat., VA Hospital and Depts. Pharmacol. & Psychiat., Loyola Univ., Hines IL.

The binding of a highly specific ligand for 5-HT uptake sites, [^{3}H] paroxetine, was studied in brain regions of normotensive Wistar Kyoto (WKY) and spontaneous hypertensive (SHR) rats. [^{3}H] paroxetine bound to a single, high affinity binding site in the brain. The affinity (K_d) and density (B_{max}) of [^{3}H] paroxetine binding were found to be similar in spinal cord, pons and medulla and cerebral cortex of WKY and SHR rats. However, in midbrain the B_{max} values of [^{3}H] paroxetine binding were significantly reduced (27.16 %) in SHR as compared to WKY rats. The K_d values were found to be similar in SHR and WKY rats. The effects of clonidine and centhaquin, centrally acting hypotensive agents, on [^{3}H] paroxetine binding were also determined and compared with imipramine, a known 5-HT uptake inhibitor. Clonidine did not displace [^{3}H] paroxetine binding at any concentration (1 X 10^{-4} to 1 X 10^{-7} M). On the other hand, centhaquin, which produces hypotension similar to clonidine, could displace [^{3}H] paroxetine binding in a concentration dependent manner. In cerebral cortex and brain stem the IC_{50} values of imipramine and centhaquin for [^{3}H] paroxetine binding were found to be similar in WKY and SHR rats. The affinity (K_i) of centhaquin to displace paroxetine from 5-HT uptake sites was 10 times lower than imipramine, while clonidine had no effect on 5-HT uptake sites. The results indicate that (1) the density of 5-HT uptake sites is reduced in the midbrain of hypertensive rats, and (2) centhaquin, a centrally acting hypotensive agent, acts on 5-HT uptakes sites. This study suggests the possiblity that 5-HT uptake mechanisms play a role in hypertension.

493.2

MESENCEPHALIC CUNEIFORM NUCLEUS PATHWAYS SERVING CARDIOVASCULAR RESPONSES DURING ADAPTATION TO STRESS. S.M. Korte*, D. Jaarsma, P.G.M. Luiten, and B. Bohus. Dept. of Animal Physiol., University of Groningen, P.O. Box 14, 9750 AA HAREN, The Netherlands.

The aim of the present study was to explore the neuroanatomic network that underlies the cardiovascular responses of reticular formation origin in the region of the cuneiform nucleus. The left iliac artery was supplied with a catheter for the measurement of systolic blood pressure. Low intensity electrical stimulation if the mesencephalic reticular formation (MRF) in the vicinity of the cuneiform nucleus (CNF) always resulted in pressor and bradycardiac responses, whereas stimulation in the parabrachial nucleus (PB) and Kölliker-Fuse nucleus (KF) led to a pressor response and a small tachycardiac response. The efferent connections of the effective stimulation sites in the CNF area, were investigated by anterograde tracing with the lectin Phaseolus vulgaris leucoagglutine (PHA-L). The CNF sends descending fibers to the gigantocellular reticular nuclei (GI), the motor nucleus of the vagus (DMNV) and nucleus tractus solitarious (NTS). These projections are probably involved in the bradycardiac response to stimulation. The descending pathway to the NTS/DMNV and GI may therefore be the parasympathetic limb of the circuit. Furthermore, the CNF sends ascending fibers to limbic forebrain areas and descending fibers to the PB-KF complex. The KF in its turn projects to the rostroventrolateral medullary nucleus (RVLM) and the intermediolateral cell column (IML). These latter projections are partly involved in producing the pressor response and thereby represent the sympathetic limb of the circuit. The widespread connections of the CNF correspond with the possible role of this area in the integration and production of fear-related immobility (orientation and freezing) accompanied by anticipatory/expectancy-related bradycardia.

493.3

EFFECT OF NEUROTRANSMITTER ANTAGONISTS IN THE PARABRACHIAL NUCLEUS ON VISCERAL INPUT TO THE THALAMUS. T.M. Saleh* and D.F. Cechetto, Robarts Research Inst./University of Western Ontario, London, Canada, N6A 5K8.

The putatative neurotransmitter(s) in ascending visceral sensory pathways was investigated by recording the changes in the response of thalamic neuronal activity evoked by vagal stimulation before and after neurotransmitter antagonist injection into the parabrachial nucleus (PB). Synaptic blockade with cobalt (10 mM) injections into the PB resulted in inhibition of both the evoked thalamic response to vagal stimulation (100%) and the spontaneous firing of thalamic neurons (86%). Injections of kynurenate (0.25 μM) or the NMDA antagonist AP5 (200 μM) effectively blocked the evoked thalamic response to vagal stimulation (94% and 100% respectively), but did not significantly change spontaneous activity. In contrast, injections of phentolamine (α-agonist; 0.1 μM) or yohimbine (α-2 antagonist; 200 μM) into the PB inhibited the spontaneous firing of thalamic units (56% and 77% respectively) but had no effect on the evoked response to vagal stimulation. Prazosin and propranolol (α-1 and β antagonists respectively) were ineffective. Bicuculine significantly enhanced spontaneous thalamic unit activity (76%) without affecting the evoked response to vagal stimulation. Atropine (0.1 μM) had no significant effects. These results indicate that an excitatory amino acid NMDA receptor is necessary for transmission of visceral input through the PB and that neurons in the PB with α-2 receptors contribute to the tonic, spontaneous firing activity of ventral basal thalamic neurons. (Supported by the Heart and Stroke Foundation of Ontario)

493.4

EFFECTS OF PEPTIDES IN THE PARABRACHIAL NUCLEUS ON VISCERAL INPUT TO THE THALAMUS. D.F.Cechetto* and T.M.Saleh. Robarts Research Inst./ University of Western Ontario, London, Ont., Canada. N6A 5K8

The role of neuropeptides in ascending visceral pathways was investigated by recording the changes in the response of thalamic neuronal activity evoked by vagal stimulation before and after peptide injection in the parabrachial nucleus (PB) in anaesthetized rats. Injection of calcitonin gene-related peptide (CGRP, 5 mM), substance P (SP, 2 mM) or cholecystokinin (CCK) into the PB significantly attenuated the vagal evoked response of thalamic neuronal activity. In contrast, vagal evoked responses were significantly enhanced (508%) by injection of neurotensin (NT, 1 mM) into the PB. Spontaneous activity of thalamic neurons was inhibited by CGRP and SP and excited by NT injections into the PB. Cholecystokinin (CCK) at low doses (0.0002 - 0.2 mM) attenuated (up to 78%), while the highest dose, 2 mM, briefly enhanced (189%) the spontaneous activity of thalamic units before inhibiting their activity. Injections of somatostatin (SOM; 1 mM) did not significantly alter the response evoked by vagal stimulation, but significantly inhibited the spontaneous firing of thalamic units resulting in a ten-fold increase in the response to background ratio. The results indicate that these peptides play a significant and specific role in the PB in modulating visceral sensory input to the ventral basal thalamus. Their function may be to relay specific visceral modalities or specific patterns of visceral sensory information to the forebrain.
(Supported by the Heart and Stroke Foundation of Ontario)

493.5

SYNAPTIC BLOCKADE OF LATERAL TEGMENTAL FIELD NEURONS DOES NOT ALTER THE CARDIOVASCULAR RESPONSES TO MUSCULAR CONTRACTION OR HYPOTHALAMIC STIMULATION. G.A. Iwamoto* and T.G. Waldrop. Depts. of Physiology & Biophysics and Veterinary Biosciences, Univ. of Illinois, Urbana, IL 61801.

Prior results have indicated that neurons in the lateral tegmental field (LTF) are involved in the generation of sympathetic drive. Our previous studies have shown that contraction of hindlimb muscles increases the discharge rate of LTF neurons and elevates mean arterial pressure (MAP) and heart rate (HR). The purpose of the present study was to determine if blockade of synaptic transmission in the LTF alters the pressor response to muscular contraction. MAP and HR responses to muscular contraction (evoked by stimulation of the L_7 and S_1 ventral roots) and caudal hypothalamic stimulation (hypo stim) were recorded before and after microinjections (100 nl) of $CoCl_2$ into the LTF in anesthetized cats. Both muscular contraction and hypo stim elicited increases in MAP and HR during control conditions. Bilateral $CoCl_2$ (100 mM) microinjections into the LTF did not alter these cardiovascular responses. Moreover, LTF microinjections of lidocaine (1%) did not have any effects upon the responses to muscular contraction and hypothalamic stimulation. Thus, synaptic transmission through the lateral tegmental fields is not required for full expression of the cardiovascular responses to muscular contraction or caudal hypothalamic stimulation. (Supported by HL 06296 and American Heart Association-IL Affiliate).

493.6

***IN VIVO* NORADRENERGIC NEURAL ACTIVITIES IN LOCUS COERULEUS OF WISTAR KYOTO RATS AND SPONTANEOUSLY HYPERTENSIVE RATS.** K.H. Ko*, Y.T. Kim, M.J. Jo and E.K. Lee. Department of Pharmacology, College of Pharmacy, Seoul National University, Seoul 151, Korea.

Purpose of the present study was to adress the question whether *in vivo* noradrenergic neural activity in locus coeruleus is coupled to the manifestation of hypertension. Two groups of animals were prepared, 1) normotensive wistar kyoto rats (WKY) and 2) spontaneously hypertensive rats (SHR). At 16 weeks of age, blood pressure (BP) and release of norepinephrine (NE) and 3,4-dihydroxyphenylglycol (DOPEG) from locus coeruleus of WKY and SHR were measured by microdialysis technique at three conditions; 1) normal; 2) α_1-agonist (phenylephrine) systemically treated; 3) α_1-, α_2-, β-receptor agonist or antagonist (phenylephrine, phentolamine, clonidine, yohimbin, isoproterenol, propranolol) treated into locus coeruleus through microdialysis probe. At normal condition, BP of WKY was 106.46 ± 2.87 mmHg and BP of SHR 180.42 ± 2.57 mmHg. Basal outputs of NE and DOPEG were 0.539 ± 0.050 pg/20min, 1.896 ± 0.050 pg/20min in WKY and 0.897 ± 0.278 pg/20min, 3.568 ± 0.500 pg/20min in SHR respectively. Following systemic application of phenylephrine, BP was increased but the release of NE was decreased in both WKY and SHR, while the level of DOPEG was decreased only in SHR. However, activation of α_1-, α_2-, β-receptor increased the level of DOPEG in WKY whereas the blockade of those receptors did not. Stimulation as well as blockade of α_1- and β-receptor consistently increased the level of DOPEG while those of α_2-receptor did not. BP and the release of NE did not change significantly in any condition of those. The result from the present study suggests that noradrenergic neural activity in locus coeruleus may not be strongly coupled to regulatory mechanism of hypertension in adult WKY and SHR.

493.7

THE ORIGINS OF NON-CATECHOLAMINERGIC VASOPRESSOR AREAS IN THE GIGANTOCELLULAR TEGMENTAL FIELD OF THE ROSTRAL PONS IN CATS. S.D. Wang*, H.T. Horng. J.C. Liu, J.S. Kuo and C.Y. Chai. Dept. of Biol. and Anat., Natl. Defen. Med. Ctr., Dept. Med. Res Taichung Veterans Gen. Hosp., and Inst. of Biomed Sci., Academia Sinica, Taiwan, Republic of China.

The present study used retrograde technique to examine the origins of the gigantocellular tegmental field (FTG) of the rostral pons, in which vasopressor responses could be induced by rectangular pulses and/or sodium glutamate. Different from other vasopressor areas in the pons and medulla, regions of FTG did not contain cells immunoreactive to catecholamines. After vasopressor response was induced at FTG, HRP was injected to the same region. After two days of survive, the animals were sacrificed to process for HRP histochemical reaction from upper thoracic spinal cord to diencephalon. HRP-labeled cells were observed at dorsomedial and ventrolateral regions of the medulla and pons. Besides, numberous HRP-labeled cells were evident at the periaqueductal gray of the midbrain. These results suggest that vasopressor neurons in the non-catecholaminergic FTG regions may work with the vasopressor neurons in other catecholaminergic areas to integrate cardiovascular functions.

493.8

BARORECEPTOR INHIBITION OF SUPRAOPTIC VASOPRESSIN CELLS: NO ROLE FOR LOCUS COERULEUS. T. A. Day* and J. R. Sibbald, Dept. of Physiology and Pharmacology, University of Queensland, Qld 4072, AUSTRALIA.

Activation of arterial baroreceptors inhibits neurosecretory vasopressin (AVP) cells but the central pathways mediating this effect remain open to dispute. We have now investigated the proposal (Banks and Harris, J. Physiol., 1984) that inhibition of supraoptic nucleus (SON) AVP cells following carotid sinus baroreceptor activation is mediated via noradrenergic neurons of the ipsilateral locus coeruleus (A6 group).

Extracellular recordings were obtained from SON AVP cells in pentobarbital anaesthetized male rats in which all baroreceptor afferents except those arising from the carotid sinus had been sectioned. Electrolytic lesions which destroyed the ipsilateral locus coeruleus and immediately surrounding tissue reduced the frequency of AVP cell inhibition following baroreceptor activation (2-10 µg metaraminol i.v.) from 93% (14/15 cells) to 35% (9/26 cells). However, this effect was not duplicated by inhibition of local neurons: injection of gamma aminobutyric acid (100 mM, 100-150 nl, i.e. 10-15 nmol) into the ipsilateral locus coeruleus had no effect on AVP cell activity or responses to baroreceptor activation, even when the contralateral locus coeruleus had been destroyed by electrolytic lesion (6/6 cells). Finally, the effects of ipsilateral locus coeruleus stimulation on AVP cell activity was tested. Electrical stimulation (100-200 µA) altered the activity of 6/30 AVP cells, inhibiting 1 and exciting 5, but chemical stimulation (200 µM glutamate, 100 nl) was without effect (8/8 cells).

These data suggest that changes in AVP cell responses to baroreceptor activation after dorsolateral pons manipulations are attributable to effects on axons of passage rather than neurons of the locus coeruleus or the adjacent area.

493.9

FASTIGIAL STIMULATION INCREASES C-FOS EXPRESSION IN SELECTED BRAINSTEM AUTONOMIC NUCLEI. X. Xu* F. Zhang and C. Iadecola, Dept. of Neurology, Univ. of Minnesota, Minneapolis, MN 55455.

Electrical stimulation of the cerebellar fastigial nucleus (FN) elicits profound cardiovascular, cerebrovascular and behavioral changes. These responses are initiated not from local FN neurons but from passing fibers that may originate in the dorsal parabrachial n. (PBd)(Brain Res 473:352, 1988). We used c-fos expression as a marker of neural activity to determine whether FN stimulation activates neurons in PBd. Rats were anesthetized (halothane 1-3%), paralyzed and ventilated. Arterial pressure and blood gases were controlled. The FN or cerebellar white matter (CWM) was stimulated for 1 hr (50-100 µA; 50 Hz; 1 sec on/1 sec off) through microelectrodes. Fos, the gene product of c-fos, was detected immunocytochemically. Fos+ cells were counted in several brainstem regions. In rats that were surgically prepared for brain stimulation but not stimulated (n=7), numerous Fos+ cells were seen in the nucleus tractus solitarii (NTS; 52±13), PBd (74±20), paraventricular hypothalamus (PVH; 192±31) and periventricular thalamus (84±25; Th-PV). In rats anesthetized and sacrificed without surgery (n=5) there were few or no Fos+ cells in these regions. Stimulation of the FN (n=5), but not CWM (n=5), increased the number of Fos+ cells ($p<0.05$) in vestibular complex (VC; +179%), PBd (+181%), intralaminar thalamus (Th-IL; +282%), Th-PV (+131%), and medial amygdala (+214%). FN or CWM stimulation did not affect the number of Fos+ cells ($p>0.05$) in parvocellular reticular n., NTS, ventral parabrachial, PVH, lateral and medial habenula. We conclude that: (1) Surgery, anesthesia and ventilation may induce c-fos expression in brainstem nuclei involved in autonomic regulation and, (2) FN stimulation increases c-fos expression in some regions receiving monosynaptic projections from FN neurons (VC, Th-IL) as well as regions that do not, most notably PBd. The increase in c-fos expression in PBd supports the hypothesis that FN stimulation activates reciprocal projections between parabrachial nuclei as they cross the midline via the subfastigial bundle. Thus, PBd may participate in the responses elicited by FN stimulation. (Supported by the American Heart Association of Minnesota)

493.10

RESPONSES OF NEURONS IN THE SUBRETROFACIAL ROSTRAL VENTROLATERAL MEDULLA (RVLM) OF THE CAT TO NATURAL VESTIBULAR STIMULATION. B.J. Yates*, T. Goto & P.S. Bolton. Lab. of Neurophysiology, Rockefeller Univ., New York, NY 10021.

There is considerable evidence suggesting the existence of vestibulosympathetic reflexes (VSR) which may be important in counteracting posturally-related changes in blood pressure (Yates, Brain Res. Rev. 1992). We previously showed that over 2/3 of the neurons in the RVLM with slowly-conducting projections to the thoracic spinal cord responded to vestibular nerve stimulation (Yates et al., Brain Res. 1991), and hypothesized that these cells are likely to mediate VSR. In the present study we determine the nature of vestibular inputs to this region by analyzing the responses of RVLM neurons to sinusoidal whole body tilts in vertical planes and to horizontal rotations.

Experiments were conducted on decerebrate cats that were baroreceptor-denervated, vagotomized and had a cervical spinal transection. Of the 38 neurons whose type of vertical vestibular inputs could be classified, the majority (27) received signals mainly from otolith organs, 4 had inputs predominantly from semicircular canals, and 7 were categorized as receiving convergent inputs from otoliths and canals. In addition, only 2 of 68 neurons responded to horizontal rotations. The presence of otolith-dominant labyrinthine inputs to the RVLM is consistent with the hypothesis that this area mediates VSR which participate in correcting orthostatic hypotension.

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493.11

HYPOVOLEMIC HYPOTENSION PRODUCES LOCALIZED GLUTAMATE INCREASES WITHIN THE CARDIOVASCULAR PRESSOR REGION OF THE CEREBELLAR FASTIGIAL NUCLEUS

T. J. Parry* and J. G. McElligott. Dept. of Pharmacology, Temple University School. of Medicine, Philadelphia, PA 19140.

The cerebellar pressor region located in the rostro-medial fastigial nucleus has been shown to be important for the regulation of blood pressure. Our previous work demonstrated that microdialysis administration of kainic acid into this area produced a dose dependent increase in blood pressure and an elevation of extracellular levels of several amino acid neurotransmitters, namely, glutamate, taurine, aspartate, glycine, and GABA. In order to determine if any of these transmitters plays a functional role in blood pressure regulation, we examined the relationship between a natural perturbation to the cardiovascular system (hypovolemic hypotension) and transmitter levels within the pressor region of the fastigial nucleus. Experiments were conducted on unanesthetized rats implanted with bilateral femoral catheters. Thus, blood pressure was monitored while being reduced from 111 ± 6 to 50 mm Hg within 5 minutes by controlled hemorrhage. Six rats that had been previously adapted to head restraint were implanted with microdialysis probes (dia. = 250 μM; tip length 1 mm) within the pressor region of the fastigial nucleus. Microdialysis samples were taken every 10 minutes before, during, and after experimentally induced hypovolemic hypotension. In these animals, there was a significant increase (42% above baseline; $p \leq 0.05$) in extracellular glutamate during this hypotension which returned to baseline following reflex recovery from hypotension. There was also a delayed increase in GABA levels (38%; $p \leq 0.05$) which reached significance when reflex recovery was complete. These GABA levels then remained elevated for the duration of the experiment. No changes were detected in the levels of the other amino acid neurotransmitters. Futhermore, reinfusion of the blood did not produce any change. In addition, no changes in amino acid neurotransmitter levels during the experimentally induced hypotension were measured in the cerebellar dentate nucleus, an area unrelated to cardiovascular control. (Supported by a grant from the American Heart Association-Southeastern Pennsylvania Chapter).

CARDIOVASCULAR REGULATION: SPINAL CORD AND MEDULLA

494.1

CAROTID SINUS NERVE STIMULATION INDUCES FOS-LIKE IMMUNOREACTIVITY WITHIN ADRENERGIC, NORADRENERGIC AND SEROTONERGIC NEURONS OF THE RAT BRAINSTEM.

J.T. Erickson* and D.E. Millhorn. University of North Carolina, Chapel Hill, NC)

Stimulation of the rat carotid sinus nerve (CSN) induces a discrete distribution of Fos-like immunoreactivity (Fos-LI) within the medulla oblongata (Erickson and Millhorn, Brain Res. 567:11;1991). In this study, we extend these observations to the pons and midbrain and demonstrate, using immunohistochemical double labeling techniques, that many of these functionally activated neurons are adrenergic, noradrenergic or serotonergic. After electrical or hypoxic stimulation of CSN afferent fibers, Fos colocalization was observed with phenylethanolamine-N-methyltransferase (PNMT), or tyrosine hydroxylase (TH) in the A1/C1 cell groups in the ventrolateral medulla and to a lesser degree within the A2/C2 cell groups in the dorsal vagal complex (DVC). Fos was also observed within serotonergic cells of raphe pallidus, raphe magnus, in the "parapyramidal region" just lateral to the pyramids, and along the ventral medullary surface. Within pons, prominent Fos-LI was observed in the lateral parabrachial complex, the Kolliker-Fuse nucleus, and within the dorsal tegmental region in and around nucleus raphe dorsalis. In addition, Fos colocalization was observed with TH in the A5 cell group and within locus coeruleus. Fos colocalization with serotonin in nucleus raphe dorsalis was relatively rare. Within the midbrain, Fos immunoreactivity was consistently observed along the midline, ventral to the cerebral aqueduct, intermixed but rarely colocalized with TH-positive cells, and within the inferior colliculus.

494.2

HEMORRHAGE INDUCES FOS-IMMUNOREACTIVITY IN AMINERGIC AND SEROTONINERGIC NEURONS IN THE RAT MEDULLA.

S. L. Dun and N. J. Dun.* Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699

In anesthetized rats 1 hr after lowering the arterial blood pressure to 60-70 mm Hg by removing 4-5 ml of blood,numerous cells containing nuclear Fos-immunoreactivity (Fos-IR) were detected in the nucleus of the solitary tract (NTS) and ventrolateral medulla (VLM). A number of Fos-IR neurons were also noted in the inferior olive, nucleus raphe obscurus and raphe pallidus. In sham-operated rats only a few Fos-IR neurons were scattered in the NTS, VLM and other nuclei. Double labeling techniques with antisera to tyrosine hydroxylase (TH), phenylethanolamine-N-methyltransferase (PNMT) and 5-HT showed that nearly all TH-positive cells in the NTS and VLM were Fos-IR. Fos-IR neurons in the rostral VLM were both TH- and PNMT-positive, whereas they were TH- but not PNMT-positive in the caudal VLM. Only a few TH-or PNMT-positive cells were Fos-IR in the C3 area. Most of the 5-HT positive neurons in the raphe obscurus and raphe pallidus were Fos-negative. The results indicate that during hemorrhage, noradrenergic neurons in the caudal VLM and caudal NTS, and adrenergic neurons in the rostral VLM, and to a lesser extent the C3 adrenergic cells and 5-HT neurons, are activated insofar as c-fos expression is concerned.

494.3

FOS EXPRESSION IN NEURAL CIRCUITS SERVING CARDIOVASCULAR AND BODY FLUID REGULATION.

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The *c-fos* immediate early gene is acutely induced in many brain regions by relevant stimuli; Fos immunoreactivity (Fos-IR) presents a useful mapping technique to identify activated neuronal systems. After presentation of various body fluid and cardiovascular stimuli, Fos-IR was observed in rat brainstem regions: nucleus of the solitary tract (NTS), area postrema (AP), ventrolateral medulla (VLM), and parabrachial nucleus (PBN); and in hypothalamic regions: organum vasculosum of the lamina terminalis (OVLT), preoptic nucleus medianus (NM), bed nucleus of the stria terminalis (BNST), subfornical organ (SFO), paraventricular nucleus (PVN), and supraoptic nucleus (SON). Hypertonic NaCl did not induce Fos in SFO and only weakly in the brainstem. Ang icvt induced Fos poorly in brainstem whereas hemorrhage or iv Ang induced Fos throughout the circuit. Hypertonic NaCl activated Fos in both dorsal and ventral SON whereas induction by hemorrhage was more focused in the ventral SON where vasopressin predominates over oxytocin. Blockade of glutamate NMDA receptors with MK-801 failed to prevent Fos-IR for any of the stimuli in any of the regions. Histochemical mapping of nitric oxide synthase with NADPH-diaphorase showed a striking overlap with Fos-IR in hypothalamus but not brainstem.

494.4

IDENTIFICATION OF DEPRESSOR NEURONS IN RABBIT VENTROLATERAL MEDULLA MEDIATING THE BARORECEPTOR VASOMOTOR REFLEX: A PHYSIOLOGICAL AND C-FOS IMMUNOHISTOCHEMICAL STUDY.

Yu-Wen Li* and R. A. L. Dampney. Department of Physiology, University of Sydney, NSW, 2006, AUSTRALIA.

Three series of experiments were carried out to identify depressor cells in the rabbit ventrolateral medulla (VLM) that mediate the baro-vasomotor reflex. In the first series, rabbits were anesthetised with urethane, paralysed and ventilated, and arterial pressure (AP) was recorded. The depressor area in the VLM was mapped by glutamate microinjection (0.02M, 20nl) in the same rabbits before and after baro-denervation. Before denervation depressor responses were only evoked from sites in the VLM caudal to the obex. After denervation, the depressor area extended more rostrally to the level just caudal to the rostral pressor area. In the second series, the effect of baroreceptor stimulation on c-fos expression in VLM neurons was studied in conscious rabbits. Raising AP 20 mmHg for 60 min greatly increased, compared to control cases, the number of c-fos immunoreactive neurons in the VLM (c-fos antibody, OA-11-823, CRB). The location of these c-fos positive neurons in the VLM corresponded closely to the depressor area mapped in baro-denervated rabbits. In the third series, rabbits were prepared as in the first series, and AP and renal sympathetic nerve activity (rSNA) were recorded. Bilateral microinjections of muscimol (5 nmol in 50 nl) into the rostral part of the VLM depressor area, but not the caudal part, virtually abolished the reflex rSNA response to AP alterations. These results suggest that (1) the VLM depressor area in the rabbit extends more rostrally after baro-denervation; (2) baroreceptor activation induces neuronal c-fos expression throughout this depressor area; (3) neurons in the rostral part of the depressor area are critical for the expression of the baro-vasomotor reflex.

494.5

MECHANISMS MEDIATING VASOPRESSIN RESPONSES TO SIMULATED HAEMORRHAGE: ELECTROPHYSIOLOGICAL AND C-FOS STUDIES. D.W. Smith*, J.R. Sibbald and T.A. Day, Dept. of Physiology and Pharmacology, University of Queensland, Qld 4072, AUSTRALIA.

Central and peripheral mechanisms mediating vasopressin (AVP) responses to hypotensive haemorrhage are not fully understood. We have now used short duration caval occlusion as a model to investigate potential mechanisms underlying haemorrhage-induced activation of rat supraoptic nucleus (SON) AVP cells.

Extracellular recordings in pentobarbital anaesthetised rats showed that intra-thoracic inferior vena cava occlusion excited most SON AVP cells within 5-15 sec. This was unaltered by sectioning of cardiac buffer nerves or cervical spinal cord, suggesting central receptor involvement.

Central mechanisms mediating the AVP response were further investigated by examining caval occlusion-induced expression of the immediate early gene c-Fos. Animals were perfused 60-90 mins after caval occlusion and tissue processed for Fos plus AVP, oxytocin (OT) or tyrosine hydroxylase (TH) immunocytochemistry using a two colour immunoperoxidase technique. SON cells showed a significant increase in Fos-like immunoreactivity (FLI), predominantly in AVP cells. The A1 cell group of caudal medulla and the central nucleus of amygdala showed mild increases in FLI but the strongest correlations with SON FLI were apparent in the lateral bed nucleus stria terminalis (BST) and at the ventral surface of the rostral medulla.

These data suggest possible involvement of BST and ventral medulla chemosensitive cells in AVP responses to haemorrhage.

494.6

TIME COURSE OF HEMORRHAGE EFFECTS ON TYROSINE HYDROXYLASE mRNA IN MEDULLARY CATECHOLAMINE NEURONS MANIFESTING FOS INDUCTION. R.K.W. Chan* and P.E. Sawchenko, The Salk Institute, La Jolla, CA 92037.

Medullary catecholamine cell groups are involved in multiple modes of cardiovascular regulation and display various indices of functional activation, including widespread *c-fos* expression, in response to hypotensive hemorrhage. Assessments of the impact of such challenges on transmitter-related gene expression are complicated by the varying degrees of biochemical and connectional heterogeneity that characterize these cell groups. We used quantitative hybridization histochemical methods to follow the effects of 15% hemorrhage on levels of mRNA encoding tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis; concurrent staining for nuclear Fos-immunoreactivity (Fos-ir) permitted comparisons between cells that ostensibly were and were not targeted by the challenge. Increased levels of TH mRNA were detected in Fos-ir neurons in all cell groups examined. Mean maximal increases ranged between 133 and 168% of control values, and were attained within 0.5-1 hr post-hemorrhage in noradrenergic (A1 and A2) cell groups, and at 2 hr in adrenergic ones (C1, C2, dorsal strip). By 4 hr after the challenge, TH mRNA levels in Fos-ir neurons in all cell groups had returned to control values. By contrast, TH mRNA in *non*-Fos-ir cells either did not change significantly over the course of the experiment (C2 and dorsal strip), or showed a very rapid and transient increase, whose magnitude was less than that seen in Fos-ir cells (A1, A2, C1 cell groups). The extent to which this early response might precede the capacity of neurons to manifest detectable Fos-ir is not clear. These findings indicate that hemorrhage up-regulates TH mRNA levels in medullary catecholaminergic cell groups which have access to adaptive neuroendocrine and/or autonomic control mechanisms. The paradigm employed here may prove of value in assessing the impact of environmental events on gene expression at a cellular level in any complex or heterogeneous cell group.

494.7

Fos IMMUNOREACTIVITY IS INDUCED IN RAT SPINAL CORD AUTONOMIC AREAS FOLLOWING LIPOPOLYSACCHARIDE (LPS) INJECTION. N.C.Tkacs* AND A.M.Strack. UCSF Dept. of Physiology, San Francisco, CA 94143-0444.

We have investigated LPS treatment as a model of increased drive to the sympathetic nervous system as indicated by Fos expression in spinal cord loci of sympathetic preganglionic neurons. Male Sprague-Dawley rats were surgically instrumented with intravenous and intraarterial lines and allowed to recover for 4-6 days. On experimental days, saline or LPS (0.2 mg/kg or 1.0 mg/kg) was administered intravenously. After three hours the animals were deeply anesthetized, then perfused with saline and paraformaldehyde; brains and spinal cords were removed and processed for Fos immunocytochemistry (sheep anti-Fos, Serotec). LPS administration resulted in a dose-related appearance of Fos-positive nuclei in spinal segments T4-T13 in the intermediolateral, intercalated, and central autonomic nuclei and the lateral funiculus. LPS also induced Fos in a few cells in the T3 spinal segment. Several stress- and autonomic-related hypothalamic and brainstem nuclei showed Fos (+) cells including SON, PVN (magnocellular and parvocellular), circumventricular organs, amygdala, locus coeruleus, parabrachial region, NTS, and cells within the rostral and caudal ventrolateral medulla. These results demonstrate activation of central autonomic regions and sympathetic preganglionic neurons by LPS treatment.

494.8

CENTRAL STRUCTURES RELATED TO RENAL NERVES IDENTIFIED BY TETANUS TOXIN C-FRAGMENT. L.P. Solano-Flores*, M.P. Rosas-Arellano* and J. Ciriello. Dept. of Physiology, Univ. of Western Ontario, London, Canada, N6A 5C1.

The afferent and efferent information conveyed by renal nerves (RN) to and from the central nervous system is thought to be an important component of mechanims involved in cardiovascular and body fluid homeostasis. The present study was done to identify the central structures that integrate RN information using the anterogradely and retrogradely trans-synaptically transported tracer, the C-fragment of tetanus toxin (TTC). The central stump of the cut left RN of anesthetized adult Wistar rats was covered by a TTC (3-5 ul; 30 mg/ml)-soaked piece of gelatin foam and enclosed with vinyl plastic. The animals were allowed to survive for 7-14 days and then perfused transcardially. Transverse or horizontal sections of the forebrain; brainstem and spinal cord were processed immunohistochemically for the visualization of TTC. Neurons and neuropil containing TTC immunoreactivity were observed in the spinal cord, commissural aspect of the nucleus of the solitary tract, area postrema, the region of the A5 cell group, arcuate nucleus, median eminence, subfornical organ, and organum vasculosum laminae terminalis. The celiac and nodose ganglia were also densely labelled. These data suggest that RN may be functionally linked to several central sites via both spinal cord pathways and the vagus nerve. (* postdoctoral fellows from Universidad Nacional Autónoma de México; support by MCR and ICCS of Canada).

494.9

HEMODYNAMIC ANALYSIS OF THE CENTRALLY EVOKED CARDIOVASCULAR RESPONSES OF ENDOTHELIN-1 (ET). M.A. Hashim* and A.S. Tadepalli, Division of Pharmacology, Burroughs Wellcome Company, Research Triangle Park, NC 27709.

Previously, we reported that central ET produces profound decreases in blood pressure (*Am. J. Physiol* 262: H1-H9, 1992). The hemodynamic effects of central ET were elaborated in this study. In anesthetized, ventilated rats, cardiac output (CO) and renal blood flow were monitored by ultrasound Doppler and skin blood flow (SBF) in the hindpaw by laser Doppler flowmetry. ET (10 pmol) applied to the IV cerebral ventricle decreased mean arterial blood pressure (40 ± 5%; peak response, mean ± SE) for up to 60 min. Cardiac output increased initially (64 ± 30%) for up to 15 min and subsequently returned to baseline level. Total peripheral vascular resistance (TPR) decreased (53 ± 12%) while SBF increased (71 ± 17%) for up to 60 min. Changes in heart rate were small and variable. These data suggest that: i) central ET-induced hypotension is due to peripheral vasodilation and ii) initial increase in cardiac output may be due to decreased output impedance. RBF decreased (33 ± 6%) concomitant with the blood pressure, but did not recover for up to 90 min; renal vascular resistance remained unchanged for up to 60 min. These observations suggest that the fall in RBF is secondary to the hypotension, probably because the blood pressure is below the autoregulatory limits of the kidney. Thus, the primary cardiovascular response of centrally administered ET appears to be a reduction in TPR, probably via withdrawal of sympathetic vascular tone. Peripherally administered ET increases blood pressure and decreases RBF; these effects are due, primarily, to the vasoconstrictor actions of ET. In contrast, this study suggests that central ET-induced hypotension is due to peripheral vasodilation; the fall in RBF is secondary to the hypotension and occurs in the absence of renal vasoconstriction.

494.10

SYMPATHOADRENAL NEURONS RECEIVE MORE TYROSINE HYDROXYLASE SYNAPSES THAN SYMPATHETIC PRE-GANGLIONIC NEURONS PROJECTING TO THE SUPERIOR CERVICAL GANGLION. I.J. Llewellyn-Smith*, J.B. Minson, P.M. Pilowsky and J.P. Chalmers, Dept of Medicine and Centre for Neuroscience, Flinders University, Bedford Park SA 5042 AUSTRALIA

Sympathetic preganglionic neurons (SPN) receive synapses from nerve fibres that contain a variety of neurotransmitters. However, it is not known if the density of synapses from different types of nerve fibres varies depending upon the target of the SPN. In this study, varicosities that directly contacted or synapsed on rat SPN projecting to the adrenal medulla or superior cervical ganglion (SCG) were evaluated for the presence of tyrosine hydroxylase (TH) immunoreactivity. Cholera toxin B subunit (CTB) was injected into either the SCG or the adrenal medulla and the rats were perfused after 2-3 days. Parasagittal sections through the lateral horn were stained simultaneously for CTB and TH. Immunoreactivity was localized with avidin-biotin-peroxidase and a nickel-DAB reaction. TH-immunoreactivity was present in 25.9% of terminals that contacted or synapsed on sympathoadrenal neurons (305/1178; range in 4 rats, 24.8%-28.1%) whereas only 11.2% of terminals on SPN projecting to the SCG contained TH-immunoreactivity (120/1073; range in 4 rats, 10.3%-12.8%). These ultrastructural findings support the physiological evidence that there is regional specificity in the control of sympathetic outflow.

494.11

BIOCYTIN-FILLED SYMPATHETIC PREGANGLIONIC NEURONS IN THE CAT LUMBAR AND THORACIC SPINAL CORD LACK AXON COLLATERALS. P.M. Pilowsky*, I.J. Llewellyn-Smith, L.F. Arnolda, J.B. Minson and J.P. Chalmers. Dept of Medicine and Centre for Neuroscience, Flinders Univ., Bedford Pk 5042, AUSTRALIA.

A few recent studies have used the technique of intracellular recording and dye-filling to assess the detailed morphology of electrophysiologically identified sympathetic preganglionic neurons (SPN). These studies have revealed that in the cat thoracic spinal cord, SPN have extensive dendritic arborisations that are mostly restricted to the nucleus intermediolateralis pars principalis (IMLp), and that their axons arise either directly from the soma, or from a primary dendrite-like process. No examples of axon collaterals have been reported. We report here results from a survey of 26 SPN from the T3 (n=13) or L3 (n=13) spinal segments that had been identified by stimulation of the appropriate white ramus, and then filled intracellularly with biocytin. Biocytin is a recently introduced marker that is particularly effective in demonstrating axon collaterals. Most of the somata were found in the IMLp. The soma of one neuron in the L3 segment was found 100μm lateral to the IMLp. There was a positive correlation between conduction velocity and soma area ($r=0.46$; $P<0.05$). The majority of SPN dendrites were found in the IMLp, although several had dendrites that projected dorsolaterally, or medially as far as the central canal. No examples of axon bifurcation or collateralization were found. These results confirm the findings of others with regard to the dendritic morphology of SPN, and demonstrate a lack of axon collaterals from SPN in the cat lumbar and thoracic spinal cord.

494.12

DISTRIBUTION OF PEPTIDES IN AUTONOMIC NUCLEI OF SPINAL CORD IN THE RAT. Y.Hong* and L.C.Weaver. John P.Robarts Res. Inst. and Dept. of Physiology, Univ. Western Ontario, London, Canada N6A 5K8

The distribution of substance-P (SP), enkephalin (Enk) and VIP in fibers and cells was examined in the lower thoracic (T) and lumbar (L) segments of the rat spinal cord. Attention was focused on the relationship between the location of the peptides and sympathetic preganglionic neurons (SPNs) contributing to the greater and lesser splanchnic nerves. To identify splanchnic preganglionic neurons, Fluorogold was applied to the left splanchnic nerve in anaesthetized rats and some of these animals received intrathecal administration of colchicine (10-15 μg each at T6, T9 and T12) 24-48 hrs before perfusion with fixative. The spinal cords (T6-L2) were sectioned at 40 μm and SP-, Enk- and VIP-like immunoreactivity (Li) in fibers and cells was detected with fluorescent immunocytochemical techniques. Most retrogradely labelled cells (90%) were located in the nucleus intermediolateralis (IML) and the rest were situated in the nucleus intercalatus spinalis (IC) and the central autonomic (CA) region of the gray matter. Terminals of fibers containing immunoreactivity to all three peptides were found in all autonomic regions. Fibers containing SP- and Enk-Li were seen projecting in the white matter to the region of the IML and extending from the IML to CA. Putative terminals containing each of the three peptides were further found surrounding the retrogradely labelled cells in the IML. Approximately 2 cells containing VIP-Li were found per section and 80% were located in the autonomic regions. Fewer cells with SP- and Enk-Li were observed (approximately 1 per section) and 70% were outside Laminae VII and X. Although SP-, Enk- and VIP-Li cells were located in all autonomic regions of the spinal cord, very few cells were doubly labelled with retrograde dye and with the antisera to either of the peptides. The data suggest that 1) SP, Enk and VIP are contained in fibers of neurons regulating preganglionic sympathetic control of the abdominal viscera and its vasculature; 2) these peptides may not be major transmitters within splanchnic SPNs. (Support: MRC Canada and Rick Hansen World Tour Society)

494.13

IDENTIFICATION OF RENAL PREGANGLIONIC NEURONS USING HERPES SIMPLEX 1 IN HAMSTERS. N.S. Dehal, G.Dekaban, A. Krassioukov, F. Picard and L.C. Weaver*. J.P. Robarts Research Institute, London, Ont., Canada.

Members of the family of Herpes viruses have been used in various animals to trace pathways from target tissues to the CNS. We used Herpes Simplex 1 (HSV1) to trace the sympathetic pathway from the kidney to the spinal cord. Initially we determined that rats (n=20) and guinea pigs (n=9) were not susceptible to infection with neurotropic strains of HSV1 after injections into visceral organs or ganglia. Hamsters were susceptible to infection with the McIntyre strain of HSV1. To verify that control of renal sympathetic nerves in hamsters is similar to that in rats, electrophysiological studies were done using anesthetized hamsters. Accordingly, blockade of firing of rostral ventrolateral medullary neurons with microinjections of glycine decreased discharge of hamster renal sympathetic nerves by 41±3% and this nerve activity was reduced by only 11±5% after cervical spinal cord transection. To identify renal neurons 5µl of HSV1 ($1x10^9$ pfu/ml) was injected into one kidney of hamsters. After 3-7 days they were perfused and ganglia, spinal cords and brains were removed. Immunohistochemistry was used to visualize the virus-infected neurons. Sympathetic preganglionic neuron (SPN) labelling was found in the ipsilateral intermediolateral cell column of the spinal cord as well as the lateral funiculus in 13 of 28 hamsters. Most infected SPNs were located in thoracic segments 7-9 (T7-9) but the virus labelled SPNs as high as T3 in hamsters with longer survival times. Infected neurons were not found caudal to T11 and rarely were found in the medulla. SPN morphology was usually normal, showing detailed dendritic arborizations, and lysis was not prevalent. Small infected cells were sometimes observed close to SPNs. Because HSV1 was not found in ganglionic neurons in these same hamsters, the polymerase chain reaction was used in different hamsters and detected viral DNA in the paravertebral ganglia ipsilateral to the kidney injected with HSV1. In conclusion, because HSV1 infects a limited number of neurons and appears to cross only one or two synapses it cannot identify all neurons in a pathway. However, the absence of lysis and lack of cellular reaction to the infection permit neurons in a select population to be studied in detail. Support:MRC Canada

CARDIOVASCULAR REGULATION: CARDIAC CONTROL AND SYMPATHETIC RHYTHMS

495.1

DIRECT VISUALIZATION OF OPIOID RECEPTORS ON PREGANGLIONIC PARASYMPATHETIC CARDIAC NEURONS IN THE NUCLEUS AMBIGUUS. D. Mendelowitz* and D.L. Kunze. Department of Molecular Physiology and Biophysics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

It is well known that administration of opiates evoke pronounced cardiorespiratory depression. However, the neurons that possess opioid receptors and are responsible for these responses are unknown. In this study we examined whether preganglionic parasympathetic cardiac neurons in the nucleus ambiguus possess opioid receptors and, therefore, could be responsible for the increased parasympathetic activity and bradycardia.

Preganglionic parasympathetic neurons were identified as described previously (*Neurosci. Lett.* 132:217-221, 1991). Rats were anesthetized with pentobarbital, respirated, and a right thoracotomy was performed to expose the heart. To label cardiac motorneurons the retrograde fluorescent rhodamine tracer (XRITC) was applied to the epicardial surface of cardiac tissue that contain parasympathetic ganglia. The incision sites were closed and the animals recovered for 3 days.

The animals were then sacrificed and the medulla was cut in sections 200 microns thick using a vibratome. Tissue that contained cardiac neurons was incubated in a solution containing 10 microM of the opioid antagonist naloxone labeled with a fluorescent fluorescein tag for 2 hours.

Parasympathetic cardiac neurons and opioid receptors were visualized simultaneously using a laser confocal scanning microscope. Opioid receptors were densely localized to the soma of cardiac neurons, as well as other unidentified neurons, within the nucleus ambiguus. Electrophysiological studies will be needed to identify the potency of different opioid agonists, and the role of opiates in mediating parasympathetic cardiac activity.

495.2

HETEROGENEOUS DISTRIBUTION OF NEUROTRANSMITTER EXPRESSING NEURONS IN THE CANINE PARASYMPATHETIC CARDIAC GANGLIA. M. Mostafa, E.J. Neafsey, R.D. Wurster and T.S. Gray*. Neuroscience Program, Loyola University Stritch Sch. of Med, Maywood, Il. 60153

The distribution of choline acetyltransferase (ChAT), dopamine beta hydroxylase (DBH), substance P, vasoactive intestinal polypeptide (VIP), neuropeptide Y and serotonin in the dog cardiac ganglia was examined using immunohistochemistry. The cardiac ganglia were immersion fixed in 4.0% paraformaldehyde in borate buffer. Frozen sections were cut and processed using the nickle-intensified DAB immunoperoxidase technique.

All large cells were intensely immunoreactive for ChAT and lightly positive for DBH. Fewer large cells contained substance P and VIP immunoreactivity and were distributed evenly throughout the various ganglia. Distinct clumps of large cells were stained positive for neuropeptide Y. Smaller neurons that resembled intensely fluorescent cells stained positively for serotonin and DBH. Many densely staining neuropeptide Y fibers and terminals were observed throughout the ganglia. The terminals appeared to contact large neurons in the ganglia. Fewer fibers stained positive for VIP and substance P. DBH immunostained fibers usually were observed outside the ganglia, but on occasion entered the ganglia.

The results demonstrate that there is heterogeneous distribution of neurotransmitter phenotypes in the parasympathetic cardiac ganglia of the dog. (Support: NIH HL 27595)

495.3

POTASSIUM (K^+) CURRENTS IN ADULT RAT INTRACARDIAC NEURONS *IN SITU*. S.X.Xi-Moy*, N.J.Dun. Dept. of Anat., Med. Col. of Ohio, Toledo, OH 43699.

Potassium currents of *in situ* parasympathetic, intracardiac ganglion neurons of 5-wk-old rats were studied using single-electrode voltage-clamp techniques. Ganglia were dissected from small fat pads on dorsal atrial wall. Intracellular Lucifer Yellow iontophoresis verified that intracardiac ganglion neurons are unipolar cells with few dendrites. Resting potentials ranged from -40 to -79 mV. Prolonged intracellular current pulse evoked either single or multiple (30-35%) spike(s). Spontaneous EPSPs (with or without spikes) or pacemaker-like action potentials were observed in approximately 40% of the neurons. An inwardly rectifying current was slowly activated by step hyperpolarization and was blocked by cesium (1 mM). This current showed a steady-state amplitude of 200-400 pA and threshold of -80 to -90 mV. Background currents evoked in a Ca^{2+}-free solution containing cesium (1 mM), TEA (10 mM) and 4-AP (1 mM) showed the characteristics of "leak" K^+ conductance. The time- and voltage-dependent outward currents activated by step depolarization in the presence of TTX (3 μM) showed reversal potentials as predicted by the Nernst equation. The depolarization-activated K^+ currents were reduced by TEA (10 mM) or Ca^{2+}-free solution, suggesting the presence of both delayed rectifying K^+ current and Ca^{2+}-activated K^+ current. Inhibition of K^+ currents increased membrane excitability and evoked spontaneous spikes, and may also increase the presynaptic release of ACh. These results underscore the important role of K^+ currents in vagal control of cardiac functions. (Supported by AHA Ohio Affiliate)

495.4

DOES SMOKING IMPAIR VAGAL CONTROL OF HEART RATE? B. Bishop*, J. A. Hirsch, and J. L. York. Dept. Physiology, SUNY/Buffalo; and Research Institute on Alcoholism, Buffalo, NY 14214.

Respiratory sinus arrhythmia amplitude (RSA) is the difference between maximum and minimum instantaneous HRs following inspiratory onset, and is accepted as an index of vagal cardiac control. We studied 33 smokers (S) and 33 non-smokers (NS), who were matched for age, race, gender, height, and blood pressure, in supine and seated positions. Mean resting HR, obtained over 30 seconds, was higher in S than NS suggesting that chronic tobacco use alters the relative sympathetic and parasympathetic contributions to cardiac control. RSAs, determined for 10 consecutive deep (>50% VC) and slow (5-7/min) breaths, were not different in S and NS in either position, but RSA was higher in the seated than in the supine position, suggesting that smoking blunts neither respiratory nor baroreflex modulation of vagal cardiac control. We concluded the higher HRs in smokers is likely the result of the sympathomimetic effects of nicotine rather than an impaired vagal control. (Supported by NIAAA RO1-AA06867)

495.5

CHOLINERGIC INFLUENCES ON BEHAVIORAL AND CARDIAC RESPONSES TO ACUTE UMBILICAL CORD COMPRESSION IN THE RAT FETUS. S. M. Umphress*, S. R. Robinson and W. P. Smotherman. Laboratory of Perinatal Neuroethology, Center for Developmental Psychobiology, SUNY-Binghamton, Binghamton, NY 13902-6000.

On day 20 of gestation, fetal rats exhibit a stereotypic bradycardia and transient increase in motor activity following experimental compression of the umbilical cord. Previous research has identified a role for cholinergic mediation of fetal cardiac responses, but not motor responses, to other forms of sensory stimulation. In this experiment, pregnant rats were prepared by chemical transection of the spinal cord to permit direct observation of fetal rats in vivo. Fetuses were fitted with paired cardiac leads for measurement of heart rate (HR) and received an ip injection of atropine (1.0 mg/kg) or the isotonic saline vehicle 9 min before testing. Real-time recordings of fetal motor behavior and HR were collected during a 1-min period prior to placement of a microvascular clamp on the umbilical cord, a 2-min period of umbilical cord compression, and a 3-min period following removal of the clamp and restoration of umbilical circulation. Saline-injected fetuses showed the typical, brief period of increased motor activity, which was predominated by lateral flexions of the body trunk, and a pronounced HR deceleration, which gradually returned to pre-clamp baseline levels after removal of the clamp. Atropine-injected fetuses exhibited a slower onset of bradycardia and more rapid return to baseline HR after removal of the clamp. Further, atropine-injected subjects showed a reduced behavioral response during the period of cord compression and exhibited a secondary increase in trunk movements during recovery. These findings suggest a role for parasympathetic mediation of the magnitude and patterning of both cardiac and motor responses to cord compression.

495.6

NORCOCAINE AND COCAETHYLENE SHARE DELETERIOUS CARDIO-ELECTROPHYSIOLOGICAL EFFECTS WITH COCAINE. H. K. Erzouki, S. R. Tella, S. R. Goldberg* and C. W. Schindler. Behav. Pharmacol. and Genetics Lab., NIDA Addiction Research Center, Baltimore, MD 21224.

To compare the electrophysiological effects of cocaine with its metabolites, three different groups of pentobarbital anesthetized, artificially ventilated rats were infused with cocaine (COC), norcocaine (NC), and cocaethylene (CE) at a rate of 1.5 mg/kg/min (n = 5-6). CE is formed when cocaine and alcohol are used in combination. If remarkable changes in cardiac electrical function were observed, $NaHCO_3$ (4 mEq/kg) was given to attempt reversal of the observed effect. Mean blood pressure (BP), sinus cyclic length (CL), PR interval, QRS duration, QT interval and blood pH were measured throughout the experiments.

	CL (ms)	PR (ms)	QRS (ms)	QT (ms)	PH
Pre-COC	202±25	50±5	20±1	75±11	7.441
COC	304±42	76±11	59±8*	147±15*	7.380
COC +$NaHCO_3$	502±83†	50±7	27±3†	91±11†	7.826†
Pre-NC	208±15	63±9	22±3	86±13	7.424
NC	299±37	104±15	53±8*	151±20*	7.300
NC +$NaHCO_3$	340±53	80±9	26±3†	152±22	7.693†
Pre-CE	165±9	52±5	18±1	58±1	7.443
CE	283±9*	97±10*	57±5*	152±15*	7.383
CE +$NaHCO_3$	305±21	69±5†	27±2†	144±11	7.813†

$p < .05$ *Pre versus Drug, †$NaHCO_3$ vs Drug Values are mean ± S.E.M.

Ventricular tachycardia was noted in all groups, but was inconsistent across animals. COC, NC and CE all prolonged CL, PR, QRS, and QT. All three agents exhibited a local anesthetic effect on the myocardium and were potent arrhythmogenic agents. $NaHCO_3$ reversed the QRS prolongation and converted the ventricular tachycardia to a regular sinus rhythm.

495.7

NEUROPEPTIDE Y PRODUCTION IN RAT MYOCYTE CULTURE IS REGULATED BY SYMPATHETIC NEURONS . K.L.Marek* and T.Niven-Fairchild, Dept. of Neurology, Yale Univ. School of Medicine, New Haven CT 06510

Sympathetic innervation of heart dramatically alters cardiac peptide production. The neural regulation of Neuropeptide Y (NPY) production and secretion has been examined in cardiac myocyte culture and in cardiac myocyte-sympathetic neuron (superior cervical ganglion) co-culture. NPY mRNA levels were quantitated by Northern analysis. NPY content was measured by radioimmunoassay and NPY processing was further analyzed by gel filtration. NPY was stable in spent medium for at least 48 hours. Cultures were maintained in complete serum free medium for up to 21 days.

NPY production and secretion was increased (by 1.5-2 fold) in atrial and ventricular cultures treated with neuron conditioned medium, but was reduced (by 2-4 fold) in SCG-atrial cultures and SCG-ventricular cultures. The changes in myocyte NPY expression were dependent on the age of the cardiac cells in culture and on the duration of treatment with neuron conditioned medium or neurons. Changes in atrium and ventricle were tissue specific.

NPY production in myocytes is regulated by SCG neurons and by SCG culture conditioned medium as myocytes develop in culture. Experiments are underway to further elucidate the mechanisms of this neuronal-target interaction and to identify the developmental significance of cardiac NPY expression. NSO1168, AHA900834.

495.8

SYMPATHETIC NERVE RESPONSE TO ACUTE AND CHRONIC MORPHINE ADMINISTRATION IN THE RAT. S.C. Baraban* and P.G. Guyenet. Dept. of Pharmacology, Univ. of Virginia, Charlottesville, VA 22908.

This study investigates the effects of acute and chronic administration of morphine sulfate (MS) on the discharge of the lumbar sympathetic nerve (SND) and phrenic nerve (PND) in urethane-anesthetized, paralyzed, vagotomized 300-350g Sprague-Dawley rats. Acute MS (5 mg/kg i.v., N=5) reduced i) resting MAP (-27mmHg, $p<0.05$) ii) resting PND amplitude (-90%, $p<0.05$) iii) the central respiratory modulation of SND and iv) the effect of carotid chemoreceptor stimulation (10% O_2, 10-15 seconds) on PND amplitude (-55%, $p<0.05$) and SND (-45%, $p<0.05$). MS also attenuated the sympathetic baroreflex and raised the resting SND (+34%, $p<0.05$). The opiate antagonist naloxone (Nal, 1 mg/kg i.v., 45-60 min. after MS) reversed the effects of MS on resting MAP, resting SND, the sympathetic chemo- and baroreflexes. Interestingly, Nal increased significantly above pre-MS control level i) the resting PND amplitude at constant 5% expired pCO_2 (+146%, $p<0.05$) and ii) the central respiratory modulation of SND.

Rats chronically treated with MS (one 75 mg pellet day 1, 1 p. day 2, 2 p. day 3 2 p. day 4, and 3 p. day 5, N=5) were compared on day 6 with controls implanted with inert pellets (same schedule). MS-treated rats had i) a higher threshold for apnea (3.8% expired CO_2) and ii) attenuated sympathetic baroreflexes. Nal (1 mg/kg i.v.) given to MS treated rats increased resting MAP (+25mmHg, $p<0.05$), PND amplitude (+344%, $p<0.05$) and the central respiratory modulation of SND, but reduced PND rate and resting SND (-45%, $p<0.05$). Nal also restored baroreflex function to normal. Nal exerted no effect in control rats. In summary, the major effects of acute and chronic MS administration on SND include perturbations of the baroreflex and changes in the respiration dependant portion of central sympathetic tone generation. Support: HL28785 and HL39841 from NIH.

495.9

NALTREXONE PRECIPITATED MORPHINE WITHDRAWAL STIMULATES C-FOS PRODUCTION IN AUTONOMIC AREAS OF RAT BRAINSTEM AND FOREBRAIN. R.L. Stornetta*, R. Norton and P.G. Guyenet. Dept. of Pharmacology, Univ. Va. Health Sci. Center, Charlottesville, VA 22908. Autonomic activity is greatly increased during morphine withdrawal. We sought to determine the specific autonomic areas that might be responsible for this increased activity by using an antibody against the nuclear protein *c-fos* in brain tissues from rats withdrawn from morphine. Five groups of 4 male, Sprague-Dawley 250-300g rats were implanted with morphine pellets (75 mg, NIDA) or placebo pellets over a 5 day regimen and injected on day 6 with either saline or naltrexone (100 mg/kg) (Rasmussen et al, 1990). Two and one half hours after injection, rats were quickly anesthetized with pentobarbital, i.p., and perfused transcardially with 4% paraformaldehyde. Coronal brain sections (40 μm) were cut on a vibratome and reacted with an antibody against *c-fos* protein kindly provided by T. Curran. After a standard PAP protocol, *c-fos*-like immunoreactivity was observed in several autonomic areas of the medulla including the nucleus of the solitary tract (NTS), caudal (CVL) and rostral ventrolateral medulla (RVL). Although some *c-fos*-like reactivity was seen in these areas in control rats (either morphine-implanted, saline injected (n = 5), or placebo-implanted, saline (n = 5) or naltrexone injected (n = 5)), a significantly higher number of *c-fos* positive cells in NTS, CVL and RVL were seen in the naltrexone injected morphine-implanted rats (n = 5). Large numbers of *c-fos*-like immunoreactive cells were also seen in locus coeruleus (LC), central gray adjacent to LC, A5 pontine noradrenergic nucleus, pontine parabrachial nucleus and paraventricular nucleus of the hypothalamus from morphine withdrawn rats. (NIDA R29 DA07353-01)

495.10

CLINICAL EVALUATION OF SYMPATHETIC ACTIVITY WITH CARDIOVASCULAR REFLEX TESTS AND SPECTRAL ANALYSIS OF HEART RATE VARIABILITY. S. Šega and T. Kiauta*. Dept. of Neurology, University Medical Centre, SLO-61105 Ljubljana, Slovenia.

Orthostatic test, handgrip test and spectral analysis of heart rate variability were performed on 70 healthy volunteers of both sexes, aged 21 to 60 years. Arterial blood pressure changes during orthostatic test, diastolic blood pressure increase during handgrip and integrals of the low- and medium-frequency bands of amplitude spectra in the standing posture were evaluated.

Blood pressure changes during orthostatic and handgrip tests did not correlate with age, whereas integrals of amplitude spectra did. Blood pressure changes during handgrip and orthostatic test did not correlate with each other or with integrals in the standing posture.

A possible explanation of these results could be that tests involving non-invasively measured ABP changes are less sensitive than amplitude spectra in the standing posture for the evaluation of sympathetic activity.

495.11

INTERACTION BETWEEN SYMPATHETIC NERVE ACTIVITY AND ARTERIAL PRESSURE IN CONSCIOUS AND ANESTHETIZED RAT. D.R. Brown, L.V. Brown, A. Patwardhan and D.C. Randall*. Ctr. Biomed. Engin. and Dept. Physiol. & Biophys., Univ. Kentucky, Lexington, KY, 40536.

Sympathetic nerve activity (SNA) and blood pressure (BP) were sampled for 9.56 min. $\geq$ 2 days after implantation of renal nerve electrodes and arterial catheters in rat (n=3) to test the premise that low frequency BP oscillations are generated by the baroreflex. The measurements were repeated after anesthetizing the animals (pentobarbital, 30 mg/kg). SNA and BP autospectra and coherence were computed. In the low frequency range the maximum coherence (0.90 ± .002, mean ± SD) and maximum SNA power occurred at 0.40 ± .0004 Hz. In contrast, the coherence computed at the frequency (0.17 Hz) for maximum BP spectral power was only 0.40 ± 0.21. After anesthesia the frequency (0.40 ± .003 Hz) at maximum coherence and coherence (0.88 ± .004) were essentially unchanged although SNA and BP spectral power decreased by 78.5 and 70.2 percent, respectively. These data suggest that BP spectral power at a frequency of approximately 0.4 Hz may be due to a SNA-baroreflex interaction and that anesthesia does not abolish this interaction. However, anesthesia apparently profoundly reduces both the SNA and BP responses at this frequency. (Supported by KY Affiliate, AHA and KY Tobacco Health Res. Inst.)

495.12

COMPARISON OF VAGAL POWER DURING PACED BREATHING AND TREADMILL EXERCISE TESTING IN CHRONIC FATIGUE SYNDROME. **W.L. Boda, S.A. Sisto, P.Z. Zhang, T.W. Findley, and W.N. Tapp, B.H. Natelson*. Kessler Institute for Rehabilitation, West Orange, NJ, New Jersey Medical School, Newark, NJ, VA Medical Center, East Orange, NJ, 07019**

Currently the etiology of Chronic Fatigue Syndrome (CFS) is unknown. It is characterized by many neuropsychological, infectious, and immunological symptoms. The most debilitating feature of CFS is fatigue that is severe enough to reduce activity by greater than 50 % for longer than 6 months. Since there is no diagnostic test for CFS, arguments exist as to whether it is medical or functional in etiology. To evaluate this problem, a static paced breathing protocol of 8, 12, and 18 breaths/min both in sitting and standing was used to compare vagal power in CFS patients and normals. Vagal power was derived through the application of heart rate spectrum analysis on the ECG and respiration signals. Paced breathing was lower in sitting for CFS patients vs. normals. Vagal power did not fall in the standing posture as compared to controls. Progressive exercise tests on a treadmill showed an increased vagal response to exercise as compared to normals. It is not clear at this point how to reconcile these differences between these two studies. Measurement of fatigue was also analyzed using biomechanical parameters through video analysis to determine if there is a correlation between physiological and functional fatigue of CFS patients and normals at similar work levels.

These results preliminarily indicate that CFS patients may exhibit a consistent organic component to their illness, that is, inappropriate vagal firing in different postures and during exercise. Comparisons of the extent to which this vagal firing affects exercise, may aid in the diagnosis of CFS or perhaps in the classification of severity of CFS.

Supported by NIH # AI32247 and NIDRR # H133F10030

495.13

AGE-RELATED COHERENCE OF EFFERENT SYMPATHETIC (SYMP) ACTIVITY IN NEONATAL SWINE. B. W. Hundley, P. M. Gootman*, G. Condemi, J. M. Sierra, H. L. Cohen, L. P. Eberle and A. P. Rudell. Dept. of Physiology, SUNY- Hlth. Sci. Ctr., Brooklyn, NY 11203.

Simultaneous recordings of cervical SYMP, splanchnic and efferent phrenic (PHR) activity were obtained in Saffan-anesthetized, paralyzed and artificially ventilated piglets (1-38 days of age) along with aortic pressure, EKG and end-tidal CO_2. Power spectra and coherence functions of SYMP discharge were obtained by a fast Fourier transform routine. PHR discharge defined the separate inspiratory (I) and expiratory (E) epochs used for gating spectral estimates. Peaks were revealed in four frequency ranges (3-7 Hz, 10-15 Hz, 18-24 Hz, 30-38 Hz). Coherence varied from insignificant values (< 0.1) in swine < 2 wks old to significant coherence values (> 0.3) in animals 19 days or older. Peak coherence values were obtained for 15 swine, separated into three age groups, for analysis of variance. The linear component of the age-coherence relationship for the 3 - 7 and 18 - 24 Hz peaks was significant for both I and E epochs ($p < .05$). The quadratic component was not significant. For the 10 - 15 Hz peak both the linear and quadratic components were significant ($p < .05$). For the 30 - 38 Hz peak, neither linear nor quadratic components were significant ($p > .05$). Correlation of age with E to I power ratios of the SYMP was only significant in the cervical SYMP (rho = 0.52). Results suggest that coherence is a good indicator of postnatal maturation occurring within the SYMP rhythm generating system(s). (Supported by NIH grants HL-20864 and HD-28931.)

495.14

HEART RATE DYNAMICS DURING SLEEP-WAKING STATES IN NORMAL INFANTS. R.K. Harper*, V.L. Schechtman and R.M. Harper. Brain Research Institute and the Dept. of Anatomy & Cell Biology, UCLA School of Medicine, Los Angeles, CA 90024.

Extent and pattern of heart rate variation, assessed by summary procedures, undergo marked developmental changes over the early postnatal period. Summary measures of heart rate variation, however, fail to demonstrate the beat-by-beat dynamics of heart rate. We examined the development of moment-to-moment changes in cardiac interbeat intervals in normal infants over the first 6 months of life.

Twelve-hour physiological recordings were obtained from 24 normal infants at 1 week and 1,2,3,4, and 6 months of age. For each recording, plots were made of each cardiac R-R interval as a function of the previous interval (Poincaré Plots) during all periods of quiet sleep, REM sleep, and waking. In each plot, the extent of dispersion of points about the line of identity was quantified at a relatively high and a relatively low heart rate. The dispersion of points at both low and high heart rates showed significant age and state effects. Analysis of covariance indicated that all state and age related changes paralleled changes in basal heart rate; only the effect of dynamic rate (heart rate during the previous beat) remained significant after controlling for differences in basal heart rate. Thus, the relationship of one cardiac R-R interval with its predecessor changes significantly with age over this period, paralleling short and long term changes in heart rate.

Supported by HD22695. Data acquisition and sleep state classification were performed under the direction of Drs. J. Hodgman and T. Hoppenbrouwers under NICHD contract HD22777.

495.15

DYNAMIC ANALYSIS OF BEAT-TO-BEAT HEART RATE CHANGES IN DEVELOPING INFANTS. V.L. Schechtman*, R.K. Harper and R.M. Harper. Brain Research Inst. and Dept. of Anatomy & Cell Biology, UCLA School of Medicine, Los Angeles, CA 90024.

Poincaré Plots demonstrate particular aspects of heart rate dynamics. However, the strong positive correlation between one **interval** and the next obscures the immediate relationship between a **change** in heart rate and the next change. The nature of this relationship was examined in 24 infants recorded at 1 week and at 1,2,3,4, and 6 months of age. In each sleep-waking state, the number of ΔRRs (the difference between two successive RR intervals) larger than 4 msec was determined as a percentage of the total number of heart beats, and each pair of successive changes was categorized based on the directions of the two changes. Analysis of variance was used to identify differences in the proportion of large ΔRRs and their temporal patterns over ages and sleep states. During all states, the proportion of large ΔRRs decreased over the first month of life and increased from 1 to 3 months of age. The reduction over the first month depended on the increase in heart rate over that period, but some subsequent changes were heart rate independent. Furthermore, during the first month of life, infants showed significantly more sustained increases in heart rate than sustained decreases, while the opposite pattern was seen in infants from 3 to 6 months of age. We speculate that the profound and enduring changes in cardiac rate dynamics occurring between 1 and 3 months of age may reflect the emergence of cardiosympathetic reflexes.

Supported by HD22695. Data collection and state classification performed under direction of Drs. J. Hodgman and T. Hoppenbrouwers under contract HD22777.

CARDIOVASCULAR REGULATION: HYPERTENSION, REFLEXES AND PERIPHERAL AUTONOMICS

496.1

HYPERTENSION INDUCED BY RANDOM AIR-JET STRESS IN THE BORDERLINE HYPERTENSIVE RATS (BHR). Guy Drolet*, Sylvie Laforest and Hélène Bachelard. Unité d'Hypertension, Centre de Recherche du CHUL, Université Laval, Québec (Qc), G1V 4G2.

The relationship between psychological stress and hypertension is regarded to be circumstantial. However, it has been suggested that a genetic predisposition for the development of hypertension might be necessary for the expression of this relationship. The present study investigated the existence of such relationship in the F1 offspring of female genetically predisposed to hypertension (SHR) and normotensive male Wistar Kyoto (WKY) rats. Five weeks old male BHR were randomly assigned to an experimental and a control group. Rats in the experimental group were placed 5 days/week for 30 minutes in a restrainer while receiving randomly-timed (interval 10-60 sec.) compressed air puffs of random duration (0.5 to 10 sec.) directed toward the snout. This treatment lasted 8 weeks. Maturational control BHR remained in their home cage and were handled daily. Weekly blood pressure measurements were made in all rats using tail plethysmography. Exposure to air puff stress for 8 weeks induced an increase of ~ 20 mmHg in mean arterial pressure as compared to the control rats. However, there were no differences neither in resting heart rate nor in basal plasma catecholamines concentrations suggesting that the activity of the autonomic nervous system was not different between stressed and control BHR, at least in resting conditions. These results suggest that randomly delivered air puff stress induced-hypertension in BHR is independent of an increase in sympathetic nervous system activity in resting conditions.(Supported by MRC, HSFQ and FRSQ)

496.2

ALCOHOL-INDUCED SECRETION OF ATRIAL NATRIURETIC FACTOR (ANF) IN RATS:POSSIBLE IMPLICATION OF ß-ENDORPHIN P. Guillaume*, C. Gianoulakis, Dept. Physiology, Douglas Hospital Research Centre, McGill University, Verdun, Québec.

It has been previously reported and confirmed by us that small quantities of ethanol decrease the age-dependent development of hypertension in rats. The objective of the present studies was to test the hypothesis that ethanol stimulates the release of Atrial Natriuretic Factor (ANF), which in turn could mediate its antihypertensive effects. Male Long-Evans rats having a catheter in the jugular vein were used. The animals were injected i.p. with various concentrations of ethanol or saline. Blood samples were withdrawn at 0, 15, 30, 60 and 120 minutes post-injection for estimation of plasma ANF, corticosterone and ß-endorphin (ß-EP). Results indicated that ethanol increased the plasma concentration of ANF, ß-EP and corticosterone. The increased plasma content of ANF could be due to a direct effect of ethanol on the heart atria, or mediated by a number of hormones the release of which is enhanced by ethanol. Since it was previously shown that opioids modulate the secretion of ANF from heart atria, ß-EP could be partially responsible for the increased ANF release following ethanol administration. Further studies using appropriate opiate antagonists could confirm this hypothesis.

496.3

THE IMMEDIATELY RELEASABLE STORE OF TRANSMITTER IN SYMPATHETIC NEURONS FROM SHR. *J.C. Magee and G.G. Schofield. Dept. Physiology, Tulane Medical School. New Orleans, LA 70112.*

A greater amplification of preganglionic nerve activity (at physiological frequencies) occurs at sympathetic ganglia of spontaneously hypertensive rats (SHR), compared with normotensive controls. However, transmission of high frequency (70-100 Hz) nerve activity is depressed in these ganglia. This relative depression could result from a greater depletion of the presynaptic transmitter store by an elevated quantal content observed in SHR. Or, less transmitter could be available for release from SHR preganglionic nerve terminals. To test this, trains of 30 supramaximal orthodromic stimuli were delivered to superior cervical ganglia (SCG) isolated from SHR and WKY rats and the resulting EPSCs were recorded from postganglionic neurons. The neurons were voltage clamped, at 31 - 33° C, using conventional electrodes and the discontinuous SEVC. The resulting trains of synaptic currents from both groups initially increased in size and then rapidly decreased to a new steady-state amplitude by the 20-25th stimulation. Plots of EPSC amplitude versus time for each neuron could be fit by a single exponential plus a constant function. The mean time constants of these fits were 5.8±0.8 ms and 5.2±0.8 ms, for SHR and WKY, respectively. The steady-state amplitudes reached were 136±10 nS for SHR and 150±26 nS for WKY. The number of quanta residing in the immediately releasable store, as calculated from these plots, was found to be 4700±200 and 4500±600 for SHR and WKY, respectively. These results demonstrate that the amount of transmitter immediately available for release is the same in SHR and WKY. An enhanced removal of transmitter from a similarly sized store by the increased quantal content of SHR preganglionic neurons could lead to a pronounced depletion of transmitter stores, reducing the ability of SHR SCG to transmit high frequency impulse activity. *Work supported by PHS Grant HL43656.*

496.4

EFFECTS OF CALCIUM AND POTASSIUM ON HYPERTENSION IN RATS. A.D.Dalhouse,* M.Moitzheim, C.Cannon, V.Streich, J.Byrne, J.Tjaden, and J.Kozel. Dept. Psyc., Moorhead State Univ. Moorhead, MN 56563

In a series of experiments to investigate the effects of diatery electrolytes on blood pressure (BP) 20 SHR and 20 WKY male rats were placed on combination diets of high and low calcium (HCa, LCa) and potassium (HK, LK) for 7 weeks after 7 days of base rate data. Pulse, BP, and urine were collected daily during base rate and once weekly during the 7 weeks on the diets. Blood was collected via heart puncture for plasma electrolytes analysis. No pre-post diet related BP differences were observed for WKYs however, SHR HCaHKs showed significant ($p<.01$) BP elevation from base rate. The HCaHK and LCaHK SHRs also showed higher BP than the WKYs ($p<.05$). The HCaHK WKYs showed lower post diet pulse than the HCaHK and LCaHK SHRs but did not differ significantly from the LCa WKYs. All groups showed post diet urine reduction ($p<.01$) in calcium, potassium and sodium with the HCaLK and LCaLK WKYs, and the LCaLK SHRs showing greatest change in urine K. The HCaHK SHRs showed significantly lower plasma Ca than the WKYs, but no other plasma differences were observed. High potassium appears to be associated with elevated BP and pulse in hypertensive rats but with lower BP and pulse in the normotensive rats.

496.5

CALCIUM DEFICIENCY INCREASES RENAL VASCULAR REACTIVITY IN SHRs BUT DOES NOT ALTER TUBULAR RESPONSES TO NERVE STIMULATION. D.C. Hatton*, Y. Qi, and D.A. McCarron. Oregon Health Sciences University, Portland, OR 97201.

SHRs fed high Ca^{2+} diets have lower BP, less BP reactivity to NE, smaller BP responses to α-1 adrenergic blockade and fewer α-1 receptor binding sites in whole kidneys than SHRs fed low Ca^{2+} diets. To determine the functional correlates of the difference in renal α-1 binding sites, tubular and vascular responses were assessed in the current study. SHRs fed either high (2.0%) or low (0.1%) Ca^{2+} diets for 8 weeks were used. While anesthetized with inactin (100mg/kg) intrarenal injections of norepinephrine (NE) were made at 10, 20, 40 or 80 ng/kg. In other animals, a bipolar Ag-AgCl electrode was placed around the renal nerve. The nerve was stimulated at a level just below that required to change blood flow. Urine was collected for 20 min before, during and after stimulation. BP was significantly higher in animals on low Ca^{2+} diets (140 vs 122 mmHg, $p<.01$) as was blood flow (7.9 ml/min/gkw vs 5.6 ml/min/gkw). Animals on low Ca^{2+} diets had greater reductions in renal blood flow at all doses of NE ($p<.005$). Nerve stimulation caused a significant antidiuresis and antinatriuresis but the change was not different between diet groups. The results suggest vascular but not tubular responses may be altered in animals on different Ca^{2+} diets.

496.6

MECHANISM OF PRESSOR RESPONSE TO RAT JOINING PEPTIDE. M. Yoshida, T. Hamakubo F. Sulser* and T. Inagami. Depts. of Biochemistry and Psychiatry, Vanderbilt Univ. Sch. of Med., Nashville, TN 37232.

Intracisternal administration of rat joining peptide (rJP), generated from the pro-opiomelanocortin, has a pressor effect especially in spontaneously hypertensive rats (SHR). To characterize this pressor response, we investigated the effect of angiotensin or adrenergic receptor blockers in conscious SHR. The pressor response to 10 nmol of rJP was abolished by intracisternal pre-administration of 5 μg of losartan (DuP 753) (34 ± 5 vs. 4 ± 1 mmHg). Similar blocking effect was observed by [Sar^1 , Ile^8] angiotensin II. The concentration of angiotensin II in CSF was increased 2.0- and 4.4-fold by 10 and 30 nmol of rJP, respectively. Pre-administration of yohimbine or propranolol did not prevent the pressor response to rJP. These results suggest the brain renin-angiotensin system may participate in the pressor response to rJP.

496.7

POTENTIAL ADVERSE EFFECTS OF LOW DIETARY COPPER AND RANDOM LIGHT/DARK CYCLES ON BLOOD PRESSURE IN NORMOTENSIVE RATS. E.S. Halas* and L.M. Klevay. Department of Psychology, University of North Dakota and USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202.

Humans who work a night shift have a significantly higher rate of heart disease than people who work a day shift. A disruption in the circadian rhythms may be a major cause of the increased heart disease among shift workers. However, other factors such as disrupted family life, an inadequate diet, lack of recreational opportunities, and reduced social activities may also contribute to the elevated heart disease. Rats were subjected to random light/dark cycles (Halas and Klevay, *Soc. Neurosci. Abstr.* 17: 1000, 1991). The 8-hour light cycle was initiated at one of three starting periods: 12:00 a.m., 8:00 a.m. or 4:00 p.m. A given cycle would last from 2 to 4 days and then it would be changed. Half of the rats were exposed to the random light/dark cycle while the other half received the standard 12-hour light/dark cycle. Half of the animals in each group were fed a purified diet (Klevay, *Am. J. Clin. Nutr.* 26: 1060, 1973) with 2.00 ppm Cu diet while the others were fed the diet with 5.00 ppm Cu. There were 15 normotensive, Sprague-Dawley male weanling rats in each of the four groups. The experiment lasted nine weeks. Significant elevation of blood pressure occurred in the 2.00 ppm Cu groups ($p<0.01$) whereas the random light/dark cycle groups did not exhibit any increase in blood pressure. These results are in contrast with a prior experiment (loc. cit.) which found that both the 2.00 ppm Cu diet and random light/dark cycle significantly increased plasma cholesterol. These results suggest that the environmental factors that control blood pressure and cholesterol may be different.

496.8

NPY IMMUNOREACTIVITY IS DECREASED IN SYMPATHETIC GANGLIA OF HYPERTENSIVE, BUT NOT HYPERACTIVE RATS. X-M. Fan, K. M. Braas, V. May, E. D. Hendley and C.J. Forehand*. Depts. of Physiology & Biophysics and Anatomy & Neurobiology, Univ. of Vermont, Sch. of Med., Burlington, VT 05405.

Four inbred rat strains (WKY, SHR, WKHT and WKHA) were used to examine whether neuropeptide Y (NPY) levels are specifically altered in sympathetic ganglia of hypertensive animals. SHRs are both hypertensive and hyperactive; WKYs are neither. WKHTs are hypertensive, but not hyperactive; WKHAs are hyperactive, but not hypertensive (Hendley & Ohlsson, Am. J. Physiol 261:H584, '91).

Immunocytochemistry was combined with computerized densitometry to quantitate relative intracytoplasmic NPY in individual sympathetic ganglion cells. Total NPY immunoreactivity was reduced in superior cervical ganglia of both SHR and WKHT rats (75% and 66%, respectively, relative to WKY). A similar reduction in NPY immunoreactivity was observed in stellate ganglia. Inferior mesenteric ganglia in the hypertensive strains showed an even greater reduction in NPY immunoreactivity (to 45% in SHR and 37% in WKHT, relative to WKY). Although both fewer NPY-immunoreactive cells/ganglion and lower NPY/cell contributed to the reduction of ganglionic NPY in hypertensive animals, a decreased level of NPY immunoreactivity in individual cells accounted for the majority of the reduction. Significant differences were not observed between WKY and WKHA rats. These studies suggest that a reduction of NPY levels in sympathetic ganglia is specifically associated with the hypertensive trait.

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496.9

NOVELTY-INDUCED GROOMING BEHAVIOUR AND DOPAMINE RECEPTORS IN THE SPONTANEOUSLY HYPERTENSIVE RAT. A.C.E. Linthorst, P.L.M. Van Giersbergen*, Th. De Boer, Tj.B. Van Wimersma Greidanus, W. De Jong and D.H.G. Versteeg. Rudolf Magnus Institute, Medical Faculty, State University of Utrecht, Utrecht, The Netherlands.

Recent work from our lab has provided further evidence for a role of the nigrostriatal dopamine (DA) system in the development of hypertension in the spontaneously hypertensive rat (SHR). It was shown that the release of DA in the caudate nucleus of SHR is lower than in that of normotensive Wistar-Kyoto rats (WKY). In addition, in the caudate nucleus of SHR a super-sensitivity of presynaptic DA D_2 autoreceptors was found. To further characterize the nigrostriatal DA system of SHR and WKY novel environment-induced grooming behaviour, regulated extensively by central DA systems, was studied. Moreover, receptor binding studies were performed to establish affinity and concentrations of DA D_1 and D_2 receptors in the caudate nucleus of both rat strains. Novelty-induced grooming behaviour scores were lower in SHR than in WKY. A dose-dependent suppression of grooming behaviour was induced by the DA D_1 antagonist SCH 23390 and by the DA D_2 agonist quin-pirole. The SCH 23390-induced suppression was less, whereas that by quinpirole was more pronounced in SHR than in WKY. No differences between SHR and WKY were found in binding characteristics of DA receptors. The results suggest that differences between SHR and WKY as found in the grooming paradigm are not related to changes in binding characteristics of caudate DA receptors. However, the stronger quinpirole-induced inhibition of grooming behaviour may correspond to the previously reported super-sensitivity of presynaptic DA D_2 receptors in the caudate nucleus of SHR.

496.10

HYPOVOLEMIA-INDUCED VASOPRESSIN SECRETION IN CHRONIC BARORECEPTOR DENERVATED RATS. A.M. Schreihofer*, E.M. Stricker, and A.F. Sved. Department of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA, 15260.

We previously demonstrated that in chronic sino-aortic denervated (SAD) rats, bilateral nucleus tractus solitarius (NTS) lesions had no effect on plasma vasopressin (VP) levels (McDonald and Sved, Neurosci. Abstr. 1991). Since these results are inconsistent with the notion that cardiopulmonary (CP) baroreceptor vagal afferents provide a tonic inhibitory influence on VP secretion, the present study sought to further examine the role of CP baroreceptor afferents in the regulation of VP secretion in chronic SAD rats. In chloralose-anesthetized, ventilated chronic SAD rats (n=4) plasma VP levels remained low following bilateral vagotomy (7±2 pg/ml), consistent with our previous results indicating a lack of tonic inhibition of VP secretion mediated via the NTS. To examine the VP response to hypovolemia, chronic SAD rats were subjected to serial hemorrhage (4 samples of 2 ml/300 g at 10-min intervals); plasma VP levels were increased similarly to control rats (SAD=148±56 pg/ml, n=5; control=146±34 pg/ml, n=6). However, rats with chronic NTS lesions (arterial and CP baroreceptor reflexes were abolished) also responded to hemorrhage with increases in plasma VP levels not significantly different from control or chronic SAD rats, indicating that hemorrhage is not specific enough to evaluate CP baroreceptor function. To elicit a more specific hypovolemic stimulus, rats were injected with a 30% polyethylene glycol solution (5 ml, sc.); the colloid was allowed to draw protein-free plasma out of the vasculature for 6 hrs, creating a 25-40% decrease in plasma volume (plasma protein concentration increased from 5.4±0.1 g/dl to 7.0-8.8 g/dl). Colloid treatment increased plasma VP levels in chronic SAD rats (n=7) similarly to control rats (n=6). However, the VP response to colloid was substantially attenuated in chronic NTS lesioned rats (n=6), suggesting that this hypovolemic stimulus acted through remaining vagal afferents in chronic SAD rats. These results suggest that although remaining CP baroreceptor afferents do not provide tonic inhibition of VP secretion in chronic SAD rats, hypovolemia is still capable of increasing VP secretion through a non-tonic excitatory mechanism mediated through the NTS.

496.11

EFFECTS OF CHEMORECEPTOR STIMULATION ON REGIONAL HEMODYNAMICS IN RATS. A.M. Hoque, R.A. Shaffer and S.J. Lewis,* Dept. of Pharmacology and Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242.

Chemoreceptor (CR) reflexes are critical in the regulation of cardiovascular function. In this study the effects of CR stimulation by sodium cyanide (NaCN 50 - 200 μg/kg, i.v.) on regional hemodynamics were examined before and after bilateral transection of the carotid sinus nerves (CSNX) in urethane-anesthetized rats. The injection of NaCN produced a fall in arterial pressure (AP), an increase in cardiac output (CO) and a reduction in total peripheral resistance (TPR). NaCN also increased hindquarter (HQF), renal (RF) and mesenteric (MF) blood flows and consequently reduced the resistances in these beds. Bilateral CSNX produced marked increases in TPR, hindquarter (HQR), renal (RR) and mesenteric (MR) resistances, a decrease in CO and no change in AP. Bilateral CSNX did not modify the hemodynamic effects of NaCN. These results suggest that stimulation of chemoreflexes produces marked cardiovascular changes and that the CSNs also provide tonic regulation of regional hemodynamics. The present study also suggests that i.v. NaCN stimulates chemoafferents other than those in the CSN. (Supported by HL14388 and HL44546)

496.12

FUROSEMIDE IMPAIRS CENTRAL AND PERIPHERALLY-INDUCED PRESSOR RESPONSES. D.S.A. Colombari; E. Colombari; W.A. Saad*; L.A.A. Camargo; A. Renzi; L.A. De Luca Jr. and J.V. Menani, Dept. of Physiology, School of Dentistry, UNESP, Araraquara, SP 14800, Brazil.

In the present work we investigated the effect of previous treatment with furosemide on the pressor responses to intracerebroventricular (ICV) angiotensin II (AII), carbachol (CARB), norepinephrine (NOR) and 2 M NaCl (HS) as well as intravenous (IV) AII, NOR and vasopressin (AVP). Normotensive rats received two injections of furosemide (Lasix, Hoechst, 30 mg/kg b.w., 12 and 1 h before the experiment). The pressor responses to ICV AII (25 ng), CARB (7.5 nmol) and NOR (80 nmol) and to IV AII (50 ng) and NOR (0.3 ug) were reduced after the treatment with furosemide. The pressor response to ICV HS and to IV AVP was not changed after furosemide. The unchanged pressor response to central HS could be more related to AVP release since the effect of IV AVP was also not changed after furosemide. The results are in accordance with the idea that furosemide impairs sympathetic-induced vasoconstriction. It is also possible that the impairment in pressor responses is a result of changes in fluid-electrolyte balance.

Research supported by FAPESP and CNPq.

496.13

INFLUENCE OF ESTROUS CYCLE ON CONTRACTILE RESPONSES TO SENSORY AND ADRENERGIC NERVE STIMULATION IN FEMALE RAT BLOOD VESSELS. Z. Li and S.P. Duckles*, Dept. of Pharmacology, University of California, Irvine, CA 92717

To study whether the estrous cycle has a significant effect on vascular reactivity to nerve stimulation, 3 month old F-344 rats in medestrous and proestrous stages (characterized by vaginal smear) were used. In the isolated Krebs' perfused mesenteric vascular bed with guanethidine present to block adrenergic nerves and methoxamine to maintain smooth muscle tone, sensory nerve activation caused by nicotine (Nic) exposure or transmural nerve stimulation (TNS) was not significantly different between medestrous and proestrous groups. In isolated tail artery ring segments which are rich in

	Perfused Mesentery Relax. (%)		Tail Artery Contraction (g)	
	Nic (3×10^{-4} M)	TNS (4 Hz)	NE (3×10^{-6} M)	TNS (4 Hz)
Med-E	49.1 ± 12.6	48.1 ± 17.5	0.28 ± 0.05	0.35 ± 0.04
Pro-E	60.4 ± 6.0	41.5 ± 3.3	0.28 ± 0.07	0.47 ± 0.15

adrenergic but lack sensory innervation, increasing frequencies of TNS caused an increase in developed force in both medestrous and proestrous groups; no significant difference was seen. Furthermore concentration response curves to norepinephrine (NE) were superimposed. In addition, there was no significant difference in the non-specific relaxation produced by higher concentrations of nicotine. We conclude that different estrous stages do not significantly influence vascular responses to sensory or adrenergic nerve stimulation.

Supported by California Tobaco Related Disease Program

496.14

ROLE OF NITRIC OXIDE (NO) IN THE CARDIOVASCULAR EFFECTS OF ACETYLCHOLINE (Ach). J.S. Simon*, R.A. Shaffer, A.M. Hoque, and S.J. Lewis. Depts. Pharmacol., Internal Medicine & Cardiovasc. Ctr., Univ. of Iowa, Iowa City, IA 52242.

It is well established that Ach releases NO from the vascular endothelium. This study examined the possibility that the effects of Ach on regional hemodynamics in urethane-anesthetized rats involves NO. Ach (0.1 - 5 μg/kg, i.v.) produced a fall in mean arterial (MAP) and pulse pressure (PP) and heart rate, an increase in cardiac output (CO) and decreases in total peripheral resistance (TPR), hindquarter (HQR) and mesenteric (MR) but no change in renal (RR) resistance. Following injection of the NO-synthesis inhibitor L-NAME (25 μmole/kg, i.v.), Ach produced exaggerated falls in MAP, no bradycardia, an exaggerated increase in CO, similar changes in TPR and HQR but larger reductions in RR and MR. However, the duration of the Ach effects were markedly reduced following L-NAME. L-NAME also abolished the Ach-induced reduction in PP. These results suggest that a) NO is involved in the Ach-induced bradycardia and the maintenance but not the initiation of the hemodynamic effects of Ach, b) Ach reduces PP (probably via a decrease in vascular compliance) by a NO-dependent mechanism. (Supported by HL14388 and HL44546.)

496.15

EVIDENCE THAT NITROSYL FACTORS (NOFS) ARE RELEASED FROM SYMPATHETIC NERVES. R.L. Davisson*, A.K. Johnson, and S.J. Lewis. Depts. Psychology, Pharmacology, & Cardiovasc. Ctr., Univ. of Iowa, Iowa City, IA 52242.

Bretylium (BRE) selectively depolarizes sympathetic nerve terminals leading to a release of neurotransmitter stores. This study examined the effects of BRE on vascular hemodynamics in conscious rats. BRE (5 mg/kg, iv) caused an increase in arterial pressure (AP), a sustained decrease in hindquarter resistance (HQR), and a gradual increase in renal (RR) and mesenteric (MR) resistances in saline-treated rats. After the α1-adrenoceptor antagonist prazosin (100 μg/kg iv), BRE produced hypotension and exaggerated decreases in HQR, RR, and MR. The NO synthase inhibitor L-NAME (25 μmol/kg iv) abolished the BRE-induced decrease in HQR in saline-treated rats and the decreases in HQR, RR, and MR in prazosin-treated rats. This suggests that BRE causes the co-release of pre-formed stores of norepinephrine and vasodilator NOFs from sympathetic nerves. This raises the possibility that NOFs are neurotransmitters in sympathetic vasodilator neurons and neuromodulators in sympathetic vasoconstrictor neurons. (Support by HLB 14388 & 44546)

497.1

IMMUNOCYTOCHEMICAL CHARACTERISTICS OF DORSAL FACIAL AREA OF THE MEDULLA IN CATS. J.S. Kuo[1], T. Chyi[2], V. Cheng[2], J.Y. Wang*. [1]Dept. Med. Res., Taichung Veterans General Hosp., Taichung, [2]Inst. Biol., Tunghai Univ., Taichung; *Dept. Physiol. and Biophysics, Natl. Def. Med. Cent., Taipei, Taiwan, Republic of China.

The so called dorsal facial area (DFA) is located at levels from 5 mm to 7 mm rostral to the obex and dorsal to the facial nucleus in the medulla in cats. Either electrical or glutamate stimulation of the DFA in the anesthetized cats induced predominantly an increase of blood flow of the ipsilateral common carotid artery, accompanied with neither change in blood flows of other vascular beds nor changes in the heart rate and blood pressure. The DFA response were mediated via the 7th and 9th cranial nerves. By measuring tissue blood flows (TQ) with radioisotope-labeled microspheres reference flow technique, we found both the intra- and extra-cranial TQ were all increased in the DFA response. In addition, the DFA vasodilation involved atropine sensitive and insensitive mechanisms. Results of immunohistochemical studies showed that 10±3 ChAT and 7±2 DBH immunoreactive soma in the DFA. The substance P and serotonin immunoreactive fibers also presented in the DFA.

497.2

INCREASES IN INTRA- AND EXTRA-CRANIAL TISSUE BLOOD FLOWS IN RESPONSE TO STIMULATION OF DORSAL FACIAL AREA OF THE MEDULLA IN CATS. T. Chyi[1], E.H.Y. Lee*, and J.S. Kuo[2]. [1]Inst. Biol., Tunghai Univ., Taichung; *Inst. Biomed. Sci., Academia Sinica, Taipei; [2]Dept. Med. Res., Taichung Veterans General Hosp., Taichung, Taiwan, Republic of China.

Microspheres reference flow techniques was used to measure regional blood flows (RBFs) of the intra- and extra-cranial tissues. Electrical or glutamate stimulation of the dorsal facial area (DFA) generally increased the intracranial RBFs of both cerebral hemispheres. Intracranial blood flow increases were enhanced after i.v. administration of atropine but reduced after physostigmine. In contrast, extracranial blood flow increases responded to both drugs in the opposite direction. Thus, DFA stimulation may cause acetylcholine release to promote extracranial RBFs increase, but to restrict intracranial RBFs increase.

497.3

CARDIOVASCULAR RESPONSE TO TREADMILL EXERCISE IS SUPPORTED BY THE ROSTRAL VENTROLATERAL MEDULLA (RVLM) IN THE CONSCIOUS DOG. K.J. Dormer* and S.R. Ashlock. Physiology Dept., Univ. Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK 73190.

Questions remain as to the physiological role of C1 adrenergic cells within the RVLM in the maintenance of arterial pressure (AP) at rest and during daily stressors. The instrumented dog has been our model for recording cardiorespiratory responses before and after incomplete lesions are made in pressor regions of RVLM by microinjection of kainic acid. Understanding the central command component of cardiorespiratory control during dynamic treadmill exercise tolerance testing (ETT) is the purpose of this study. Mongrels (n=15) were conditioned to run a modified Bruce protocol up to 4 mph, 16 % grade during which heart rate (HR), AP, aortic blood flow and cardiac output (CO), internal thoracic arterial flow (IT) and total peripheral resistance (TPR) were monitored. Instrumentation included solid state pressure transducers (Konigsberg) and pulse-transit time flowmeter (Transonic) and 20 MHz Doppler probes. With a unilateral lesion (100 nl, 100mM), in the transition region between the compact division of n. ambiguus and retrofacial n. the cardiovascular pattern response to exercise was unchanged. AP was significantly decreased ($P < 0.01$) and did not even attain pre-lesion resting values during the ETT. TPR was significantly lower at all workloads except 3/0. The CO, peak systolic aortic flow, and IT were consistently lower than pre-lesion values but not significantly. When observing behavioral and motor control there was no discernable difference in the pre- to post-lesion treadmill performance. Lesions causing these results were relatively small, approximately 1mm diameter, and within the rostral portions of the C1 cell column as revealed by immunocytochemical staining for PNMT. We conclude: portions of the C1 area are universally important in the maintenance of AP during rest and exercise. (Supported by NIH grant HL 39105)

497.4

ANATOMICAL RELATIONSHIPS AMONG THREE POPULATIONS OF MEDULLARY EFFERENTS: RETICULOHYPOTHALAMIC, RETICULOVAGAL, AND RETICULOSPINAL NEURONS. S.G.P. Hardy*. Departments of Physical Therapy and Anatomy, University of Mississippi Medical Center, Jackson, MS 39216.

Within the medulla are neurons that project to the hypothalamus, the vagus nerve, and the spinal cord. The primary purpose of the present study was to identify those regions which contain representatives from each of these neuronal populations. (The long term hypothesis being that these areas, containing a diversity of efferents, may serve to integrate medullary control over vital functions.) For this purpose, a variety of retrogradely-transported tracers were made into either the postero-lateral hypothalamus, vagus nerve or thoracic spinal cord, in a series of anesthetized rats. Subsequently, the locations of labeled medullary neurons were plotted.

The three neuronal populations, mentioned above, were observed in close apposition within the ventrolateral (CVL) and dorsomedial (CDM) aspects of the caudal medulla. In the CVL, the three populations of neurons were observed in the vicinity of the nucleus ambiguus (NA). Vagal projections originated from the dorsomedial aspect of NA, whereas spinal projections originated from the ventrolateral aspect of NA. Hypothalamic projections did not originate from the NA, but rather from an area immediately ventral to it. In the CDM, the three neuronal populations were observed in the vagal-solitary complex.

497.5

NUCLEUS TRACTUS SOLITARIUS (NTS) HYPERTENSION IN GUANETHIDINE-SYMPATHECTOMIZED RATS. E. E. Benarroch*, J. D. Schmelzer, K. K. Nickander and P. A. Low. Neurophysiology Laboratory, Dept. of Neurology, Mayo Clinic, Rochester, MN 55905

Guanethidine (GU) produces chronic postganglionic sympathectomy (GUSX) in adult rats. We sought to determine the effects of chronic sympathectomy on the development of hypertension induced by kainic acid (KA) injection into the nucleus of the tractus solitarius (NTS). Guanethidine (40 mg/kg i.p.) was administered daily for 5 weeks. Controls received saline. Six to eight weeks after treatment, rats were anesthetized (urethane), cannulated and ventilated. Arterial pressure (AP) and heart rate (HR) were continuously monitored. Plasma norepinephrine (NE) and epinephrine (E) were measured by HPLC and vasopressin (AVP) by radioimmunoassay. KA (1 nmol) was injected bilaterally into the NTS. Basal AP, HR, and plasma NE and E were slightly but not significantly lower in GUSX. KA injection into the NTS produced hypertension in both controls and GUSX rats, but increase of AP was higher in controls (ΔAP: 94±5 vs. 40±9 mm Hg, $p<0.01$). In control, but not GUSX rats, NTS hypertension was associated with significant elevation of NE (from 1.8±0.3 to 15±4 ng/ml, $p<0.05$) and E (from 2.5±0.3 to 7.6±1.5 ng/ml, $p<0.05$). Plasma AVP was similar between groups. GUSX attenuates, but does not prevent, NTS hypertension. Expression of NTS hypertension may reflect denervation supersensitivity of vascular targets and sparing of prevertebral ganglion neurons, as well as pressor effects of circulating AVP.

497.6

BRAINSTEM PROJECTIONS OF Na^+ SENSITIVE SITES IN THE PARAHYPOGLOSSAL AREA. S. L. Hochstenbach* and J. Ciriello. Department of Physiology, University of Western Ontario, London, Canada, N6A 5C1.

We have previously shown that microinjections of hypertonic phosphate buffered saline (PBS) solutions into the dorsal vagal complex and the region immediately ventral to it elicits changes in both arterial pressure and heart rate in the rat. In this study, the efferent pathways that may be involved in mediating the pressor responses from Na^+ sensitive sites in the parahypoglossal area (PHA) were investigated using the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L) combined with either tyrosine hydroxylase (TH) or phenylethanolamine-N-methyltransferase (PNMT) immunohistochemistry. In male Wistar rats under equithesin anesthesia PHA-L (2.5%) was iontophoresed at sodium sensitive sites in the PHA that were identified with microinjections of 150 mM NaCl in a PBS solution. After a survival period of 7-12 days the rats were perfused transcardially and transverse sections of the brainstem were processed using the double labelling immunofluorescense technique for the identification of PHA-L, TH and PNMT. PHA-L labelled fibers and presumptive nerve terminals were observed bilaterally within the nucleus of the solitary tract, area postrema, the rostral and caudal ventrolateral medulla in association with catecholaminergic neurons, nucleus ambiguus, in the region of the A5 noradrenergic cell group, locus coeruleus, inferior olive, lateral paragigantocellular nucleus and parabrachial nucleus. These data provide the neuroanatomical substrate by which sodium sensitive pressor sites in the PHA can alter the regulation of the cardiovascular system. (Supported by the Heart and Stroke Foundation of Ontario.)

497.7

AREA POSTREMA STIMULATION EXCITES THEN INHIBITS MEDULLARY NEURONS IN THE BAROREFLEX PATHWAY. A.C. Bonham*, M-A. Obaidi, K. Ravi and J.A. Stewart. University of California, Davis, Davis, CA 95616.

Neurons in the area postrema augment baroreflex-mediated sympathoinhibition. We have shown previously that this augmentation may occur in the nucleus tractus solitarius (Soc Neurosci 16:220, 1990). The current study was aimed to determine if area postrema neurons also modulate the activity of neurons in the rostral ventrolateral medulla (RVLM) in the baroreflex pathway. We performed experiments in α-chloralose-anesthetized, paralyzed and ventilated rabbits in which renal sympathetic nerve activity (RSNA), arterial pressure, ECG and tracheal pressure were recorded. Extracellular single unit activity was recorded in the RVLM and tested for discharge patterns locked to RSNA; for baroreceptor input by injecting iv phenylephrine (6 - 20μg/kg) and nitroprusside (6 - 20μg/kg); and for responsiveness to single electrical pulses delivered to the area postrema (25 - 50μA, 1Hz, 0.3 - 0.7ms). Six of seven cells which discharged in phase with RSNA, were inhibited by increases in arterial pressure (4 - 41mmHg), and were excited by decreases in arterial pressure (5 - 19mmHg), also received a biphasic excitatory/inhibitory input from the area postrema. The early excitatory phase had a mean peak latency of 31.7 ± 4.0ms ($\bar{x}$±SD)(range = 24.9 - 36.9ms); the subsequent inhibitory phase had a mean onset latency of 60.8 ± 17.7ms (range = 41.6 - 91.2ms) and a duration of 99.4 ± 51.4ms (range = 40.0 - 168.0ms). The remaining cell was excited (peak latency of 30.7ms) but not inhibited by area postrema stimulation. These findings suggest that area postrema neurons may modulate in a complex manner the activity of medullary sympathetic neurons in the baroreflex pathway. Supported by AHA 9-138.

497.8

A1 AREA NEURONS WITH PROJECTION TO THE SUPRAOPTIC NUCLEUS ARE EXCITED BY ELECTRICAL STIMULATION OF THE ABDOMINAL VAGUS NERVE. Z.J. Gieroba* and W.W. Blessing. Centre for Neuroscience, Flinders University, Bedford Park, SA 5042, Australia.

Neurons in the A1 area of the medulla oblongata excite vasopressin-secreting cells in the hypothalamic magnocellular nuclei. Vasopressin neurons in the hypothalamus are affected by stimulation of the abdominal vagus. We have now tested, in urethane-anesthetized rabbits (1.5 g/kg, i.v.), whether the discharge rate of A1 area neurons, identified with extracellular tungsten electrodes and antidromic activation from the supraoptic nucleus, meeting collision criteria, is affected by electrical stimulation of the abdominal vagus (cuff electrode at the level of the diaphragm, 0.5 ms, 200 Hz, 1-3 cathodal pulses). Peristimulus time histograms were constructed using an ITC16 interface and a Macintosh IIfx computer programmed with IGOR. Of 58 neurons identified, 42 (72%) were excited by stimulation of the vagus. No neurons were inhibited. The latency to maximum excitation was 251 ± 11 ms (conduction velocity 0.52 m/s). Excitation of neurons was followed by inhibition lasting approximately 50 ms. Of the 30 neurons excited by the vagus, 26 were inhibited by activation of baroreceptors using intravenous phenylephrine. We have searched for a physiological stimulus which might activate these neurons. Infusion of hypertonic saline into the portal vein did not affect the discharge of the A1 cells. Nor did distension of the stomach with a balloon. Nor did stimulation of the central end of all nerves entering the hilus of the kidney. These results provide evidence, that nearly all neurons in the A1 area with projections to the supraoptic nucleus are excited by electrical stimulation of the abdominal vagus. The relevant physiological stimuli activating the cells via abdominal vagal afferents are not yet identified.

497.9

Cardiovascular effects of oxytocin injections into areas of the medulla oblongata receiving oxytocinergic innervation.
R.E.Gomez, M.A.Cannata, M.Anwar* and D.Ruggiero. ININCA,UBA,Bs.As. and Div. of Neurobiol., CUMC,NY

We examined the distribution of oxytocinergic terminals and the effect on arterial pressure (AP) of oxytocin microinjections into the nucleus tractus solitarii (NTS) and the reticular formation of the caudal (CVL) and rostral (RVL) ventrolateral medulla. Immuno-cytochemical labeling with an antiserum against a synthetic oxytocin conjugate revealed axons and punctata with oxytocin-like immunoreactivity in NTS and RVL. In combined retrograde transport immunofluorescence studies deposits of Fluoro-Gold into NTS labeled larger numbers of neuro-peptidergic neurons in the parvicellular paraventricular hypothalamic nucleus than RVL deposits. Changes in AP (mmHg) after bilateral microinjections in anesthetized rats occured within the first minute after injection:

Oxytocin	NTS	CVL	RVL
10 pmol	3±1	-3±7	6±1
20 pmol	2±2	-0.3±3	15±0.5

A similar dose of oxytocin injected intravenously produced no change in AP. The data suggest that oxytocin may modulate AP via receptors in the medullary reticular formation.

497.10

TRACING OF SINGLE FIBERS DEMONSTRATES THAT MIDBRAIN PERIAQUEDUCTAL GRAY NEURONS HAVE COLLATERALIZED PROJECTIONS TO SOMATIC AND CARDIOVASCULAR RELATED REGIONS IN THE MEDULLA.
P. Carrive, R. Bandler and M. Christie* Departments of Anatomy and Pharmacology, University of Sydney, NSW 2006, Australia.

The midbrain periaqueductal gray (PAG) plays a crucial role in the integration of somatic and autonomic components characteristic of different patterns of defensive reactions. These components are mediated by descending projections to autonomic and somatic regions of the lower brainstem, but the extent of collateralization within these projections is not known. We report here the existence of collateralized projections to distinct somatic and autonomic regions of the medulla and cervical cord.

Biocytin was used as an anterograde tracer. It was applied iontophoretically at sites located in the intermediate third of the lateral PAG of 3 rats and 2 cats. Ten fibers labelled with biocytin were followed from section to section and reconstructed along their entire medullary course.

The results show that 6 of 8 fibers terminating in the vasopressor region of the rostral ventrolateral medulla also have collaterals terminating (i) in the dorsomedial part of the facial nucleus (control of ear musculature; 3 fibers), (ii) in the nucleus retroambigualis (control of expiratory musculature; 3 fibers), and (iii) in the ventral horn of the upper cervical spinal cord (control of neck muscles; 2 fibers). Each region mediates a characteristic component of the defensive reaction evoked from the intermediate third of the lateral PAG. Such collateralized projections may well play a significant role in the integration of the autonomic and somatic functions characteristic of this region of the PAG.

497.11

MODULATION OF THE CARDIOVASCULAR DEFENCE RESPONSE BY THE MEDULLARY RAPHE NUCLEI. T.A. Lovick. (SPON: Brain Research Association). Dept. of Physiology, University of Birmingham, Birmingham B15 2TT, U.K.

Electrical stimulation (10sec, 80Hz, 30-100μA) in the lateral area of the anterior hypothalamus (LAAH) or dorsal periaqueductal grey matter (PAG) evoked a pressor response with tachycardia and vasodilatation in hindlimb muscle in rats anaesthetised with Saffan. Activation of perikarya in nucleus raphe magnus (NRM) and nucleus raphe obscurus (NRO), by microinjection of 10-15nmol D,L-homocysteic acid (DLH) attenuated all components of the PAG-evoked cardiovascular defence response whereas individual components of the LAAH-evoked response were modified selectively. Stimulation in NRM reduced the LAAH-evoked vasodilatation by 64%, converted the tachycardia to a bradycardia and had no significant effect on the pressor response. Activation of cells in NRO also produced a reduction (38%) in the LAAH-evoked vasodilation but potentiated the tachycardia and pressor response by 110% and 35% respectively.

The results suggest 1) that the medullary raphe may differentially modulate activity in the descending pathways from the hypothalamic and midbrain defence areas and 2) that functional differences exist between NRM and NRO with respect to control of the LAAH-evoked response.

497.12

LONGITUDINAL COLUMN OF CARDIOVASCULAR SYMPATHOINHIBITORY CELLS IN CAT MEDULLA EXTENDS MEDIALLY INTO LATERAL TEGMENTAL FIELD. C. W. Dempesy*, D. E. Richardson, and C. J. Fontana. Lab. of Neurosurgery, Tulane University School of Medicine, New Orleans, LA 70112.

We have previously shown (Neurosci. Abst. 17: 994, 1991) that cardiovascular sympathoinhibitory cells in cat medulla are distributed in a longitudinal column running parallel and lateral to the longitudinal array of cells comprising the ambiguus nucleus. The column extends 4.5 mm from its posterior end in the caudal ventrolateral medulla (CVLM) to its anterior end above the rostral ventrolateral medulla (RVLM). In contrast, Gebber and Barman have reported in cat a similar columnar distribution of cells displaying cardiovascular sympathoinhibitory activity, but lying entirely medial to the ambiguus nucleus in the lateral tegmental field (J. Neurophysiol. 54: 1498, 1985). We now report that a survey of the medullary region lying medial to our lateral column, using microinjection of excitatory amino acids, verifies the existence of a continuum of cardiovascular sympathoinhibitory cells distributed from 2 to 4 mm laterally and encompassing the region of the ambiguus nucleus. Chemical inhibition of small areas of this continuum yields hypertension, tachycardia, and partial loss of baroreflex, with these effects weakening in the more medial and caudal aspects of the array.

497.13

MODULATION OF THE NEURONAL FIRING IN THE NUCLEUS TRACTUS SOLITARIUS BY ELECTRICAL STIMULATION OF THE HYPOTHALAMIC DEFENSE AND VIGILANCE AREAS IN RABBITS. Y-F. Duan*, R.W. Winters, P.M. McCabe, E.J. Green, Y. Huang, and N. Schneiderman, Neuroscience Program and Department of Psychology, University of Miami, Coral Gables, FL, 33124.

Stimulation of the hypothalamic defense area (HDA) elicits a cardiorespiratory response that is thought to prepare the animal for "fight or flight". This response is characterized by increases in cardiac output, blood pressure (BP), heart rate (HR), hindlimb blood flow and hyperventilation. The cardiorespiratory response elicited by stimulation of the hypothalamic vigilance area (HVA) is associated with the inhibition of movement and is characterized by a BP increase, HR decrease and brief inspiratory apnea or a shallow tachypnoea. The present study was conducted to determine if HDA and HVA make functional connections with NTS in rabbits.

Adult New Zealand albino rabbits were anesthetized with isoflurane. The HDA and HVA stimulation was delivered to the posterior dorsomedial hypothalamus via a bipolar stainless electrode (200-300 uA,100 Hz, 0.5 ms duration, 0.1-5 sec train). Extracellular unit recordings were made in NTS with stereotrode electrodes during the hypothalamic stimulation.

Most of the NTS neurons affected by electrical stimulation of the HVA showed a decrease in firing rate. Approximately half of the NTS neurons that responded to the HDA stimulation showed an increase in firing rate. Many of these NTS neurons were found to receive barosensory information as well. A small number of the NTS neurons recorded did not respond to either the HVA or HDA stimulation. These findings suggest that both HVA and HDA make functional connections with the NTS. (Supported by NIH HL 36588 and HL 07426).

497.14

USE OF PSEUDORABIES VIRUS IN DEFINING THE CONNECTIONAL CIRCUIT OF THE MEDULLARY BARORECEPTOR VAGAL REFLEX IN THE RAT *J.A. Escardo, L. Enquist, M. Whealy and J.S. Schwaber*, DuPont Co & Dupont-Merck, Wilm., DE 19880.*

Experiments using Cholera Toxin-Horseradish Peroxidase (CT-HRP) as a tracer have delineated the region of baroreceptor and cardiac afferent input as well as the distribution of vagal preganglionic cardiomotor neurons (Escardo et al., 1991). However, the location, extent and number of interneuronal groups subserving the medullary component of the baroreceptor vagal reflex is unknown. In order to address these questions, in the present study we have used the attenuated Bartha strain of the pseudorabies virus (PRV) as a retrograde trans-neuronal (putatively trans-synaptic) tracer. PRV was injected into two distinct cardiac sites shown by injections of CT-HRP to receive vagal preganglionic innervation: between the superior vena cava and the aorta and at the crossing of the jugular vein, left ventricle and pulmonary artery. The earliest labeling of neurons in the medulla was present at 48 hours and was confined to members of the rostral and caudal nucleus ambiguus (NA) vagal cardiac efferent populations, as defined by location and morphology seen earlier in the CT-HRP studies. With longer survival times the picture became much more complicated, but we will focus here on just a few relevant cell groups. At 68-72 hours survival time numerous non-vagal motor neurons were labeled with PRV in the vicinity of the caudal NA group, basically in the area of the caudal ventro-lateral medulla or A1 catecholaminergic population. Labeled neurons were scattered along an arc through the dorsal medullary reticular field and a few scattered neurons were labeled in the nucleus tractus Solitarii (NTS), particularly in its ventral subdivisions. At 94 hours+ this later labeling became more abundant, but in addition a new, densely packed group appeared in the dorsal NTS within the regions receiving baroreceptor and cardiac afferents. We interpret these results to suggest that the simplest baroreceptor vagal reflex involves at least two interneurons.

497.15

PHYSIOLOGICAL CHARACTERIZATION OF RHYTHMICALLY BEATING NEURONS IN THE CARDIORESPIRATORY NUCLEUS OF THE SOLITARY TRACT (crNTS) IN THE RAT. *J.F.R. Paton* & J. S. Schwaber,* Neural Computation Group, E.I. DuPont Co., Wilmington, DE 19880-0323.

We previously characterized two groups of rhythmically beating cells at 5Hz (autoactive & synaptically driven) in the crNTS *in vitro* (Paton et al. *J.Neurophysiol.* **66**: 824-838, 1991). The present study sought to determine whether such neuronal activity existed *in vivo* and, if so, to physiologically characterize cells based on the visceral origin of synaptic input(s). Extracellular recordings were made from 28 rhythmically discharging single units in the crNTS in anesthetized rats (chloral hydrate: 375 mg/kg & pentobarbital: 75 mg/kg; i.p.). All cells fired a single spike regularly (mean 6.5 Hz) and were divided into two groups based on their firing relationship with the R wave of the ECG. One group of neurons (n=10) discharged with a relatively constant phase angle to the R wave whereas the second group was less tightly coupled to the cardiac cycle but fired at a similar frequency as heart rate. Both groups of cells showed a peak in the R wave triggered histogram (mean latency: 18ms). Of 18 cells tested, 16 received an excitatory synaptic input following electrical stimulation of the ipsilateral vagus nerve (mean latency 20 ms) and 3 neurons were also excited by aortic nerve stimulation (latency range: 15-18ms). Brief periods of cardiac arrest, produced by electrically stimulating the peripheral end of the cut vagus nerve, decreased the firing frequency and, following recovery of heart rate, disrupted the phase relationship with the R wave of the ECG. Veratridine (5-20 μg; i.v. bolus) strongly excited all 4 rhythmically firing cells tested. These data indicate the presence of neurons beating rhythmically at the cardiac rate in the crNTS that receive cardiac afferent synaptic inputs.

497.16

ENCODING OF ARTERIAL BLOOD PRESSURE CHANGES BY PUTATIVE SECOND ORDER BARORECEPTIVE NEURONS IN THE NUCLEUS OF THE SOLITARY TRACT IN THE RAT. *R.F. Rogers, J.F.R. Paton, W.F. Herblin,* & J. S. Schwaber,* Neural Computation Group, E.I. DuPont Co., Wilmington, DE 19880-0323.

There is a paucity of information concerning the response patterns of second order NTS neurons to graded arterial pressure changes. Thus, the present study examined the firing response characteristics of putative second order baroreceptive neurons during increases in arterial pressure following i.v. administration of phenylephrine (PE; 5-20 μg). Anesthesia was induced in rats using halothane followed by an infusion of Saffan (12 mg/kg/hr i.v.). Extracellular recordings were made from 19 single NTS neurons receiving a short and invariant excitatory synaptic input following ipsilateral aortic nerve stimulation (range of latency to spike: 1.8-4.0 ms). From a total of 19 cells tested 10 were silent at baseline mean arterial pressure of 90-120 mmHg. The response of these neurons to PE injection was characterized by either a single action potential or a transient burst of activity (2-7 spikes) at a specific level during the rising phase of the blood pressure response. As blood pressure continued to rise past this point firing ceased until the same blood pressure level was reached during the falling phase, at which time a similar pattern of discharge occurred. The remaining 9 cells showed ongoing activity (mean pressure range 90-120 mmHg) which increased and decreased proportionally during the rising and falling phases of the blood pressure response to PE injection respectively. These results show a heterogeneous population of putative second order baroreceptive NTS neurons. It is suggested that while some NTS neurons reflect mean arterial pressure in their individual firing frequencies, other neuron types may contribute to population encoding of mean arterial pressure and changes in blood pressure.

497.17

IONIC CURRENT MODEL OF DELAYED EXCITATION IN NEURONS OF RAT MEDIAL NUCLEUS TRACTUS SOLITARIUS (mNTS). M.C. Andresen*, S. Khushalani, J.H. Schild, M. Yang, D.L. Kunze, and J.W. Clark. Oregon Health Sciences Univ., Portland, OR 97201, Rice Univ. and Baylor College of Medicine, Houston, TX 77251.

Anatomical studies suggest that aortic arch baroreceptors synapse within a limited area of mNTS. Our studies focused on intracellular recordings from medullary slices including this area and on patch clamp work with cells acutely dispersed from mNTS. In slices, depolarizing current injection resulted in a rapid increase in spike rate followed by spike frequency adaptation (SFA). Pre-hyperpolarization induced a prolonged delay (DE) before resuming spiking and eliminated SFA in all neurons. In isolated mNTS neurons, two hyperpolarization-activated, transient-outward potassium currents were found with time constants matching the DE time course. We developed a comprehensive ionic current model with thirteen coupled, first-order, non-linear differential equations (Hodgkin-Huxley) to represent 8 ionic conductances, two ATPase pumps (Na-K and Ca), a Na/Ca exchanger, and calcium balance within the neurons. With a single fixed set of fit parameters, the model effectively predicted neuronal responses to a wide range of multi-step current injection protocols over tens of seconds. The voltage dependence of DE, SFA, and changes in action potential shape were successfully reproduced. The model demonstrates the dynamic interaction of a family of voltage dependent conductances, emphasizes the pivotal role of transient potassium currents in shaping neuronal responses and suggests a valuable tool for integrating information from isolated cells with more intact preparations.

497.18

THE INTERRELATIONSHIP OF RENIN-ANGIOTENSIN SYSTEM AND ADENOSINE IN THE BRAINSTEM NUCLEI OF RATS. H.C. Lin, C.S. Tung, C.T. Yen*, C.J. Tseng, Depts. of Pharmacology, Physiology, and Zoology, National Defense Medical Center and National Taiwan University, Taipei, Taiwan, R.O.C.

The purpose of this study is to determine the possible interaction of adenosine and renin-angiotensin system in the brainstem nuclei of the rat. Male Sprague-Dawley rats were anesthetized with urethane. Adenosine, angiotensin (ANG) II, ANG III, and their antagonist 1,3-Dipropyl-8-p-sulfophenylxanthine (DPSPX) and Sar^1,Ile^7 ANG III were microinjected into the nucleus tractus solitarii (NTS) and area postrema (AP) of rats. Our results demonstrated that microinjection of DPSPX significantly attenuated the depressor and bradycardia effect at low dose (9.6 pmol) of ANG II and ANG III. Whereas, the same dose of DPSPX significantly potentiated the pressor effect at high dose (480 pmol) of ANG II and ANG III in the NTS and AP. The depressor and bradycardia induced by high dose ANG II and ANG III were slightly inhibited by DPSPX. On the other hand, when ANG antagonist were microinjected 10 minutes prior to the injection of adenosine, we found that the Sar^1,Ile^7 ANG III can affect the depressor and bradycardia effects of adenosine in the AP, not in the NTS. In conclusion, the endogenous adenosine may influence the renin-angiotensin system in the brainstem nuclei of rats. Precisely modulatory mechanisms remain to be clarified.

497.19

REGULATION OF ANGIOTENSINOGEN mRNA EXPRESSION IN THE MEDULLA OBLONGATA IN HYPERTENSIVE AND NORMOTENSIVE RATS. A. Milsted*, C.H. Block, K.B. Brosnihan, Z.R. Rodriguez and C.M. Ferrario. Cleveland Clinic Fndn, Research Institute, Cleveland, OH 44195.

A critical role for the dorsal and ventral medulla oblongata in cardiovascular regulation is well established. Further, angiotensin (Ang) peptides and receptors have been demonstrated in these cardiovascular regions. We showed an increase in expression of angiotensinogen (Aogen) mRNA in aortic-ligated hypertensive rats, compared to sham-operated controls (Nishimura et al., Hypertension, 1992). To further define more discrete changes in Aogen mRNA expression in these medullary areas, we carried out *in situ* hybridization histochemistry to both localize and quantitate changes in Aogen mRNA. Aogen-specific probe was a 30-base long synthetic oligodeoxynucleotide, 3'-labeled with ^{35}S-ATP, complementary to the nucleotides encoding Ang I [anti-sense]. Non-specific [sense] probe was its reverse complement. Following autoradiography, signals were quantitated by microdensitometry to measure relative film density in the area postrema, nucleus tractus solitarius, dorsal motor nucleus of the vagus (DMNX), hypoglossal nucleus, reticular formation, and inferior olive. Expression of Aogen mRNA was increased by approximately 50% in the DMNX and the hypoglossal nucleus six days after aortic ligation, at a time when circulating and central renin-angiotensin systems are activated and plasma renin activity and Ang II levels are elevated. Twenty-four days after aortic ligation, no apparent changes in Aogen mRNA expression were found in the regions examined, suggesting homeostasis in Aogen mRNA expression. This return to normal levels of Aogen mRNA expression in the medulla parallels changes in plasma renin activity and Ang II levels which have returned to near normal levels, despite persistently elevated blood pressure. Results of these studies confirm a role for the DMNX during the development of hypertension, and further indicate that compensatory homeostatic systems are likely to assume a more prominent role in regulating expression of Aogen mRNA in the medulla oblongata during the chronic stages of hypertension. (Supported in part by NIH HL-6835).

497.20

MICROINJECTION OF AN ENDOTHELIN ANTAGONIST INTO THE NUCLEUS TRACTUS SOLITARII FACILITATES BAROREFLEX ACTIVATION. R. Mosqueda-Garcia*, T. Inagami, M. Appalsamy and R.M. Robertson. Departments of Medicine and Biochemistry, Vanderbilt University, Nashville, TN 37232.

Previous reports have documented that central administration of endothelin-1 (ET-1) affects BP, HR and baroreflex sensitivity. Interpretation of these effects, however, has been complicated by the potential non-specific effects of this peptide. We have sought to circumvent this limitation with the use of a specific ET-receptor antagonist.

Glass micropipettes were placed in the nucleus tractus solitarii (NTS) of urethane anesthetized Sprague-Dawley rats. Baroreflex function was evaluated with bolus administration of phenylephrine. After control baroreflex slopes were obtained, the animals were divided into groups that received saline or different doses of the specific receptor ET_A-antagonist BQ-123 (1,4,8 nmols/60 nl). Ten minutes after intra-NTS administration of the drugs, baroreflex slopes were again evaluated. Bilateral microinjection of BQ-123 into the NTS decreased BP and HR in a dose-dependent manner (8 nmols, -30±4 mmHg and -80±21 bpm) and increased baroreflex slope.

DRUG	n	CONTROL SLOPE	DRUG SLOPE
SALINE (60 nl)	4	2.6 ± 0.4 mmHg/sec	2.2 ± 0.3 mmHg/sec
BQ-123 (1 nmol)	6	1.3 ± 0.2	2.3 ± 0.5*
BQ-123 (4 nmol)	12	2.0 ± 0.2	3.5 ± 0.8*
BQ-123 (8 nmol)	16	2.0 ± 0.2	2.5 ± 0.4*

Values are mean ± sem; * indicates significant difference from control ($p<0.5$).

These results suggest that ET-1 has an important modulatory effect on baroreflex activation which are mediated by specific receptor subtype.

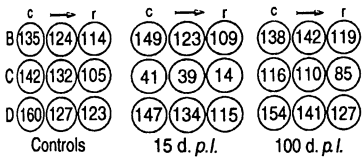
SUBCORTICAL SOMATOSENSORY PATHWAYS: TRIGEMINAL

498.1

QUANTITATIVE STUDY OF THE PATTERN OF REINNERVATION OF VIBRISSAL FOLLICLES AFTER PARTIAL DENERVATION OF THE WHISKERPAD OF ADULT MICE.

M-E.Corthésy, E.Welker, H.Van der Loos, B.M.Riederer*.
Institute of Anatomy, University of Lausanne, 1005 Lausanne, Switzerland.

Partial denervation of skin in adult animals leads to changes in the central representations of neighboring skin areas. We have quantified the peripheral innervation of the caudalmost whisker follicles of rows B, C & D at varying intervals after denervation (double ligation followed by transection of the nerve) of follicles of row C in 9 adult mice. Controls show a caudo-rostral (c→r) gradient of innervation (figure). Numbers are medians based on counts in 3 mice per group. At five days *post lesionem* (*p.l.*) Wallerian degeneration is complete: there are no fibers in the follicles of row C. At 15 days *p.l.* reinnervation appears, and from about 60 days *p.l.* onwards the number of nerve fibers reaches a plateau. The c→r gradient of innervation is restored, although fiber numbers are smaller than in controls. There were no changes in the innervation of the neighboring, intact follicles. These observations provide evidence of a numeral regulation of the reinnervation of vibrissal follicles and will be used in elucidating the mechanisms underlying adult plasticity in the somatosensory pathway. Support: Swiss NSF 31-30932.



498.2

TIME COURSE OF SEROTONERGIC AFFERENT PLASTICITY IN RAT SPINAL TRIGEMINAL NUCLEUS AFTER INFRAORBITAL NERVE CUT. B.G. Klein*, W.D. Blaker, C.F. White and B.R. Misra. Dept. of Biomedical Sciences, College of Veterinary Medicine, VA Tech, Blacksburg, VA 24061, Dept. of Biology, Furman University, Greenville, SC 29613.

Non-peripheral serotonergic afferents to rat spinal trigeminal subnucleus interpolaris (SpVi) exhibit an increased concentration of serotonin (5-HT) and an increased density of 5-HT immunoreactive (5-HTIR) varicosities >76 days following infraorbital nerve (ION) transection in adults (Klein & Blaker, 1990, Brain Res., 536:309-314). To delineate the time course of this change, high performance liquid chromatography with electrochemical detection (HPLC-ED) and immunocytochemistry were used to examine serotonergic afferent plasticity within SpVi between 3 and 79 days following ION transection. For HPLC-ED, samples from the infraorbital (IO) region of SpVi on the lesioned side were compared to similar samples on the intact side and to samples in normal rats. For post-lesion survival times from 3-49 days, no change in 5-HT concentration was observed on the lesioned side. Also, at 49 days after ION cut, no change in density of 5-HTIR varicosities was seen in the IO region on the lesioned side. In rats surviving 76-79 days post-lesion, we replicated our previous finding of a significant increase (38%) in 5-HT concentration on the lesioned side. It appears that the anatomical and biochemical changes in 5-HT afferents to SpVi following ION transection exhibit a slow or delayed development, that does not reach detectable proportions until >7 wks following the cut. Such data will help to interpret the role of 5-HT afferent plasticity in the somatosensory sequelae observed following ION damage. Support: NIDR DE08966 to BGK.

498.3

CENTRAL EFFECT OF DECIDUOUS TOOTH PULPECTOMIES. [1,3]M.A. Henry* and [1,2,3]L.E. Westrum. Depts. [1]Neurol. Surg., [2]Biol. Struct., and [3]Restor. Dent., University of Washington, Seattle, WA 98195.

We are studying the response of the trigeminal nuclei (TN) to injury. Previous studies have shown argyrophilia and degenerative alterations of axons and terminals within the TN following tooth pulp lesions performed in the adult cat. The effect of similar lesions performed on deciduous teeth in kittens has not previously been reported. Pulpectomies and gutta percha obturation of the canals were performed on the left maxillary and mandibular deciduous cuspids of 8-10 week old kittens (8 subjects) with survival periods ranging from 1 day to 3 weeks. Tissue from throughout the TN was reacted with the Fink-Heimer stain and in addition laminae I and II of pars caudalis/medullary dorsal horn was examined with the electron microscope (EM) in regions where dental afferents are known to terminate. Examination of the Fink-Heimer preparations showed a lack of argyrophilia. Sections examined with the EM revealed axonal and terminal alterations suggestive of degeneration and less commonly of regeneration or recovery and the latter were characterized by the presence of agranular reticulum. Most of the terminal alterations were electron lucent and included flocculent forms with reduced synaptic vesicles often surrounded by enlarged glial profiles or terminals with inclusions including glycogen particles. The lack of argyrophilia and the presence of fibers with agranular reticulum suggest a greater potential for recovery in the immature feline trigeminal system following injury. (Supported by NIDR Grants DE00219 & DE04942. L.E.W. is a research affiliate of the CDMRC).

498.4

NERVE GROWTH FACTOR RECEPTOR (NGFR) PATTERNS IN NORMAL SPINAL TRIGEMINAL NUCLEUS AND AFTER DEAFFERENTATION: LIGHT AND ELECTRON MICROSCOPY. [1,2,4]L.E. Westrum*, [1,4]M.A. Henry, and [3]M.A. Bothwell. Depts. of [1]Neurol. Surg., [2]Biol. Struct, [3]Physiol. & Biophys. and [4]Restorative Dentistry, University of Washington, Seattle, WA 98195.

Light and electron microscopy (LM, EM) are being used to study localization of immunoreactivity (IR) to NGFR in the brainstem spinal trigeminal (V) nucleus of normal adult felines and after retrogasserian rhizotomy (10 and 30 days). The antibody was raised against human NGFR and presumably recognizes both high and low affinity sites. The LM-IR occurs as either dense pockets of label or as a more homogeneous label, greater than background, that outlines the nuclei. Lamina II in pars caudalis shows dense NGFR-IR. EM of this region shows NGFR-IR in small unmyelinated or medium-sized myelinated axons and in terminals that form asymmetric synaptic contacts, some of which are a part of a glomerulus. Trigeminal rhizotomy results in a complete loss of NGFR-IR (both types) throughout the nuclei, but spares a few dense pockets of IR compatible with inputs from VII, IX and X. The findings show that NGFR-IR clearly delineates the boundaries of the V nucleus, represents primary afferents, some of which are associated with nociception (dense pockets) and that spared non-V input may represent nociceptive input from VII, IX and X. (Supported by NIH Grants DE00219, DE04942 and NS23343. L.E.W. is a research affiliate of CDMRC).

498.5

RESPONSES AND THALAMIC PROJECTIONS OF THERMORECEPTIVE NEURONS IN RAT SUPERFICIAL MEDULLARY DORSAL HORN (MDH). W.D. Hutchison* and J.O. Dostrovsky, Dept. Physiology, Univ. of Toronto, Toronto, Canada M5S 1A8

Little is known concerning central processing of innocuous temperature inputs in the rat. The aim of the present study was to determine the characteristics and projection targets in thalamus of neurons in rat MDH that respond to innocuous thermal stimuli applied to the orofacial region.

Recordings were made from 58 cold cells in the superficial MDH of chloralose/urethane-anesthetized rats. Cells were identified by responses to cold probes and radiant warmth and their responses further quantified with a Peltier thermode. The receptive fields of most of the neurons were on the upper lip (54%) or tongue (27%). Many (45%) of the neurons responded only to innocuous cooling, whereas the remainder could also be activated by mechanical stimulation although these responses were small compared to those produced by a rapid thermal change. Of these mechanically sensitive neurons, 57% responded to touch, 25% only to noxious pinch and 17% to both touch and pinch stimuli. Many (56%) of the 27 neurons tested responded also to noxious thermal stimuli. Cooling steps (5°C) produced mostly tonic responses in 40% of the 15 cells tested, mostly phasic responses in 27% and both types in the remaining 33%.

Antidromic mapping was performed for six MDH cold cells. Stimulation was delivered at 250μm steps as the bipolar electrode was driven through thalamus. Multiple tracks at mediolateral intervals of 0.5 mm were made. The neurons were antidromically activated from thalamus with currents of 9-50 μA (0.2ms, 1Hz) at latencies of 2.0-2.4 ms (mean conduction velocity 6.6 m/s +/- 0.9 S.D.). All 6 neurons were activated from the ventrobasal complex; 2 of the neurons were also activated from sites that were in medial thalamus, one within Sm. (supported by NIH DE05404)

498.6

DISTRIBUTION OF TRIGEMINAL NEURONS PROJECTING TO SPECIFIC CEREBELLAR CORTICAL REGIONS IN THE RAT. J.J.A. Arends*, T.J.H. Ruigrok & M.F. Jacquin, Dept. of Anatomy & Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104; Dept. of Anatomy, Erasmus University, 3000 DR Rotterdam, The Netherlands.

Single and multiple retrograde tracing studies using WGA-HRP, Fluorogold, Diamidino-yellow, and FITC- and TRITC-labeled latex bead injections into different "orofacial" areas of the cerebellar cortex were used to study the distribution of trigeminal neurons that project to specific or widespread cerebellar regions. Injection targets included the vermis/paravermis (uvula and lateral lobules II-V) and the hemispheres (paramedian lobule, Crus I and II, simple lobule). In 22 rats, labeled cells were seen in trigeminal subnuclei principalis, oralis and/or interpolaris, as well as the trigeminal ganglion. There was a clear relationship between the dominant receptive field type within the cerebellar injection site (vibrissa vs. non-vibrissa; from Welker, '87) and the numbers and distribution of retrogradely labeled trigeminal neurons. For example, injections in regions with predominant vibrissa representations (Crus I, uvula) produced the largest numbers of labeled cells in vibrissa regions of ventral principalis and interpolaris, with moderate numbers in dorsal oralis following uvula injections. Tracer deposits in other non-vibrissa cortical regions produced labeled cells primarily in non-vibrissa regions of dorsal principalis, oralis and interpolaris. Similarly, contralateral and bilateral projections were primarily to non-vibrissa cerebellar areas and originated in rostral interpolaris, caudal oralis and rostral principalis. Labeled ganglion cells were seen in all cases except those involving the paramedian lobule. Only a small % of the trigeminal mossy fibers branched to terminate in more than one cerebellar region. NIH NS29885, DE07734, DE07662.

498.7

DIFFERENTIAL FOCI OF TRIGEMINAL PRINCIPALIS AND INTERPOLARIS PROJECTIONS TO THALAMUS. M.N. Williams* & M.F. Jacquin, Anat/Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

The thalamic VPM nucleus receives inputs from all 4 trigeminal brainstem subnuclei. We (Williams et al., Neurosci. Abstr. 17, '91) have reported that principalis (PrV) terminals are more likely to synapse on more proximal portions of VPM dendrites than terminals from interpolaris (SpVi). To further assess the extent to which PrV and SpVi inputs to thalamus are complementary or overlapping, adult rats each received HRP injections in PrV and rhodamine- or fluorescein-dextran deposits in ipsilateral SpVi. Only those 8 cases where HRP-TMB reaction product spanned all of PrV and revealed a complete whisker-like projection pattern in VPM were considered. PrV's most robust projection was to the barreloid region of VPM. SpVi's most robust projections were to non-barreloid regions of thalamus, including the VPM "shell" that encapsulates the barreloid area, posterior thalamus, and nucleus submedius. In the barreloid region, SpVi projections were relatively sparse and terminal swellings occurred most frequently in the peripheral fringes of individual, HRP-labeled, whisker-related patches. In nucleus submedius, SpVi inputs were dense and patchy. A dense SpVi projection was also revealed within ventral and caudal VPM that intrudes in a "finger-like" manner into the barreloid region. We therefore conclude that PrV and SpVi have, for the most part, complementary projection foci in thalamus. In conjunction with our prior indications of a differential synaptic organization of PrV and SpVi inputs to VPM dendrites, the present results suggest additional anatomical bases for the relative efficacies of these 2 parallel pathways in dictating thalamic responses to trigeminal stimuli.
Support: NIH DE07662, DE07734, NS29885.

498.8

RECEPTIVE FIELD SYNTHESIS IN RAT NUCLEUS PRINCIPALIS: SPINAL TRIGEMINAL CONTRIBUTIONS. D.W. Doherty*, H.P. Killackey & M.F. Jacquin, Psychobiology, Univ. Calif., Irvine, CA 92717; Anatomy & Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Most cells in trigeminal nucleus principalis (PrV) have single whisker receptive fields (RFs) with phasic responses lacking direction sensitivity. As a first step in assessing how spinal V inputs impact on PrV RFs, 79 PrV cells (66 thalamic-projecting, 13 local circuit) were recorded in 11 barbiturate anesthetized rats before and after infusions (0.037 - 0.53 μl) of glutamate (0.5 M) or lidocaine (2%) into most of subnuclei caudalis and interpolaris. The projection status of PrV cells was determined with antidromic and intracellular labeling methods. Glutamate-induced changes in PrV RFs were seen in 19 of 43 thalamic-projecting cells, where 13 exhibited smaller RFs or became less responsive to peripheral stimuli and 6 displayed larger RFs or more spikes per whisker deflection. Glutamate-induced changes were seen in 5 of 6 local circuit neurons, all of which became less responsive to peripheral stimuli. In 3 projection neurons with phasic responses to sustained whisker deflection, glutamate infusion induced tonic responses.

Lidocaine-induced changes were seen in 12 of 23 thalamic-projecting cells, where 3 exhibited smaller RFs or fewer spikes per stimulus, 7 displayed larger RFs or more spikes per stimulus, and 2 shifted their RF to another whisker(s). Lidocaine-induced changes in local circuit neurons were only seen in 2 of 7 cells, where 1 expressed a RF shift and the other showed a larger RF. Lidocaine infusion also affected directional sensitivity in 3 projection neurons and altered adaptation properties in 4 others. Thus, it would appear that spinal V neurons have robust influences on the RFs of many PrV cells. DE07662, DE07734, BNS87-19311.

AUDITORY SYSTEM: COCHLEA

499.1

IS AVIAN BASILAR MEMBRANE TRULY LINEAR? A.N. Temchin and G. Moushegian*. Callier Center, University of Texas at Dallas, Dallas, TX 75235.

The only available measures of avian basilar membrane (BM) intensity functions indicate that its behavior is linear in pigeon (Gummer et. al., Hear. Res., 29:63-92, 1987), implying passive cochlear mechanics. Evidence is presented of the existence of three main types of rate-versus-level functions (saturating, slope-saturating, and linear) at the characteristic frequency (CF) of pigeon primary auditory fibers. These functions were obtained with 2dB steps between 0 and 90 dB SPL. Saturating fibers have had broad dynamic ranges (up to 40-45 dB) and sometimes were hardly distinguished from slope-saturating. Unlike mammals, type of function does not depend on the spontaneous rate or threshold at CF. Correlation between CF-threshold and spontaneous rate was small (r=-0.06) and statistically insignificant. Clear break points of sloping-saturated fibers within narrow CF region in the same animal occur over a narrow range of intensities and may be found between 35-45 dB SPL in different animals. The results, along with the earlier described phenomenon of single-tone suppression, which could occur at SPLs even slightly below the CF-threshold may be explained by the nonlinearities of BM and active mechanics in avian cochlea.

499.2

CHARACTERIZATION OF MINERALOCORTICOID (TYPE I) RECEPTORS IN THE INNER EAR BY COMPETITION STUDIES WITH SPIRONOLACTONE. D.Z. Pitovski[1]*, E.C. Burton[1], and D.G. Drescher[1,2], [1]Department of Otolaryngology, [2]Laboratory of Bio-otology, Wayne State University School of Medicine, Detroit, MI 48201.

Spironolactone, a clinically important anti-mineralocorticoid, is thought to compete with aldosterone at mineralocorticoid (Type I) receptor sites in target tissues. Since Type I receptors are present in the inner ear (Pitovski et al., Soc. Neurosci. Abstr. 17: 1107, 1991), it was of interest to investigate the antagonistic action of spironolactone in auditory and vestibular fractions. Microdissected inner ear tissues of male Hartley guinea pigs were incubated with various concentrations of spironolactone (4×10^{-7} to 4×10^{-5} M) in the presence of 4×10^{-9} M ^{3}H-aldosterone. A 500-fold excess of RU 28362 (a highly specific glucocorticoid agonist) was included to minimize binding to glucocorticoid (Type II) receptors. Paired control incubations were performed with ^{3}H-aldosterone, RU 28362, and a 2,000-fold excess of unlabeled aldosterone to determine non-specific binding. Specific binding was calculated from the difference between total binding in the presence of spironolactone (experimental) minus non-specific binding (control, opposite ear). Binding values were also corrected for ^{3}H-aldosterone present intercellularly, monitored with ^{14}C-sucrose and normalized to tissue dry weight. The IC_{50} values for spironolactone in the lateral wall of the basal cochlear turn and the ampulla of the semicircular canal were 4.1×10^{-6} and 4.6×10^{-6} M, respectively, while the corresponding values of K_i were 2.0×10^{-6} and 1.9×10^{-6} M.

Inhibition studies performed with spironolactone showed a dose response reduction of ^{3}H-aldosterone binding, implying that in inner ear tissues this compound behaves as an aldosterone antagonist. (Supported by NIH Clinical Investigator Development Award DC 00046-01 to D.Z.P., NIH Grant T32 DC 00026, and ONR Contract N00014-88-K-0067.)

499.3

DEVELOPMENTAL CHANGES IN THE RESPONSE OF THE PERIPHERAL AUDITORY SYSTEM TO TWO-TONE STIMULI. J.L. Fitzakerley*, J. McGee and E.J. Walsh. Boys Town National Research Hospital and Creighton University, Omaha, NE 68131.

Auditory nerve rate suppression studies have been used to characterize the mature cochlear transduction process, which is nonlinear in nature. In contrast to the adult, some immature peripheral auditory neurons exhibit facilitation, rather than suppression (Fitzakerley et al., ARO 14:422, 1991). This study was designed to investigate several of the stimulus parameters and neural factors influencing the expression of facilitation and suppression during postnatal development. Single unit responses of peripheral auditory neurons were examined using standard extracellular recording techniques. Rate differences were evaluated for test tones placed both above and below characteristic frequency (CF), with probe tones placed at CF. Facilitation was observed throughout the first postnatal month, although its incidence declined with increasing age, while suppression was observed as early as 7 postnatal days. Although all test/probe tone ratios tested were capable of producing facilitation in the youngest animals, above-CF test tones produced facilitation in a higher percentage of units than below-CF test tones. Magnitude of suppression was directly correlated to the degree of frequency selectivity, and facilitation was less likely to be expressed in units showing mature tuning characteristics. The ability of the system to produce action potentials appeared be one of the factors limiting the magnitude of facilitation, while suppression seemed to be more dependent upon probe tone rate than facilitation. (Supported by NIDCD #DC1007).

499.4

ORGAN OF CORTI PROTEIN II SEQUENCE. K. E. Isenberg*, P. K. Robeff, I. Thalmann, and R. Thalmann. Departments of Psychiatry and Otolaryngology, Washington University, St. Louis, MO 63110.

The distinctive structure and function of the organ of Corti probably requires the expression of unique proteins. Two such proteins, designated OCPI and OCPII, have been isolated (Thalmann et al., Laryngoscope 100:99-105, 1990). OCPII was purified by separation of 2-dimensional polyacrylamide gel electrophoresis, electroblotted to PVDF membranes, and subjected to amino acid sequencing. The determination of internal sequence was performed by subjecting OCPII to limited proteolysis with chymotrypsin, papain, subtilisin, or V8 protease, separation of the peptide fragments by one-dimensional polyacrylamide electrophoresis and sequencing the resulting bands. The sequence of OCPII, although incomplete, does not resemble the sequence of any previously described protein. A synthetic peptide corresponding to OCPII residues 3 to 16 was used to raise polyclonal antisera; the polyclonal antisera recognizes OCPII on Western blots. An oligonucleotide was designed to correspond to OCPII sequence and found to detect unique genomic fragments on Southern blotting. The oligonucleotide was used to screen a cochlear cDNA library (provided by Dr. A. F. Ryan); positive clones are being tested with the polyclonal sera against OCPII. The molecular characterization of OCPII will permit the elucidation of the role this protein plays in organ of Corti function.

499.5

INFORMATION RATES AND CODING EFFICIENCIES OF AUDITORY NERVE FIBERS. DA Bodnar*, FM Rieke, RR Capranica, and W Bialek, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, and NEC Research Institute, 4 Independence Way, Princeton, NJ, 08540

Changes in either the amplitude or phase spectrum of a complex sound produce changes in both the spike rates and the synchronization characteristics of auditory nerve fibers in the bullfrog. Because of the simultaneous variation in these two coding parameters it is difficult to assess how complex sound stimuli are represented based on solely on spike rate and synchronization patterns. Therefore, we have applied a new decoding analysis method [Bialek et al.(1991), *Science*, 252:1854], to examine additional coding properties of peripheral auditory units. With this method, an estimate of the stimulus waveform can be made from the spike train response of a neuron. In addition, this method provides measures of the information rate and coding efficiency of a fiber's spike output. Thus, although a unit may have the same average spike rate in response to two different sound stimuli, the information rate and coding efficiency of its spike output may differ – in particular, "naturalistic" stimuli may be coded with higher information rates and efficiencies than white noise stimuli. In this study, we have examined how changes in the amplitude and phase spectra of an acoustic signal affect the information rate and coding efficiency of the spike outputs of auditory nerve fibers in response to complex sound stimuli. This research is supported by NIH Grant #NS-09244 to RRC and NEC Research Institiute.

499.6

EFFECTS OF CHANGES IN THE AMPLITUDE AND PHASE SPECTRA OF COMPLEX SOUND STIMULI ON THE PHASE SENSITIVITY OF AUDITORY NERVE FIBERS. RR Capranica* and DA Bodnar, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

In a recent study, we have demonstrated that auditory nerve fibers in the bullfrog (*Rana catesbeiana*) exhibit changes in their spike outputs in response to changes in the relative phase angle of a single excitatory harmonic component in a multi-harmonic stimulus [Bodnar and Capranica (1991), *Soc Neurosci Abstr* 17: 1107]. In the present study, we have examined the effects of changes in the relative amplitudes or phase angles of components in a multi-harmonic stimulus on the phase sensitivity of peripheral auditory units. For studies of changes in relative amplitudes we have compared cases in which the stimulus components have equal amplitudes or relative amplitudes comparable to the power spectrum of the bullfrog advertisement call. For studies of changes in the phase spectrum we have compared the cases when all the harmonic components have either a relative starting phase of 0°, 180°, or random. Results from these experiments indicate that the phase sensitivity of a unit can be influenced by the relative amplitudes and phase angles of the other components in a stimulus. In addition, we have found that units which exhibit two-tone suppression are sensitive to changes in the relative phase angle of the suppressor component and this phase sensitivity is also affected by changes in the relative amplitudes and phase angles ofother stimulus components. This research is supported by NIH Grant #NS-09244 to RRC.

499.7

EXPRESSION OF mRNAs ENCODING FOR ALTERNATELY SPLICED SEGMENTS OF FIBRONECTIN IN THE DEVELOPING RAT COCHLEA. N.K. Woolf*, D.V. Jacquish, F.J. Koehrn and V.L. Woods. Depts. of Otolaryngology and Medicine, UCSD Medical Center and Veterans Administration Medical Center, La Jolla, CA 92093-9112.

Previously we demonstrated the distribution of fibronectin-like protein within the developing inner ear of rats. From embryonic day 18 through day 1 postpartum, intense, discrete immunoreactivity was observed in the cochlea immediately beneath the inner and outer hair cells, sites of auditory nerve fiber growth and nerve-cell synaptogenesis. Fibronectin was also found to be a major structural component within the basilar membrane throughout development. In order to define the cellular site(s) of fibronectin synthesis and the role of functionally distinct forms of fibronectin, we are performing in situ hybridizations on rat cochlear tissue with segment-specific mRNA probes (kindly provided by Dr. R.O. Hynes, MIT), which distinguish three alternately spliced segments of fibronectin. Data will be presented demonstrating ontogenetic changes in cochlear fibronectin mRNA expression.

Supported by DC139, DC386 & the VA Research Service.

499.8

SIMULTANEOUS MEASUREMENTS OF TUNING IN THE ANTEROVENTRAL COCHLEAR NUCLEUS AND DISTORTION PRODUCT OTOACOUSTIC EMISSIONS: EFFECTS OF ENDOCOCHLEAR POTENTIAL VARIATION. R. Rübsamen, D.M. Mills*, E.W. Rubel Hearing Development Lab., Dept. of Otolaryngology - Head & Neck Surgery, Univ. of Washington, Seattle, WA 98195.

The objective of this study was to precisely evaluate the relationship between tuning characteristics and the thresholds of cochlear afferents and the properties of distortion product otoacoustic emissions (DPOE). Response areas of multiunit clusters in the anteroventral cochlear nucleus and DPOEs at a range of input stimulus levels were simultaneously measured in the adult gerbil during furosemide-induced changes of the endocochlear potential. Intraperitoneal injection of 75 mg/kg furosemide has only a minor effect on the high level DPOEs (stimulus intensity 75-80 dB SPL), but the low level DPOEs (stimulus intensity 50 dB SPL) are reduced from approximately +20 to -10 dB SPL within 15 min. Emissions fully recover after about 60 min. Characteristics of the frequency threshold curves (FTC) after furosemide injection were: (1) threshold at the best frequency (BF) increased by about 30-40 dB, (2) threshold of the FTC-tail (one octave below BF) was elevated by 10-20 dB, and (3) the FTC-bandwidth 10 dB above threshold *widened from 0.2-0.4 octaves to 0.6-0.8 octaves. The dynamics of FTC deterioration and recovery are the same as for the DPOE* reduction and recovery. This congruence indicates that tuning characteristics of cochlear afferents are dynamically regulated by the same biological mechanism that regulates otoacoustic emissions.

499.9

DISTRIBUTION OF OLIVOCOCHLEAR NEURONS IN THE CHINCHILLA. J.M. Weekly, W.B. Warr* and B.J. Morley. Boys Town National Research Hospital, Omaha, NE 68131

Although the chinchilla is a commonly used animal in auditory research, very little work has been done on the origins and distribution of its cochlear efferents. The purpose of this study was to define and quantify these distributions using retrograde fluorescent tracer techniques. Four adult chinchillas were anesthetized and 10 µl of 4% w/v FluoroGold (Fluorochrome Inc.) was infused into one cochlea. After a survival time of 6-10 days, the animal was fixed by intracardiac perfusion of 4% paraformaldehyde, the brain removed and sectioned at 35 µm in the coronal plane (N=3) or in the horizontal plane (N=1) and studied under fluorescence microscopy. We could readily classify labeled OCN into two groups: lateral (LOC) and medial (MOC) olivocochlear neurons, as is the case in most mammals studied. Labeled OCN averaged a total of 1268 (s.d.=143) and were located mainly in the neuropil of the lateral superior olivary nucleus (LSO) ipsilaterally (mean=965:76% of total, s.d.=118) and in the contralateral dorsomedial periolivary region (DMPO) (mean=231:18% of total, s.d.=68). OCN were present in fewer numbers in the ipsilateral DMPO (mean=50:3.9% of total, s.d.=25) and contralateral LSO (mean=22:1.7% of total, s.d.=14). Although the general distribution of OCN in the chinchilla is similar to that known in other rodents, the restriction of MOC neurons to the DMPO is unique among rodent species so far studied. [Supported in part by NIH-NINDC/P60 DC00982]

499.10

EXPRESSION OF NMDA-RECEPTOR mRNA IN THE RAT AUDITORY SYSTEM. H. Kuriyama[1], R. Albin[2], S. Shiosaka[3] and R.A. Altschuler[1]*. Kresge Hearing Research Institute[1], Dept. Neuroscience[2], Univ. of Michigan, Ann Arbor MI, 48109 and Dept. Neuroanatomy, Osaka Univ.[3], Japan

There is now considerable evidence suggesting that glutamate or a related excitatory amino acid is the transmitter of auditory pathways and that it acts on an excitatory amino acid receptor. The NMDA receptor has been known to play a key role in many excitatory amino acid CNS synapses, however its role in the cochlea and auditory brainstem has not been well established. Recently two sequences for the NMDA-receptor have been reported (Moriyoshi et al., 1991 and Kumar et al.,1991), each with different properties. Expression of NMDA receptor mRNA in the rat auditory system was examined using *in situ* hybridization histochemical techniques. For *in situ* hybridization studies one oligoprobe was made of antisense codons (2225-2269) of Moriyoshi's sequence (NMDAR-1) and another one was made of the antisense codon (1107-1151) of Kumar's sequence (NMDAR-2). These probes were end labeled with α-^{35}S dATP (NEN) and applied to cryostat sections of rat brainstem and decalcified cochlea. Sections were hybridized overnight followed by rinse and dip in emulsion. Slides were then exposed for 1-3 weeks and developed. A density of silver grain considerably over background level was seen over spiral ganglion cells in the cochlea, and over neurons in cochlear nuclei and superior olivary complex in the brainstem with both NMDAR-1 and NMDAR-2 probes. This pattern of staining represents expression of NMDA receptor mRNA in these neurons. In spiral ganglion cells, the signal seen for NMDAR-1 was much stronger than for NMDAR-2. The specificity of the hybridization signal was confirmed by using sense probe and RNAase as a control. In all control specimen, the hybridization signals were virtually absent. These results suggest that NMDA-receptor is a component of excitatory amino acid synapses in the cochlea and auditory brainstem. Immunohistochemical studies will be necessary to determine the specific synaptic localizations of these receptors in the cochlea and brainstem auditory nuclei.

supported by NIDCD grants DC00383 and DC00078

499.11

HSP 72 INDUCTION WITH ACOUSTIC OVERSTIMULATION IN THE RAT COCHLEA AND AUDITORY BRAINSTEM. H.H. Lim, O.H. Jenkins, J.M. Miller*, M. Myers and R.A. Altschuler. Kresge Hearing Research Institute, Department of Otolaryngology, University of Michigan, Ann Arbor, MI, 48109.

Heat shock proteins (HSPs) are induced after the exposure of cells to various metabolic and environmental stresses. In the auditory system, induction of the 72 kD HSP has been shown with heat in the rat (Kim et al., 1991) and guinea pig (Thompson et al., 1992) cochlea , as well as with hypoxia in the rat cochlea (Myers et al., 1992). The purpose of this study was to determine if high intensity acoustic stimulation would induce HSP 72 in the rat cochlea and auditory brainstem. Sprague-Dawley rats were exposed to 110 dB SPL broad band noise for 1.5 hours and sacrificed 4, 6 and 8 hours after stimulation. Control animals did not receive any stimulation. Immunocytochemical and western blot analysis were performed using monoclonal antibodies against HSP 72 (Amersham, StressGen). Tissue for western blot analysis came from unfixed dissected cochlea, cochlear nucleus and inferior colliculus. Immunoperoxidase immunocytochemistry was performed on vibratome sections of the rat brainstem, cryostat sections of cochlea and cochlea with the bony shell removed, the latter yielding surface preparations.

Western blots showed an intense 72 kD band in the noise exposed animals compared to a very light band in control animals. Immunocytochemical results in the cochlea revealed noise induced HSP 72 immunoreactive (IR) staining of outer hair cells. The maximal IR staining was observed 6 hours after noise exposure. Only a few IR stained inner hair cells were seen and spiral ganglion cells were not stained. In the brainstem, noise induced HSP 72 IR labeling of neurons was observed in cochlear nucleus, medial nucleus of the trapezoid body, lateral superior olive, but not in inferior colliculus.

These results indicate that acoustic overstimulation can induce the expression of HSP 72 in the rat cochlea and auditory brainstem nuclei. HSP 72 immunolabeling may serve as a marker for cellular stress and potential damage. Further studies may also help to determine a protective function for HSP 72 in the auditory system.

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499.12

CONTRALATERAL SOUND SUPPRESSES DISTORTION PRODUCT OTOACOUSTIC EMISSIONS VIA CHOLINERGIC MECHANISMS. S.G. Kujawa, T.J. Glattke, M. Fallon and R.P. Bobbin*, Dept. of Speech & Hearing Sciences, Univ. of Arizona, Tucson, AZ 85721 and Kresge Hear. Lab., LSUMC, New Orleans, LA 70112.

Contralateral sound suppression (CSS) of distortion product otoacoustic emissions (DPOAEs) involves the activation of olivocochlear efferent fibers which synapse on outer hair cells (OHCs) (Puel & Rebillard, JASA, 76:1713, 1990). One neurotransmitter of these fibers is acetylcholine (ACh). We studied the effects of ACh antagonists on CSS of DPOAEs to determine if ACh is involved and the receptor type. Urethane anesthetized guinea pigs with sectioned middle ear muscles were used. Perilymph spaces of cochleae were perfused with artificial perilymph (AP) and drugs at 2.5 µl/min for 10 min. After each period of perfusion the $2f_1$-f_2 DPOAE at 5 kHz (f_1 = 6.25 kHz; f_2 = 7.5 kHz; 60 dB SPL) was measured before, during and after a contralateral wideband noise (70 dB SPL). CSS of DPOAEs was 1.59 ± 0.15 dB. AP did not alter CSS of DPOAEs. Strychnine (10 µM), curare (10 µM) and atropine (10 µM) reversably blocked CSS of DPOAEs. Results support the involvement of both nicotinic and muscarinic receptors in CSS of DPOAEs. (Supported by NIH grant DC-00722).

499.13

MIDDLE EAR TRANSFERFUNCTION AND DIRECTIONAL CHARACTERISTIC IN CLAWED FROG AND ZEBRA FINCH.

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Acoustic properties of the middle ear were studied in the clawed frog *Xenopus laevis* and in the zebra finch *Taeniopygia guttata*. During the experiments the frog was positioned 10 cm below the water surface on a small turntable in the center of a circular 4m pond. A probe microphone was inserted into each middle ear. Reference hydrophones were placed on both sides of the frog's head. The zebra finch was positioned inside a sound proof-chamber, on a turntable, with similar positioning of middle ear and reference microphones. In both cases acoustic stimulation consisted of continuous white noise delivered at every 10° rotation angle, up to 360° azimuth. Sound pressures received by all the microphones were processed by means of a special program for signal analysis. Transfer functions between freefield and middle ear and between the middle ears of both sides were established for the two species. These experiments were performed under experimental conditions of intact ear and various systematic manipulations of ear structures.

Morphological analysis of the internal connection between both ears consisted of three-dimensional computer reconstructions on the basis of serial sections of the head regions involved. Shapes and dimensions of the cavities between both ears were also made visible by filling with plastic material. While the interaural-pathway of the clawed frog consists of only one direct connecting with an opening to the mouth cavity the zebra finch possesses a more diverse internal connection that has no orifice. The eustachian tube of the frog opens in the medial corner of the roof of the mouth; the larynx lies just beneath this opening. Acoustic measurements revealed a certain frequency range around 5 kHz in the zebra finch and 3 kHz in the clawed toad without an acoustic crosstalk. These are also the frequency ranges, where sound pressure differences between the two ears occur, which are large enough for directional hearing. On the basis of the morphological and experimental results a theoretical model was designed for the description of a bird's and a frog's ear.

499.14

GROWTH PHASES OF THE COCHLEAR DUCT IN THE OPOSSUM, *MONODELPHIS DOMESTICA*. F.H. Willard*, Department of Anatomy, University of New England, Biddeford, ME 04005.

Two distinct growth phases were observed in the mammalian cochlear duct using quantitative light microscopy and image analysis (Sigma-Scan) of Nissl-stained sections. The ***first phase*** featured the linear growth of the cochlear duct but with no concomitant increase in its absolute volume. From birth (13 days after conception) the cochlear duct, a cul-de-sac whose spiral has only 3/4s of a turn, expands over the next 8-10 days to reach one and 3/4s turns without a detectable increase in absolute volume. From birth to postnatal day (PND) 6, numerous mitotic figures (MFs) were seen in the epithelium along the full length of the duct (2-6 MFs per cross-sectional profile of the duct). By PND-8 the mitotic figures, although plentiful (2-4 MFs per profile), were confined to a bud in the apex of the duct. The ***second phase*** of growth was initiated between PND 8-10 when the linear growth and cell division in the cochlear epithelium diminished (less than one MF per profile) and volumetric expansion of the duct began. The growth curve for the volume of the duct is sigmoidal, reaching its asymatote by PND-20. During this time the cross-sectional profiles of the duct increased in size, however, there are very few mitotic figures present in the cochlear epithelium. Hypertrophy of the cartilaginous posterior wall of the duct begins around PND-13 and ossification follows around PND-15. The zone of ossification sweeps rostrally (from base to apex) finally ossifying the anterior wall of the duct around PND-20. Thus two phases of growth are present in the mammalian cochlear duct, the first phase involves linear growth with cell division present in the cochlear epithelium. The second phase involves volumetric expansion with little evidence of cell division. The second phase may be limited in duration by ossification of the duct walls.

499.15

EFFERENT TERMINATIONS IN THE NEONATAL HAMSTER COCHLEA. N.B. Mansdorf, D.D. Simmons*, and K. Bell. Dept. of Biology and Brain Research Institute, UCLA, Los Angeles, CA 90024-1606.

In adults, lateral olivocochlear (OC) neurons terminate below inner hair cells (IHCs) while the medial OC neurons terminate only on outer hair cells (OHCs). Although both lateral and medial OC neurons are presumably present in the cochlea at birth it is unclear which system matures first, innervates hair cells first and whether they maintain separate innervations of IHCs and OHCs. Our investigations have attempted to characterize the development of OC terminations using light- and electron-microscopic techniques in the postnatal hamster. Biocytin was injected into the crossed OC bundle using an *in vitro* brainstem preparation. At postnatal day (P) 6, efferent fibers gave rise to at least two types of endings: large diameter swellings that terminated directly on IHCs, and smaller diameter varicose swellings that terminated below the IHCs. The majority of efferent endings were found on the modiolar side of the IHC. No terminal endings were observed on or below the OHCs. The developmental expression of calcitonin gene-related peptide (CGRP), a marker specific for lateral OC neurons, was also immunocytochemically characterized. Immunoreactivity could not be detected on or before P 6 but was readily observable at P 9 as a dense plexus of CGRP-positive terminals only under IHCs. Our data are consistent with the hypothesis that the medial OC efferents terminate on IHCs before OHCs.

(Supported by the Alfred P. Sloan Research Foundation)

499.16

DEVELOPMENT OF THE TUNNEL OF CORTI IN THE RAT.
L.P. Rybak*, C. Whitworth. Department of Surgery, SIU School of Medicine, Springfield, IL 62794-9230.

The rat is an altricial animal which has proven to be a useful model for the study of auditory development. The purpose of the present study was to study the surface development of the organ of Corti using scanning electron microscopy (SEM). Rat pups from 1-30 postnatal days of age were anesthetized with pentobarbital and the cochleas were removed and processed for SEM using a modification of the TOTO procedure (Davis and Forge, J Microsc 1987; 147:89-101). The specimens were viewed with a Hitachi S500 Scanning Electron Microscope. The tunnel of Corti was found to undergo dramatic changes between 9 and 11 days after birth. The tunnel of Corti was extremely narrow prior to 9 days of age. A base to apex gradient of development of the tunnel width was observed. At 9 and 10 days of age the tunnel width increased in the lower middle turn and the hook region. By 11 days of age the tunnel of Corti was well developed throughout the cochlea. These morphological findings correlated with the onset of CAP response which was first detected at 13 days of age. It appears that opening of the tunnel of Corti coincides with a series of developmental phenomena which must occur prior to onset of sound-evoked responses in the cochlea.

(Supported by NIH (NIDCD) #5R01 DC321).

AUDITORY SYSTEM: ANATOMY II

500.1

INPUTS TO THE SUPERIOR PARAOLIVARY NUCLEUS. N. Kuwabara* and J.M. Zook. *Dept. of Biological Sciences and OUCOM, Ohio University, Athens, OH 45701.*

Projections to the superior paraolivary nucleus (SPN) were studied at the single cell level in the gerbil, mouse and big brown bat, *Eptesicus fuscus*, using intracellular labeling in a brainstem tissue slice preparation.

An unexpected major source of SPN input was traced from principal cells (PC) of the adjacent medial nucleus of the trapezoid body (MNTB). This input was in the form of collateral projections off of the main PC axon as it travels to the lateral superior olive (LSO). MNTB projections generally ramified across the entire dorsoventral extent of the SPN and the terminal arborizations formed bands as extensive or more extensive than those seen in the MNTB projections to the LSO. Most of the fine *en passant* and terminal boutons characteristic of this projection may be associated with dendrites of SPN cells, but this has not been established conclusively. The MNTB projection to SPN is topographically organized. Principal cells located in the medial MNTB projected to the medial SPN, while those located in the lateral MNTB projected to the lateral SPN.

Other sources of input to the SPN were also labeled. Trapezoid fibers, presumably from the ipsilateral cochlear nucleus, sent branches to SPN which terminated in thin sheets parallel to the MNTB projection. The extent and pattern of these projections may distinguish the SPN from the dorsomedial periolivary nucleus (DMPO) which is recognized in the cat and mustache bat. The DMPO, but not the SPN, is a major target of collateral branches of the calyciferous axon projection to the MNTB (Morest '68; Kuwabara et al., '91). (Supported by NIDC01303 and OUCOM)

500.2

A CONNECTIONIST MODEL OF THE OWL'S SOUND LOCALIZATION SYSTEM. D.J. Rosen, D.E. Rumelhart, E.I. Knudsen and T. Masino*. Depts. of Psychology and Neurobiology, Stanford University, Stanford, CA 94305.

We describe a connectionist model of the barn owl's auditory localization system. Simulated sound is fed into an inner ear model whose output is used to generate spike activity in frequency tuned units (auditory fibers). These units feed into paired models of the nucleus magnocellularis, containing units sensitive to input phase, and the nucleus angularis, containing units sensitive to input level. Output from the magnocellular nuclei feeds into a cross-correlation model of the nucleus laminaris whose units are therefore tuned to interaural phase differences (IPD). Time averaged samples of laminaris activity, representing a range of interaural time differences, are fed into a model of the inferior colliculus (IC) core. In parallel, outputs from the angular nuclei feed into a subtraction model of the VLVp whose units are sensitive to interaural level differences (ILD). The network integrates signals from the IC core and the VLVp in its model of the IC lateral shell, which in turn feeds into the external nucleus of the IC (ICx).

Before training, VLVp units were fully interconnected to lateral shell units of similar frequency tuning. Lateral shell and ICx units were fully interconnected. Only these two sets of connections were modifiable. The network was trained by backpropagation to create ICx units with discrete auditory spatial fields as well as lateral shell units with realistic tuning properties.

By manipulating learning algorithms, sites of plasticity and training procedures we can use the network to explore details of the structure and development of the owl's localization system.

Supported by McDonnell Pew Program in Cognitive Neuroscience.

500.3

MULTIPLE AUDITORY PATHWAYS INTO HVc.
E.S.Fortune*and D.Margoliash. Dept. of Org. Bio. and Anat, Univ. of Chicago, Chicago, IL, 60637

We examined the organization of auditory inputs to the caudal nucleus of the ventral hyperstriatum (HVc), a neostriatal site of sensory-motor interaction in the pathway for song learning and production.

Neurons in the field L complex, a thalamorecipient group of cytoarchitectonically distinct auditory nuclei, project to HVc, the 'shelf' ventral to HVc, and surrounding caudal neostriatum (NC). Injections of biotinylated dextrans into these areas retrogradely label cells throughout L1 and L3 of the field L complex. These two nuclei form the major pathways for auditory input to HVc. Cells in L1 and L3 have morphologies similar to types 1 and 2 cells seen in Golgi preparations. Occassionally type 3 oriented cells were labelled within L2a. Injections into HVc which did not invade the shelf appear to label more cells in L1 than in L3 whereas injections into NC ventral to HVc label more cells in L3. Injections that included the shelf appear to label a greater number of cells in field L than those that did not.

The majority of NIf cells that were labelled by injections into HVc have dendritic arbors which are within the borders of the nucleus. Some cells, however, have denritic arbors that extend into adjacent L1 and thus may have access to auditory information.

Some HVc cells have dendrites in the shelf. These include cells that have large somata with thick, spiny dendrites and elongate dendritic arbors, which were labelled by injections into the field L complex and presumably project to area X. Small cells with thin, short, sparsely spined dendrites and large cells with thick, densely spined dendrites that were labelled by injections into the dorsal archistriatal tract and presumably project to RA, also have dendrites which invade the shelf.

Most injections into HVc labelled nearly all of the cells in NIf whereas the numbers of cells labelled in the field L complex appears to be dependent on the size of the injection site. We are investigating the axonal organization of these projections. Supported by NIH grant NS25677 to DM.

500.4

ON THE ROLE OF THE DORSAL NUCLEUS OF THE LATERAL LEMNISCUS IN ACOUSTICALLY-EVOKED INHIBITION IN INFERIOR COLLICULUS NEURONS C.L. Faingold*, C.A. Boersma Anderson and M.E. Randall. Dept. Pharmacol., So. IL Univ. Sch. Medicine, Springfield, IL 62794.

Acoustically-evoked GABA-mediated inhibition is observed in neurons in the central nucleus of inferior colliculus (ICc). The dorsal nucleus of the lateral lemniscus (DNLL) is a GABAergic nucleus that projects to ICc. The present study examined the effects of stimulation and/or blockade of the DNLL on ICc neuronal firing. Rats were anesthetized (pentobarbital, 40mg/kg ip). A cannula or bipolar concentric electrode was placed into the DNLL, and the effects of stimulation and/or microinjection on contralateral ICc neuronal firing were evaluated. Microinjection of a local anesthetic, lidocaine (2%), or a GABA-A agonist, THIP, (10 nMol, 0.5 µl) into DNLL blocked acoustically-evoked binaural or intensity-induced inhibition in most ICc neurons. The spontaneous activity of ICc neurons exhibited a dramatic reversible increase following DNLL blockade. Trains of electrical stimulation of DNLL resulted in reduced acoustically evoked firing in most ICc neurons, which mimicked binaural or intensity-induced inhibition. The degree of firing reduction was dependent on the intratrain frequency. Effects of DNLL stimulation were blocked by microinjection of THIP into the site of stimulation, suggesting mediation of the effects of stimulation by direct actions on DNLL cell bodies. These data support the idea that contralateral GABAergic input from the DNLL is inhibitory to ICc neurons. Thus, binaural, intensity-induced and tonic inhibition in ICc neurons may be mediated, in part, by the GABAergic projection from the contralateral DNLL. (Support NIH NS 21281).

500.5

TOPOGRAPHICAL ORGANIZATION OF THE MOTONEURON POOLS INNERVATING THE MUSCLES OF THE PINNA IN THE FACIAL NUCLEUS OF THE CAT. Luis C. Populin* and Tom C. T. Yin. Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

The external ear of the cat filters the acoustic signal in a manner dependent upon the location of the sound source, and thus may play an important role in sound localization. Unlike humans, cats can move and change the shape of their pinnae extensively, thus they are able to control the filtering properties of the pinna.

As an initial step in a study of the role of the pinna in sound localization, we have characterized the topographical organization of the motoneuron pools innervating pinna muscles in the cat's Facial Nucleus (FN). Individual muscles were dissected, isolated from neighboring tissue, and injected (30-65 μL) with horseradish peroxidase conjugated to the B subunit of cholera toxin (CTB-HRP; List Laboratories, 0.1%). After survival times of 48 to 72 hours, brainstems were cut into 70 μm frozen sections, treated with tetramethyl benzidine, and counterstained with cresyl violet. Using camera lucida the contour of the nuclei and the positions of the labeled motoneurons of most pinna muscles have been reconstructed.

Retrograde labeling indicates that each muscle is innervated by a column of motoneurons extending along the rostrocaudal axis of the nucleus. These vary in the number of cells and in their length along this axis. In the coronal plane, each motoneuron pool occupies a discrete position in the medial division of the nucleus. There is little overlap among motoneurons that innervate large muscles, but the pattern is not as sharply defined for those innervating smaller muscles. Motoneurons innervating muscles that pull the pinna rostrodorsally (e.g., the Adductor Auris Inferior m.) are located dorsolaterally, and those that move the pinna caudally (e.g., the Levator Auris Longus m.) are located ventromedially in the medial division of the FN.

Supported by NIH Grant DC0016

500.6

STRUCURAL BASIS OF BINAURAL PHASE PROCESSING IN N. LAMINARIS: POSSIBLE FUNCTION OF BIPOLAR DENDRITES. W. E. Sullivan *, Dept. of Ecology and Evolutionary Biology, Princeton Univ., Princeton, N.J. 08544

For horizontal sound localization, binaural synaptic inputs time locked to the sound wavefronts at each ear activate neurons sensitive to time differences between these inputs. Maximum firing occurs for coherent bilateral inputs, suggesting that these cells are "coincidence detectors". These neurons in chicken nuc. laminaris have long bipolar dendrites. Their homologues in barn owls, which respond to higher frequencies, do not have dendrites (Carr and Konishi 1990).

Possible functions for dendrites in phase comparison were explored using compartmental modeling. The results suggest that dendrites can enable selectivity for synchronous bilateral inputs in the face of large variations in synaptic input, due to fluctuations in spike rate or to changes in sound level. The mechanism requires electrical isolation between bilateral inputs and makes use of the fact that post-synaptic voltage can approach but not exceed the synapse's equilibrium potential. If synaptic conductances are large, a single input will locally depolarize the dendrite near to saturation. Thus, another input at that location will be less effective at the soma than a separate input on another dendrite. This can produce a virtually all-or-none selectivity for bilateral coincidences over unilateral ones. This function cannot be achieved in an iso-potential, soma-only model. The model's properties can be used to predict several features of laminaris cells such as dendrite length, branching pattern and number (Smith and Rubel, 1979). Cells tuned to higher frequencies have shorter, more numerous dendrites which can be related in the model to constraints of pre- and post-synaptic processing at different frequencies.

500.7

THREE DIMENSIONAL ATLAS OF THE LATERAL NUCLEUS OF THE TRAPEZOID BODY IN GUINEA PIG. R. Bawa and G.A. Spirou*, Department of Otolaryngology, West Virginia University School of Medicine, Morgantown, WV 26506.

The lateral nucleus of the trapezoid body (LNTB) is the lateral most of a group of nuclei surrounding the major cell groups of the superior olivary complex (SOC). The proximity of the LNTB to the cochlear nucleus (CN) indicates that LNTB neurons could rapidly exert significant effects on signal processing in the CN. Topographical relationships between LNTB and CN neurons may be a function of cell position in the LNTB and morphological characteristics of LNTB neurons. We have constructed an atlas of Nissl stained LNTB neurons using transverse sections from two animals. Cells were projected at 560X and drawn using camera lucida. LNTB boundaries extend from the caudal pole of the superior olive to the rostral LSO, encompassing the caudal 50% of the SOC. The total number of LNTB neurons in each animal was 638 and 603. The greatest number of cells per 50μm section, as many as 43 cells, were found caudal to the LSO; the least number of cells per section, as few as 7 cells, were found through the middle region of the LSO. The distribution of cell areas was unimodal with a peak between 50-150μm^2 and included cells as large as 600μm^2. Neuron size was similarly distributed in each section in one animal; the largest neurons were clustered rostrally in the other animal. Over half of the LNTB neurons are caudal to a plane passing through the caudal auditory nerve root region of the CN and are positioned to exert effects in the posterior CN if interactions are largely confined to the transverse plane. Supported by NIDCD grant DC01387.

500.8

SENSORY PROPERTIES OF NEURONS IN THE ACOUSTIC THALAMUS THAT PROJECT TO THE AMYGDALA. F. Bordi*, and J.E. LeDoux. Center for Neural Science, New York Univ., NY, NY 10003.

Cells in the medial division (MGm) of the medial geniculate body (MGB) and adjacent areas of the posterior thalamus are known to respond to both auditory and somatosensory stimuli. Cells in this region also project to the amygdala. In the present study we investigated the sensory properties of single cells in the MGm nucleus and underlying posterior intralaminar nucleus (PIN). Antidromic stimulation of lateral amygdala (AL) was also carried out in some experiments to characterize the sensory properties of the neurons that project to amygdala. Rats were anesthetized and single units were recorded in the posterior thalamus during stimulation with white noise (80dB), isointensity tones (1-30 kHz, 0-90 dB), or footshock (0.3 mA). Cells responded to white noise with initial onset latencies of 7-8 ms. These units showed either phasic or sustained responses. Some cells had clear frequency preferences but even these tended to be more broadly tuned than units in the nearby pars ovoidea of the ventral division (MGv) of MGB, where narrow tuning and a tonotopic organization was found. The tonotopic organization was preserved in MGm but with broader tuning characteristics. Unlike cells in MGv, some cells in MGm and PIN responded to both auditory and somatosensory stimulation. Some multimodal cells showed frequency preferences. Cells identified by antidromic stimulation of AL were localized in MGm and PIN, but never in MGv. These cells exhibited similar response to those described. Thus, thalamo-amygdala projection neurons respond to auditory and somatosensory stimulation and some are relatively tuned with respect to frequency. Supported by MH38774 and MH46516.

500.9

OPTICAL IMAGING OF FUNCTIONAL ARCHITECTURE OF THE GUINEA PIG AUDITORY CORTEX. **I. Taniguchi*, J. Horikawa, T. Moriyama and M. Nasu. Dept. of Neurophysiology, Med. Res. Inst., Tokyo Med. and Dent. Univ., Tokyo 101, Japan.**

Three tonotopically organized auditory cortical fields (field A, DC and S) have been identified in the guinea pig with a microelectrode technique. Fields A and S have within them a rostral-to-caudal, low-to-high-frequency gradient, while field DC has a mirror-image relationship to field A with respect to the frequency gradient. Functional difference between fields A and DC has not yet been clarified. We studied functional organization in fields A and DC with an optical method using a 144-channel photodiode array and the potential sensitive dye (RH795). Guinea pigs were anesthetized with Nembutal (30 mg/kg) and the temporal brain field was exposed. Tone-bursts were delivered as stimuli from the contralateral ear with a closed system. The 12x12 channel optical responses were recorded for different frequencies and sound pressure levels. To obtain the spatio-temporal pattern of neural activity, the potentials of topological responses were color-coded and transformed to sequential images. Our results showed the tonotopical organization in field A, but not clearly in the smaller field DC. On stimulation, enhanced activity was found first in field A and propagated towards field DC. This suggests functional difference between fields A and DC in sound information processing.

500.10

PNA BINDING PATTERNS IN THE DEVELOPING DORSAL COCHLEAR NUCLEUS. G. H. Riggs and L. Schweitzer*. Dept. of Anatomical Sciences and Neurobiology, Univ. of Louisville Sch. of Med., Louisville, KY 40292.

Various studies have suggested that glycoconjugates may influence connectivity and lamination in the developing CNS. This project used peanut agglutinin (PNA) to investigate glycoconjugate distribution in the dorsal cochlear nucleus (DCN) of the developing hamster, a nucleus whose development is well characterized. Laminar differences were found, and within laminae a dorsomedial to ventrolateral gradient was observed. Specifically, PNA is most dense in the DCN's molecular layer, with label evident on PND 3, beginning to recede on PND 13 and virtually disappearing by PND 23. PNA did not differentially label the fusiform and deep cell layers at any age. Within the deep and fusiform cell layers, a pattern of trabeculation was observed beginning dorsomedially on PND 3 and extending ventrolaterally with development. By PND 14, trabeculation was less distinct dorsomedially, giving way to a more homogenous distribution of label. This homogenous pattern of labeling progressed ventrolaterally with age. The last evidence of trabeculae appeared at the ventrolateral-most limit of the DCN on about PND 23. These patterns of PNA binding parallel other events in normal development. Ingrowth of primary cochlear afferents, for example, follows the same spatiotemporal gradient as the initiation of trabeculation, occurring during PND 3-10 and following a dorsomedial to ventrolateral pattern of development. Descending inputs also grow into the deep DCN at this time. PNA label in the molecular layer diminishes concurrently with the maturation of granule cell axons, GAD-positive inputs and apical dendritic processes of fusiform cells in that layer. Unlike laminar patterns of PNA observed in other systems, the distribution of label in the DCN does not delineate all three cell layers. Therefore, changing PNA patterns are not simply a function of cell sorting in the DCN, but rather may reflect afferent ingrowth.

This work was supported by NIH Grant DC00233.

500.11

PARALLEL PATHWAYS FOR SOUND LOCALIZATION IN THE FOREBRAIN AND MIDBRAIN OF THE BARN OWL. E.I. Knudsen* and P.F. Knudsen. Dept.Neurobiology, Stanford University, Stanford, CA 94305.

We tested the hypothesis that the forebrain and the optic tectum (superior colliculus) are each capable of mediating sound localization independently of the other. The species studied was the barn owl, a species with highly developed sound localization abilities rivaling those of humans. The behavioral assay for sound localization was orientation of gaze. (Lesions can cause the expression of more complex behaviors, such as moving to a source, to be lost even when accurate localization persists, as revealed by simpler behaviors.) Moreover, both the optic tectum and the forebrain have direct access to the premotor circuitry that controls gaze.

The strategy was to interrupt the tectal pathway at the level of the optic tectum and interrupt the forebrain pathway at the level of the auditory thalamus, a nucleus called ovoidalis in birds (medial geniculate in mammals). The ability of 5 trained owls to orient gaze toward sound sources was assessed with one or both structures either lesioned or inactivated with muscimol (0.12-0.25 µg in ovoidalis; 1.0 µg in tectum). Localization responses to sources located in the contralateral (affected side) hemifield were compared with those to sources in the ipsilateral hemifield. Unilateral inactivation of the tectum caused a decrease in the probability of response, a decrease in accuracy and precision and in some owls an increase in latency. Unilateral inactivation of ovoidalis alone had little effect and, if anything, caused a slight increase in accuracy. However, unilateral inactivation of both structures left the animal unresponsive to sound sources located contralaterally. Thus, information essential for sound localization is processed in parallel in the tectum and forebrain. The pattern of deficits is remarkably similar to the deficits in gaze responses to visual stimuli observed in primates following tectal and/or forebrain lesions.

This work was supported by a grant from the NIH (DC 00155-12).

500.12

SPATIO-TEMPORAL IMAGING OF AUDITORY CORTEX OF GUINEA PIG HEARING SOUND WITH VOLTAGE-SENSITIVE-DYE. K.Fukunishi,N.Murai,H.Uno,H.Kawaguchi*. Advanced Research Laboratory,Hitachi Ltd.,Hatoyama,Saitama 350-03,JAPAN.

The neural evoked responses which are the outputs of dynamical brain system working for each specific purpose maintain useful information utilized for the brain system identification. Optical recording seems to be an important measurement method of the neural responses which could lead to identify the brain system. A 128-channel optical recording system was developed to measure the neural responses of a animal brain for hearing sounds. The voltage sensitive dye (RH795) was applied for the probe. The temporal fields of anesthetized guinea pigs (male) were observed after click and tone burst stimuli respectively. A boomerang-shaped spatial pattern due to the movement of the response parts on the auditory field was observed for the click. This kind of movement pattern was invisible for the tone bursts. The direction of the movement of the response parts for the click crossed the response parts for the tone burst. The frequency selectivity of the cortical neuron was observed but depending on the latency. This characteristics was also clarified by focusing to the strong response parts for each tone burst. The cross-correlation among the responses of each observed section revealed the existence of the modular unit of the neural information processing on the cortical field for the complex sound as the click.

500.13

CHANGES IN HEARING AND SOUND-INDUCED 2DG-PATTERN IN THE AUDITORY SYSTEM OF DEVELOPING TREE SHREWS. E. Zimmermann*, H. Binz, H. Rahmann. Deutsches Primatenzentrum, 3400 Göttingen and Institute of Zoology, University Stuttgart-Hohenheim, 7000 Stuttgart 70, FRG.

The development of hearing and sound perception in mammals has received increasing interest due to the scarce knowledge we have about the influence of epigenetic factors on differentiation.

Our present study was designed to map and compare the representation of physically defined sounds with different biological significance in the auditory system of tree shrews by means of the 2DG-method, to analyze if and at which age these sounds induce an altered metabolic response, if the evoked patterns are immutable or variable, at which age they become stimulus-specific and at which age they correspond to the pattern of adults. Besides, the development of auditory thresholds from birth to nutritional weaning was determined by psychophysical methods.

Tree shrews are born deaf, their hearing capability develops postnatally and their hearing and sound communication range is quite similar to those of higher primates (Binz et al. Behav. 109, 1989; Zimmermann, Cambr. Enc. Hum. Evol. 1992). Discrete stimulus - specific 2DG-patterns were discerned in the core area of the auditory cortex, the central nucleus of the inferior colliculus and the dorsal and ventral part of the cochlear nucleus in adult tree shrews. They imply a tonotopic organization. First sound-induced patterns of 2DG-uptake - were visualized at the time the external meatus opens and sound-induced behaviors could be evoked. However, it was not before weaning that auditory sensitivity and sound representation correspond to those of adults. These findings suggest that experience may be involved in shaping sound perception in tree shrews. Supported by the DFG (Zi 345/1-3).

500.14

FUNCTIONAL REORGANIZATION IN THE INFERIOR COLLICULUS FOLLOWING ACUTE COCHLEAR TRAUMA. R.J. Salvi* and S. Saunders Hearing Research Lab, SUNY at Buffalo, Buffalo, NY 14214.

The tonotopic organization and response properties of neurons in the auditory cortex can be dramatically altered when a segment of the cochlea is damaged. However, it is unclear how neurons distal to the cortex contribute to this reorganization. In order to explore this issue, we recorded from neurons in the inferior colliculus (IC) immediately after damaging a region of the cochlea with acoustic overstimulation. The frequency of the traumatizing tone was located above the unit's excitatory response area in order to selectively eliminate inhibitory inputs originating above CF. Units with monotonic rate-level functions were unaffected by this type of exposure; however, the response properties of units with non-monotonic discharge rate-level functions were dramatically altered. Non-monotonic units responded to a broader range of low-frequency stimuli and thresholds in the tail of the tuning curve sometimes improved by more than 30 dB after the exposure. Saturation discharge rates also increased significantly (100-200%). These results suggest that the traumatizing tone can selective inactivate regions of the cochlea which drive the inhibitory inputs to these cells and significantly alter the unit's excitatory response area and level of excitability.

500.15

LOCATION OF AUDITORY CORTEX IN THE RAT AND FERRET: A DOUBLE-LABELING STUDY USING ^{18}F- AND ^{14}C-2-DEOXYGLUCOSE. M.N. Wallace* and D. Roeda *Dept of Biomedical Sciences, AB9 1AS and Dept of Bio-medical Physics, AB9 2ZD, University of Aberdeen, Scotland, UK.

The number and location of auditory areas in the rat and ferret neocortex is uncertain. We used the deoxyglucose method to study the cortex of control and deafened animals. Commercial ^{14}C-2-deoxyglucose was injected (i.p.) (555 kBq/100g rat or 370 kBq/100g ferret) and the animals exposed to normal laboratory sounds for 45 min.. Some were then deafened under ether(rat) or ketamine(ferret) anaesthesia by bi- or unilateral desruction of the tympanic membrane. Two hours later ^{18}F-2-deoxyglucose produced with the University cyclotron was injected (i.p.) (37 MBq/100g). The brains were sectioned at 50 µm in a cryostat and exposed using Kodak X-Omat-L film for 8 hours (^{18}F) and then after 2 days for a further 16 days (^{14}C). Two areas of high deoxyglucose uptake were found in the rat auditory region (area 41) and three areas on the ferret ectosylvian gyrus. This activity was not altered by bilateral ablation but was reduced contralateral to a unilateral ablation.

Supported by the Nuffield Foundation.

500.16

THE FUNCTIONAL ANATOMY OF MIDDLE LATENCY AUDITORY EVOKED POTENTIALS: THALAMOCORTICAL CONNECTIONS S. DI* and D. S. BARTH *Department of Psychology, University of Colorado, Boulder, Colorado 80309 USA*

Recent studies in our laboratory have demonstrated that by using high resolution epicortical potential mapping in combination with numerical methods of spatiotemporal analysis, it is possible to identify and separate putative neural generators of the middle latency auditory evoked potentials (MAEP) complex in the region of auditory cortex. The object of the present study was to compare the click evoked epicortical MAEP complex with potentials evoked by direct electrical stimulation of the ventral and dorsal divisions of the medial geniculate body (MGV and MGD). Epicortical responses to click stimuli replicated earlier findings. The responses consisted of a positive-negative biphasic waveform (P1a and N1) in the region of primary auditory cortex and a positive monophasic waveform (P1b) in the region of secondary auditory cortex. A linear combination of these patterns was sufficient to explain from 90-94% of the variance of the evoked potential complex at all latencies. In the same animals, epicortical responses to electrical stimulation of the MGV and MGD were also localized to areas 41 and 36, respectively. A linear combination of potential patterns from these separate stimulation conditions was sufficient to explain from 80-93% of the variance of the original click evoked potential complex at all latencies. These data provide functional evidence for anatomically defined topographical thalamocortical projections to primary and secondary auditory cortex. They suggest that short latency cortical evoked potentials (10 - 60 msec post-stimulus) are dominated by parallel thalamocortical activation of areas 41 and 36.

501.1

SELECTIVE STAINING OF OLFACTORY AFFERENT FIBRES IN FISHES USING ANTIBODIES RAISED AGAINST APGW-NH_2.

R.Y.S. Lo and R.P. Croll*, Department of Physiology & Biophysics, Dalhousie University, Halifax, Nova Scotia Canada B3H 4J7.

APGW-NH_2 (Ala-Pro-Gly-Trp-NH_2) has recently been suggested as an important neuropeptide with roles involved in, but probably not restricted to, reproduction in molluscs (Kuroki et al, 1990, *Biochem. Biophys. Res. Comm.* 167:273; Smit et al, in press, *J. Neurosci.*). The AKH and RPCH peptides found in arthropods have common C-termini (X-PGW-NH_2 and X-GW-NH_2, respectively), thus suggesting a family of related peptides which spans phyla. In the present study, we found that polyclonal antibodies (Croll & Van Minnen, in press, *J. Comp. Neurol.*) raised against APGW-NH_2 selectively label the afferent fibres entering the olfactory bulbs of the guppy, damselfish and zebrafish. These fibres appear to be homogenously distributed in the olfactory nerve. In the olfactory bulb, they form glomeruli located in the entire lateral half and the rostral part of the medial half of the olfactory bulb. In some cases, diffused fibers are also found converging toward the anterior commissural area in the forebrain. While the projection patterns and immunoreactivity are reliably reproduced in the guppy and damselfish, immunoreactivity varies in the zebrafish and appears to be absent in the goldfish. Preliminary studies also suggest the absence of immunoreactivity in the olfactory bulb of the rat. Current studies in progress attempt: i) to double label receptor somata in the olfactory epithileum using antibodies and retrograde tracers, ii) to determine the specificity of the antibodies and characterize the immunoreactive substance(s), and iii) to screen for immunoreactivity in other classes of vertebrates.

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501.2

A NEW STRUCTURE, THE "PIT ORGAN", IN HUMAN AND MONKEY OLFACTORY MUCOSA.

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A new whole mount immunocytochemical method (Feng et al. 1991) was used to study the olfactory receptor neurons (ORNs) on the surface of the olfactory mucosal sheet. Studies with neuron-specific tubulin antibody, J1, of 26 specimens of human olfactory mucosa taken at autopsy from patients ranging in age from 2d to 83 yrs revealed a structure not previously described, an olfactory pit organ. In 2 infants (2d and 2mo of age), the olfactory pits appeared in 2 groups of 8-12 openings (diameter ≅ 50-70 μM) within the olfactory epithelium (oe) located on the anterior lateral and septal walls of the nasal cavity. The openings of the pits can be oriented in different directions. In mature specimens, most of the pits were distributed near the roof of the nasal cavity. However, the number, morphology and distribution of these pit organs varied among different individuals. A detailed analysis of the structure of the pit organ was carried out by rehydrating and sectioning the whole mount specimens. The pit organ is a blind pouch lined with ORNs which appears as an invagination of oe into the connective tissue (depth of the invagination ≅ 150-200 μM). In some sections, a thick axon bundle emerging from the bottom of the pouch was observed. The extension and termination of this axon bundle in the CNS has not been explored. In specimens from aged and Alzheimer's patients, degenerative changes were found both in the oe lining the surface of nasal cavity and in the pit organs. ORNs may be sparse and the oe is often replaced by respiratory epithelium or squamous epithelium. Some pit organs have been found in monkey olfactory mucosa, but have not been observed in rodents. The function of the pit organ is unclear. The blind pouch may prolong odorant association with the ORNs, or it may contain specialized neurons that cannot yet be distinguished morphologically or immunocytochemically. NIH AG9200 and ADRDA IIRG-89-041

501.3

OLFACTORY MUCOSA DEGENERATION FOLLOWING TREATMENT BY THE CHELATING AGENT DIETHYLDITHIOCARBAMATE (DDTC).

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DDTC is a potent chelating agent proposed as a rescue agent in chemotherapy with cisplatin. It is hypothesized to bind excess cisplatin and facilitate its removal from tissue. As part of a study of neurotoxicity of cisplatin, we examined the effects of DDTC on olfactory mucosa.

To date, five adult wistar rats have received a single 600mg/kg dose of DDTC (s.c.). Within three days, substantial sloughing of olfactory mucosa was observed with relative preservation of the respiratory mucosa. In addition, glial response in glomeruli was substantial.

These observations suggest that DDTC is toxic to olfactory receptor neurons. However, the mechanism of this toxicity is unknown but may be related to carnosine. Carnosine, a di-peptide chelating agent, is greatly enriched in olfactory receptor neurons, although its function is unclear. Carnosine has been suggested to play a role in membrane stabilization and free radical scavenging. Perhaps DDTC disrupts functioning of carnosine resulting in olfactory mucosal degeneration.

501.4

TWO TRANSDUCTION MECHANISMS IN OLFACTORY NEURONS

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Olfactory receptor neurons in channel catfish, Ictalurus punctatus, were investigated with the whole-cell patch-clamp method. L-alanine, L-norvaline, L-arginine and L-glutamic acid were used as odorants, since these stimuli predominantly bind to different receptor sites. Two major types of odorant responses occurred. Inward currents were evoked by application of amino acids in 10 of 50 olfactory neurons tested. Receptors for different amino acids were colocalized in the membrane of these neurons. A fundamentally different way of odorant detection was found in 12 of the remaining cells. Arginine and glutamic acid acted on membrane receptors to trigger events which did not result in conductance increase, but led to modulation of voltage-gated potassium channels. In the absence of odorants, depolarization elicited potassium current which contained an inactivating component. After exposure to arginine or glutamic acid, the potassium current was enhanced and did not inactivate. Thus, olfactory neurons use at least two different transduction mechanisms: a depolarization that is attributed to odorant-dependent opening of transduction channels, and a hyperpolarization due to modulation of potassium channels.

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501.5

MICROSCALE MEASUREMENT OF ODOR PLUMES WITH ELECTROCHEMICAL MICROELECTRODES: CONSEQUENCES FOR THE CODING OF SIGNALS FOR RECEPTOR ORGANS.

P.A. Moore*, R.K. Zimmer-Faust, M.J. Weissburg, S. BeMent, J.M. Parrish and G.A. Gerhardt, Depts of Pharmacology and Psychiatry, UCHSC, Denver, CO; Dept of Biology, USC, Columbia, SC; Dept of EE., UM, Ann Arbor, MI.

The dispersal of chemicals (and hence stimulus structure) is due to the fluid dynamics of a particular marine environment. To quantify the smallest spatial scales associated with chemical patches, we measured the structure of chemical signals under turbulent flow simultaneously with carbon-based and a novel semiconductor-based, multisite, microelectrochemical electrode.. Simultaneous recordings showed that patch sizes may be as small as 200 microns. Plume parameters such as pulse height and pulse slope had spatial distributions that could provide directional or distance information about the odor source to an orienting animal. Such differences in chemical signal structure over small spatial scales might be important to animals that use olfactory orientation. We propose two alternate ways in which organisms might deal with these fine scale differences in odor concentration. Animals much larger than the microscale patches may have evolved elongated olfactory organs to smooth variations in sensory input, whereas smaller animals may be able to capitalize on microscale variation to extract directional information from turbulent odor plumes.

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501.6

MOLECULAR CLONING OF PUTATIVE OLFACTORY SPECIFIC GENES FROM CATFISH.

E. Bettini, N. Dahmen, H.L. Wang, F. Margolis*, Department of Neurosciences, Roche Institute of Molecular Biology, Nutley, NJ 07110.

To identify genes selectively transcribed in olfactory tissue we have used multiple strategies including differential library screening. Most recently we have reported on the use of functional expression of transcribed RNA in *Xenopus* oocytes in a search for olfactory receptor cDNAs (Dahmen; Wang Soc. Neurosci. 1991). Recent advances in automated DNA sequencing coupled with computerized access to data bases facilitate the direct use of DNA sequencing for this purpose.

Plasmid DNA was isolated from 162 clones selected from a subpool of a directional cDNA library prepared from catfish olfactory mRNA. The inserts were sequenced from both the 5' and 3' directions using fluorescent tagged dideoxy nucleotides and an ABI automatic DNA sequencer. The obtained sequence data were analyzed and compared to Genbank, EMBL and Swissprot data bases.

Thirty independent sequences were present in the pool whose abundance varied from 1-28 copies. The three most abundant sequences were apparently novel and not similar to any previously reported sequence. An additional 14 were highly homologous to cloned sequences from other species. Among these were a homologue of a β-subunit of a G-protein and a human tag sequence. In addition, we have identified a clone coding for a novel protein with putative EF-hand calcium binding domains and one with a basic-HLH (helix loop helix) domain suggesting it may be a novel gene transcription factor. Experiments are underway to determine the tissue specificity and functional significance of these various clones.

501.7

CELL DYNAMICS OF OLFACTORY BASAL CELLS. J.M.T. Huard* and J.E. Schwob. Dept of Anatomy and Cell Biology, SUNY Health Sci Ctr, Syracuse, NY 13210

In adult rats, the olfactory epithelium has the capacity to generate new sensory neurons during normal life and after experimental injury as a consequence of the division of olfactory basal cells and the differentiation of their daughters into replacement neurons. However, the basal cell population is poorly characterized with regard to the regulation and maintenance of the neurogenic process. As a first step, we are investigating the kinetics of the mitotic cycle of basal cells and the fraction of the basal cell population that retains the capacity to re-enter the mitotic cycle. The duration of the S-phase was determined in normal adult rats (n=3) using sequential pulse-labeling with iododeoxyuridine (IdU) followed by bromo-deoxyuridine (BrdU) and was found to be 5.9 hours. Using this new information, animals were labeled with a pulse injection of ^{3}H-thymidine and subsequently by a 12 hour infusion of BrdU that began 12 hrs after the administration of the thymidine. In both normal and methyl bromide-lesioned adult animals (4 days post exposure), basal cells labeled with both thymidine and BrdU were found, indicating that some daughter cells generated by the division of olfactory basal cells can re-enter the mitotic cycle. Experiments are underway to further characterize the dynamics of the stem cell and neuronal progenitor populations. Supported by NIH R29 DC00467.

501.8

HSP 70 EXPRESSION IN RAT OLFACTORY RECEPTOR NEURONS. V.McM.Carr* and A.I.Farbman. Dept. of Neurobiol. & Physiol., Northwestern Univ., Evanston, IL 60208.

In a study of stress protein expression in rat olfactory epithelium (OE) we have observed olfactory receptor neurons (ORNs) immunoreactive for the 70kDa heat shock protein family (hsp 70). IR ONRs are widely and predictably scattered in the OE, occurring most densely in posterior and ventral septum and turbinates. More anterior regions of rostral turbinates show them also, but they are virtually nonexistent in the dorsal half of the septum. While total numbers vary among animals, IR ORNs occur in approximately equal numbers on both sides of the OE.

Following olfactory bulbectomy (OB-X) these hsp70 IR ORNs persist in equal numbers in ipsi- and contralateral OE through 7 days post-op, even though most other ipsilateral ONRs die several days earlier. Subsequently, ipsilateral IR ORN numbers also decline dramatically.

These IR ORNs may be responding to some as yet unknown physiological stress. Their disappearance after OB-X indicates a certain degree of maturity is required for hsp70 expression. Autoradiographic birthdating studies show this maturity is achieved as early as 2 wks after neuronal 'birth'. Supported by NIH grants DC 00347 and DC 01593.

501.9

TRANSFER OF MOLECULAR INFORMATION FROM OLFACTORY RECEPTORS TO MITRAL CELLS: ANALYSIS BY A COMPUTATIONAL MODEL. David A. Berkowicz*# and Gordon M. Shepherd (Section of Neurobiology and #Interdisciplinary Neuroscience Program, Yale University School of Medicine, New Haven, CT 06510)

Mitral cells in the olfactory bulb show differential responses to related members of a homologous series of fatty acid molecules (Mori et al., 1992). The fatty acid sensitive cells are concentrated in one region of the olfactory bulb, implying that olfactory receptor neurons bearing receptors preferentially tuned to these molecules converge their axons onto glomeruli in this region. In order to analyze these complex relations we constructed computational models of olfactory neurons and connected them to a model mitral cell via multiple synapses. The model olfactory neurons contain an odor ligand-gated conductance sited in the cilia and voltage-gated Na+ and K+ conductances in the soma. The ligand-gated conductance displays a differential response to stimuli based upon the number of C-atoms in the fatty acid side chain. Each olfactory neuron thus responds independently in a manner reflecting the affinity of its receptor for structurally related odor molecules. One hundred olfactory neurons send axons which converge on the glomerular tuft of a single mitral cell. The mitral cell has compartments for synaptic inputs and outputs and for voltage-gated conductances. The temporal aspects of the afferent input to the glomerular dendrites can be precisely controlled, as can the time course of the synaptic current. We have tested two models: either the receptor neurons express one type of receptor, or the receptor neurons are divided into 3 unequal populations, each expressing receptors with different affinities for members of the fatty acid series. With the homogeneous receptor neuron population, the receptor neuron responses map relatively directly onto the mitral cell responses. By contrast, the mitral cell responses to the mixed sensory neuron population show complex patterns of spike discharge to related molecules, similar to the physiological recordings. The results are a first step toward constructing more realistic models of the initial processing of molecular information in the peripheral olfactory pathway.
HHMI fellowship to (DAB) and research grants from ONR and NIDCD to (GMS).

501.10

SEXUALLY DIMORPHIC OLFACTORY SENSITIVITY TO THE BOAR PHEROMONE, ANDROSTENONE, IN PIGS. K.M. Dorries[1], M.J. Thomas[2], B.P. Halpern[1] and E. Adkins-Regan[1]* Depts. of Psychology[1] & Animal Science[2], Cornell Univ., Ithaca, NY 14853.

Adult domestic pigs (Sus scrofa) show sexually dimorphic behavioral responses to the steroidal pheromone 5α-androst-16-en-3-one (androstenone). Androstenone is attractive to, and facilitates expression of a standing mating posture in estrous females. Adult males show neither response to the steroid. Olfactory sensitivity to androstenone is sexually dimorphic in humans: Adult females have lower androstenone detection thresholds, and adult males are more likely to be specifically anosmic to the odor. We measured androstenone detection thresholds in adult pigs to determine whether olfactory sensitivity to androstenone is also dimorphic in this species. To measure detection thresholds, we trained pigs to perform a modified go/no-go operant task. Data from 3 females and 3 males indicated that adult female pigs' detection thresholds in our apparatus are a five-fold dilution lower than adult males' (1.47×10^{-3} M in mineral oil vs. 2.93×10^{-4} M). In a second experiment (N=4) that included a control odorant, geraniol, these results were confirmed, and it was found that geraniol detection thresholds for males and females do not appear to differ. The finding of a sex difference in sensitivity to this specific stimulus in pigs may prove invaluable in understanding the neural bases of odor quality coding in mammals.
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501.11

NITRIC OXIDE AND OXYGEN CHEMORECEPTION OF THE CAROTID BODY. N. R. Prabhakar*, M. Haxhiu and H. Cao. Department of Medicine; Case Western Reserve University, Cleveland, Ohio 44106, USA.

Recent evidence suggests that Nitric Oxide (NO) can be synthesized in mammalian cells and that it acts as an intracellular messenger in a number of neuronal and non-neuronal tissues. NO is synthesized from Arginine by the enzyme NO synthase. Carotid bodies are sensory organs that detect the changes in arterial oxygen. Hypoxia stimulates the sensory discharge of the carotid body. The purpose of the present study is to investigate whether NO synthase is present in the chemoreceptor tissue, and if so, whether alterations in endogenous NO influence carotid body responses to hypoxia. Experiments were performed on anaesthetized adult cats. In the first group of experiments, carotid bodies were fixed in 4% paraformaldehyde and sectioned for histological examination of NO-synthase using NADPH-Diaphorase method. Many nerve fibers enveloping the glomus cells were found to be positive for NO-synthase. In addition, some glomus cells also display positive reaction for NO-synthase. The effects of blockade of NO-synthase activity on chemoreceptor responses to hypoxia was examined on isolated perfused, superfused carotid bodies (n =6). NO-synthase inhibitor, (L-NNA; 100-300 μM) enhanced the hypoxic response by 54 ± 12% of controls. L-Arginine (500 μM) reversed L-NNA -induced potentiation of the hypoxic response. In fact, in 4 of the 6 experiments, L-Arginine attenuated the hypoxic excitation by 63 ± 7% of the controls. These results demonstrate that (1) the carotid body can synthesize NO and (2) NO is inhibitory modulator of the chemosensory response to hypoxia.
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501.12

NITRIC OXIDE SYNTHASE IN THE CAROTID BODY. Z.-Z. Wang*, D.S. Bredt, S.H. Snyder, S.J. Fidone and L.J. Stensaas. Dept. Physiol., Univ. Utah Sch. Med., Salt Lake City, UT 84108; and Dept. Neuroscience, Johns Hopkins Univ., Baltimore, MD 21205

Nitric oxide (NO) has recently been identified as a novel neurotransmitter involved in glutamate toxicity and long-term potentiation. Our previous studies demonstrated that a NO-producing agent, sodium nitroprusside, stimulated cGMP production in the carotid body (Histochemistry 96: 523-539, 1991). However, the role of NO in chemoreception is unknown. The present immunocytochemical study utilized an antibody to nitric oxide synthase (NOS) to determine whether an endogenous source of NO exists in the rat carotid body. NOS immunoreactivity was found to occur in an extensive plexus of nerve fibers innervating parenchymal cells and blood vessels. These immunoreactive axons disappeared following transection of the carotid sinus nerve (CSN), but were unchanged following removal of either the superior cervical or nodose ganglia. NOS was also present in the somata of petrosal ganglion neurons whose axons could be shown to project to the carotid body by retrograde tracers. The results indicate that the carotid body is innervated by a large contingent of NOS positive nerve fibers derived from the petrosal ganglion and that NO may play a role in modulating the functional activity of carotid body chemoreceptors. Supported by NIH grant 12636 and 07938.

501.13

EFFECTS OF HYPOXIA INDUCED BY $Na_2S_2O_4$ ON K^+ AND Ca^{2+} ACTIVITIES OF CULTURED CAROTID BODY GLOMUS CELLS. X.-Q. Zhang, L. Pang and C. Eyzaguirre.* Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108

Carotid bodies were removed from anesthetized rats or mice for mechanical dissociation and glomus cell culture. After 2-7 days, cells were superfused with oxygenated physiological saline (pH 7.43 at 30-36°C) and viewed with phase-contrast. Rat glomus cells appeared either clustered or isolated, whereas all mouse cells appeared isolated. All cells were impaled with two microelectrodes (tip <1 μM) for recording E_m (filled with 3 M KCl) and V_{ion} (K^+ or Ca^{2+}, filled with an exchanger or ionophore). Clustered rat cells had a mean a_iK^+ of 73 mM (a_oK^+, 6 mM; E_K, -62 mV). In isolated cells, the mean a_iK^+ was about 32 mM (a_oK^+, 3-5 mM; E_K, -41 to -61 mV). The mean a_iCa^{2+}, measured only in 16 mouse cells, was 83 nM (a_oCa^{2+}, 0.3 mM; E_{Ca}, 211 mV). After the controls, 1-1.25 mM $Na_2S_2O_4$ was applied to reduce saline PO_2 to 30-5 Torr. Hypoxia depolarized most clustered rat cells and all mouse glomus cells. Most isolated rat cells hyperpolarized. When cells were depolarized, a_iK^+ decreased by 31 mM in the clusters and by 27 mM in isolated cells. During hyperpolarization, $[K^+]_i$ increased by a mean of 6 mM. Hypoxia had biphasic effects on $[Ca^{2+}]_i$. As PO_2 fell, the mean $[Ca^{2+}]_i$ decreased to 41 nM, followed by an increase to 254 nM. Changes in a_i were accompanied by shifts in E_{ion}. When cells depolarized, the mean E_K shifted positively by 16 mV in the clusters and by 42 mV in isolated cells. When cells hyperpolarized, the mean E_K shifted negatively by 3 mV. E_{Ca} became less positive (188 mV) when mouse cells depolarized. Supported by NS grants 05666 and 07938.

501.14

FURTHER STUDY ON ELECTROTONIC COUPLING BETWEEN GLOMUS CELLS OF RAT CAROTID BODY. L. Monti-Bloch* V. Abudara and C. Eyzaguirre, Dept. Physiol., Univ. Utah Sch. Med., Salt Lake City, UT

Many carotid body glomus cells are electrotonically, and bi-directionally, coupled at rest. This report describes the effects of dopamine, lactic acid, hypoxia (induced by N_2 or $Na_2S_2O_4$), hypercapnia, temperature and cholinergic agents on intercellular coupling. Carotid bodies were excised from 50 g anesthetized rats and superfused with oxygenated physiological saline, pH 7.4 at 30°C. Seventy-one adjacent pairs of glomus cells were simultaneously impaled with 3 M KCl-filled micropipettes. The mean resting potential (E_m) was -25.2 ± 0.8 (SE) mV. Current pulses of either polarity (0.1-1.0 nA), alternatively delivered through both electrodes, measured the input resistance (R_o = 68.3 ± 6.6 MΩ), the coupling coefficients (K_c = 0.3 ± 0.2) and coupling resistance (R_c = 833.6 ± 371 MΩ). Applications of the different agents most commonly (55-65%) depolarized both cells in the couplets, reduced their R_o, uncoupled them and their R_c increased. Fewer cells (15-25%) showed dual hyperpolarization, increased R_o and K_c, and reduced R_c. A similar low proportion of pairs showed opposite effects, namely, depolarization of one cell and hyperpolarization of the other, uncoupling of one cell and increased coupling of the other. There was a significant ($p=0.001$) and negative correlation between ΔR_c and ΔK_c. Results suggest that intercellular coupling of glomus cells is not always symmetrical. Coupling may be accomplished by different types of connexons in the unions, or the same connexons may occasionally react differently when acted upon by the chemosensory stimuli. Supported by NS grant 07938.

501.15

FACTORS INFLUENCING CYCLIC GMP FORMATION AND CHEMOSENSORY INHIBITION IN THE RABBIT CAROTID BODY. B. Dinger*, L. He, J. Chen and S. Fidone. Dept. Physiol., Univ. Utah Sch. Med., Salt Lake City, UT 84108

Recent studies in our laboratory have shown that atrial natriuretic peptide and its analog, atriopeptin III (APIII), increase cyclic GMP (cGMP) formation in chemosensory type I cells in the carotid body. Furthermore, APIII and cell permeant forms of cGMP inhibit carotid sinus nerve (CSN) activity evoked by hypoxia. Earlier studies established that hypoxia depresses basal cGMP levels in the chemosensory tissue while increasing the content of cAMP; low pH, another natural stimulus for the carotid body, likewise elevates cAMP content. The association between cAMP and carotid body excitation was further suggested by the finding that the adenylate cyclase activator, forskolin, potentiates CSN activity evoked by hypoxia. In the present study, we have examined the influence of hypoxia, low pH and cAMP on the elevated cGMP content and CSN inhibition produced by APIII.

Rabbit carotid bodies superfused *in vitro* in physiological media equilibrated with 100% O_2 (pH 7.4) contained 69.7 ± 4.3 ($\bar{X}$ ± SEM) pmol cGMP/g tissue. Incubation for 10 min in 100 nM APIII (100% O_2-media) elevated the cGMP content 41-fold. In media equilibrated with 10% O_2, basal levels of cGMP were reduced by 19% and the response to APIII was decreased by 48% ($p<0.005$). Basal levels of cGMP were unaffected at pH 6.8 (100% O_2-media), but the APIII response was again diminished by 44%. The combination of pH 6.8 in 10% O_2-media resulted in a total 67% decrease in the effect of APIII. In contrast, forskolin (10 μM) potentiated the APIII induced generation of cGMP by 2-fold. Nevertheless, recordings of CSN activity *in vitro* showed that forskolin (10 μM) completely reverses APIII related inhibition. Our data suggest interactive influences between cAMP and cGMP, and that chemoreceptor activity is a consequence of their combined actions. Supported by USPHS grants NS12636 and NS07938.

501.16

THE EFFECT OF HYPOXIA ON TYROSINE HYDROXYLASE GENE EXPRESSION IN THE *IN VITRO* RAT CAROTID BODY. J. Chen*, B. Dinger and S. Fidone. Dept Physiol, Univ Utah Sch Med, Salt Lake City, UT 84108

Previous neurochemical and immunocytochemical studies established that tyrosine hydroxylase (TH) activity in the carotid body is elevated 24-48 hr after breathing hypoxic gas mixtures. However, evidence for the induction of TH-mRNA in the carotid body has only recently been obtained using *in situ* hybridization techniques, and little is known regarding the cellular mechanisms which regulate TH expression in the chemosensory tissue. In the present study, we have exposed carotid bodies *in vitro* to chemoreceptor stimuli in an effort to identify the experimental conditions which are responsible for TH induction.

Carotid bodies and superior cervical ganglia (SCG) exposed for 3 hr to superfusion media equilibrated with either 10% O_2 (hypoxia) or 100% O_2 (normoxia) were rapidly frozen on dry ice and processed for reverse transcriptase-polymerase chain reaction (RT-PCR) in accord with the method of Singer-Sam et al. (Nuc. Acids Res. 18: 1255, 1990) which is designed to measure the relative accumulation of specific transcripts. The size and amount of a putative 234 bp TH-DNA product was evaluated using HPLC and agarose gel electrophoresis, and the results expressed per mg protein. Hypoxia elevated the total RNA in the carotid body 2.49 ± 0.50-fold ($\bar{X}$ ± SEM), while the TH mRNA increased 3.63 ± 0.84-fold. In contrast, these parameters were unchanged in SCG similarly exposed to hypoxic media. Incubation of carotid bodies in zero Ca^{++} superfusates greatly attenuated the increase in TH mRNA evoked by hypoxia (1.39 ± 0.34-fold increase; $p<0.025$ compared to normal Ca^{++} group). Our results suggest a direct role for hypoxia in TH mRNA induction in the carotid body, and that Ca^{++} is an important component of the signal transduction pathway. Supported by USPHS grants NS123636 and NS07938.

501.17

NON-RADIOACTIVE *IN SITU* HYBRIDIZATION ANALYSIS OF TYROSINE HYDROXYLASE GENE EXPRESSION IN THE CAROTID BODY. L. Zhang and L.J. Stensaas*, Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the synthesis of dopamine, an important carotid body neurotransmitter. We have shown that exposure to hypoxia leads to a rapid increase in the level of TH in the carotid body. In the present study, *in situ* hybridization with a digoxigenin nonradiolabeled oligonucleotide probe was employed to determine the effect of hypoxia on TH gene expression at the single cell level. Chronic hypoxia experiments involved the maintenance of rats in a hypobaric chamber at half atmosphere. In normoxic animals, and following 3 hr of chronic hypoxia, TH is expressed at uniformly low levels throughout the CB. After exposure to hypoxia for 6 hr and 2 days, TH mRNA levels progressively increased in type I cells near the periphery of the CB. Intensely labeled cells were scattered throughout the CB at 7 days; high levels of TH mRNA were expressed in most type I cells at 2 weeks. In studies involving acute hypoxia, rats were exposed to a gas mixture of 5% O_2, 95% N_2 for three 30 min periods followed by 20 min of room air. High TH mRNA levels occurred in most type I cells of the CB. These observations indicate a different mode of response in TH mRNA levels to chronic and acute hypoxia.

501.18

FREQUENCY-RESPONSE CHARACTERISTICS OF CO_2 SENSITIVE PRIMARY AFFERENTS IN THE REPTILIAN LUNG. Richard. D. Tallman, Jr.*[1], and Michael C.K. Khoo[2] [1]Allied Medical Professions, Ohio State University, Columbus, OH and [2]Dept. of Biomedical Engineering, Univ. of So. Calif, Los Angeles, CA.

Receptors have been identified within the gas exchange regions of the lungs of various reptiles and birds which are inversely sensitive to airway CO_2. These intrapulmonary CO_2 receptors (IPC), send their afferent fibers via the vagus nerve. The purpose of the present study was to characterize the dynamic response characteristics of IPC in the bull snake (*Pituophis melanoleucus*). To accomplish this, we utilized a binary random function known as a pseudo-random binary sequence (PRBS) as the input signal. CO_2 was randomly switched between two levels during unidirectional ventilation while IPC activity arising from a single unit was recorded. IPC responses to three CO_2 step responses were measured, (3-1%, 2-1% and 1-0%). The receptor transfer functions were derived from the frequency-domain characteristics of the best-fit ARX (auto regressive with exogenous input) model to the data. The results indicated the presence of significant rate-sensitivity in receptor dynamics. However, the linear ARX model accounted for only 40-60% of the actual nerve responses. Further analysis suggested that unidirectionality in rate-sensitivity and half-wave rectification are important nonlinearities that account for the remainder of the observed dynamics. (Supported in part by NIH grants HL-02536 and RR-01861).

502.1

BETA-ADRENERGIC RECEPTOR KINASE-2 AND BETA-ARRESTIN-2: LOCALIZATION TO OLFACTORY RECEPTOR NEURONAL CILIA. *G.V. Ronnett[1]*, T.M. Dawson[1], J.L. Arriza[2] and R.J. Lefkowitz[2]. [1]Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205, [2]Howard Hughes Med. Inst., Duke Univ. Med. Center, Durham, NC 27710.*

Desensitization of receptor systems, notably G-protein-linked receptors, is regulated by receptor phosphorylation. The β-adrenergic receptor kinase (βARK) phosphorylates the agonist-occupied β-adrenergic receptor (βARK) to promote rapid ($t_{\frac{1}{2}}$=15 sec) receptor uncoupling from G_s. More recently, a novel protein, β-arrestin (βARR) has been shown to quench phosphorylated β_2AR-coupling to G_s.

Olfactory signal transduction likewise proceeds via interaction of odorants with G-protein-coupled receptors and is capable of rapid (5-10 sec) desensitization. Using affinity-purified antibodies we have localized by immunocytochemistry βARK2 and βARR2, two isozymes of each protein to olfactory receptor neuronal cilia and dendritic knobs (the site of the initial steps of olfactory signal transduction). Immunoreactivity for βARK2 and βARR2 is depleted upon bulbectomy, which results in olfactory receptor neuronal (ORN) degeneration. Primary cultures of ORN's demonstrate immunoreactivity for βARK2 and βARR2. The presence of these two specific isoforms of βARK and βARR suggests that olfactory signal desensitization may involve ligand-mediated phosphorylation of olfactory receptors.

502.2

PERIPHERAL DENERVATION INDUCES SELECTIVE CHANGES IN THE RAT OLFACTORY BULB. S.R. King* and J.H. McLean. Div. Basic Med. Sci., Memorial Univ. of Nfld., St. John's, NF, Canada A1B 3V6.

Lesion-induced plasticity of neurotransmitter systems is observed in many regions of the CNS. However, the response of different neurotransmitter systems to lesions may not be identical. The olfactory bulb (OB) receives direct inputs from the periphery. In addition, the connections and neurotransmitter content of the OB has been well characterized. Thus, the OB may provide a useful cortical model of lesion-induced plasticity. In this study, dopamine beta-hydroxylase, serotonin (5-HT), glial fibrillary acidic protein and S-100 immunocytochemisty was performed to elucidate the response of different transmitter systems and non-neuronal elements to peripheral denervation of the OB.

Deafferentation of the right OB was achieved by application of $ZnSO_4$ to the olfactory epithelium (OE) of young adult male rats. Following survival of at least 3 weeks, the rats were sacrificed by perfusion. The effectiveness of the lesion was determined by the degree of the loss of tyrosine hydroxylase (TH) immunoreactivity in the periglomerular cells which require the presence of the OE in order to express the TH phenotype (Baker *et al.*, 1983). The deafferentation resulted in a decrease in the density of 5-HT fibres in the OB but had no significant effect upon the noradrenergic innervation. The density of the glial cells did not appear to be affected by the lesion. However, there was an increase in the number of astrocytes that express S-100, a 5-HT growth factor. These results suggest that the lesion did not induce sprouting in the 3 week time period. In contrast, 5-HT fibres were selectively decreased for an unknown reason, perhaps due to selective neurotoxicity of zinc to 5-HT. Supported by the MRC of Canada.

502.3

TOPOGRAPHIC COURSES OF INDIVIDUAL AXONS WITHIN THE OLFACTORY NERVE. Charles A. Greer* and Christine Kaliszewski. Sections of Neurosurgery and Neurobiology, Yale University School of Medicine, New Haven, CT 06510.

The topography of olfactory receptor cell axons originating in the olfactory epithelium (OE) and terminating in the glomeruli of the olfactory bulb (OB) is not well understood. Though the rules that govern the distribution of axons to their glomerular targets remain uncertain, it is evident that a significant rearrangement occurs between the OE and OB. That is, axons from adjacent receptor cells in the OE do not necessarily maintain a neighborly topography in the OB. In this vein, Daston et al. (Brain Res. 537: 69) reported that axons maintained neighbors over long distances in the olfactory nerve (ON) until they reach the OB. To investigate further the rearrangement of the ON we followed the courses of small populations of axons in mesaxons using electron microscopy of serially sectioned ON.

Turtles (*Pseudemys scripta*) were prepared for routine thin section electron microscopy. Segments of the ON, approximately 4-5 mm in length, were blocked into sections proximal to either the OB or OE. Serial sections (10-50) were collected on slot grids, photographed with a JEOL 1200 and printed at a magnification of 37,500X. The topographic relations between populations of 20-30 axons in mesaxons were reconstructed for 3D representation.

Axon diameter ranged from 0.104 - 0.312 μm. The topography of axons in ON mesaxons was reasonably stable proximal to the OE. However, proximal to the OB a fluid topography was found; the axons bordering any given axon were more likely to change proximal to the OB. Moreover, existing axons left the subpopulation being reconstructed while new axons entered.

These data appear consistent with the hypothesis that the OB contains signals that contribute to the sorting of axons toward glomerular targets.

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502.4

CARBOCYANINE DYES AS TRACERS IN THE ELASMOBRANCH OLFACTORY SYSTEM

*L. Dryer *and P.P.C. Graziadei, Florida State University, Tallahassee. FL 32306*

One of the obstacles in tracing neural pathways in sharks of the order carcharhiniforms is the difficulty in keeping specimens alive in captivity. We have previously shown using biocytin as a tracer *in vitro* that the primary olfactory projections are segregated into a sectorial arrangement onto the bulb. But the mechanisms of biocytin transport remain unknown, and we wished to confirm this observation using other tracing methods. Carbocyanine dyes are known to be excellent tracers of neural pathways in higher vertebrates, both in living and fixed tissues, but previous attempts to apply this method to elasmobranchs have failed. We have succeeded in tracing the olfactory pathway using DiI in fixed elasmobranch preparations. The results confirm our previous observation that the primary olfactory fibers project in a straight fashion onto the bulb. In bonnethead sharks, where the olfactory bulb is made of successive swellings (sub-bulbs) along the crescent-shaped organ, each sub-bulb receives afferents from 5-6 lamellae without the convergence or divergence observed in mammals. The elasmobranch olfactory bulb is clearly compartmentalized into several units, possibly serving different purposes. The resolution of carbocyanine dyes in this preparation is superior to biocytin, and the passive mode of transport of DiI makes it an excellent tracer in any direction. Supported by NS-20699.

502.5

LESIONS OF THE OLFACTORY NERVE AFFECT TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN THE OLFACTORY BULB OF *XENOPUS LAEVIS* TADPOLES. E. O'Campo, J.B. Angevine, Jr.*, and G.D. Burd. Depts. of Molecular & Cellular Biology and Anatomy, Univ. of Arizona, Tucson, AZ 85721.

Deafferentation of the olfactory bulb results in a reversible loss of tyrosine hydoxylase (TH) expression in the olfactory bulb of mammals (Baker et al. 1983. J.Neurosci. 3:69). The purpose of this present study was to investigate whether TH immunoreactivity in the olfactory bulb of *Xenopus* tadpoles is similarly dependent on afferent innervation. Unilateral and bilateral lesions of the olfactory nerve were performed on tadpoles at stage 58 (the beginning of metamorphic climax). Two weeks following the lesions, the animals were fixed, and the brains and nasal capsules were processed for immunocytochemistry. E7, an antibody that was raised against *Xenopus* nasal capsules and that recognizes olfactory receptor cells (Matheson and Burd. 1991. Soc. Neurosci. Abstr. 17:230), was used as a marker to confirm the presence of olfactory axons in the olfactory bulb and to test the extent of the lesion in the olfactory epithelium. The TH positive cells were present in the glomerular layer, mitral cell/external plexiform layer, and the superficial granule cell layer. Bulbs with unilateral lesions demonstrated a decrease in number of TH immunoreactive neurons relative to the unlesioned side. Bilateral deafferentation significantly reduced the number of cells expressing TH on both sides. We also noted that staining with the E7 antibody was lost in the olfactory epithelium when the olfactory nerve was cut, and that the staining failed to return within the 14-day survival period. In summary, TH expression in the developing olfactory bulb of *Xenopus laevis* is significantly reduced following sensory deafferentation. Supported by DC 00446.

502.6

EFFECTS OF DOPAMINE ON RESPONSES TO ELECTRICAL STIMULATION IN THE SALAMANDER OLFACTORY BULB: INITIAL RESULTS. K.A. Hamilton* and M.R. Gurski. Department of Cellular Biology and Anatomy, LSU Medical Center, Shreveport, LA 71130.

Recent evidence suggests that the afferent input to the olfactory bulb from the olfactory nerve may be modulated by dopamine (DA) (Nickell *et al.*, *Neuroreport*, 2:9, 1991). As a first step in investigating the function of putative dopaminergic cells that give rise to glomerular dendrites in the salamander bulb (K.A. Hamilton and S.S. Foster, *Neurosci. Abstr.*, 17:637, 1991), we have examined the effects of exogenous DA and the DA antagonist fluphenazine on field potentials evoked by electrical stimulation of the olfactory nerve and tracts.

Recordings were obtained from the granule cell layer in isolated hemibrain preparations during superfusion with normal medium, 1-10 min after superfusion with medium containing 0.05-100 μM DA or 5-100 μM fluphenazine, and 1-10 min after reperfusion with normal medium. The amplitude and latency of the large, positive wave was measured. With DA concentrations below 1 μM, significant effects were not generally observed (t-tests, α=0.05). With 1-100 μM DA, however, the amplitude of the nerve response was reduced by 21-79% and the latency increased by 4-24%. By contrast, the amplitude of the tract response was reduced by only 7-26% and there was little if any change in latency. With all concentrations of fluphenazine, the nerve response was blocked, but the tract response was apparently unaffected. During reperfusion following DA exposure, the nerve response did not recover as fully as the tract response. Following fluphenazine exposure, the nerve response showed no sign of recovery.

In agreement with the results of Nowycky *et al.* (*Neuroscience*, 8:717, 1983), the results indicate that exogenous DA suppresses olfactory bulb responses to electrical stimulation. The results also provide initial support for the hypothesis that DA may modulate the afferent input to the salamander bulb from the olfactory nerve.

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502.7

IMAGING ODOR-EVOKED ACTIVITY IN THE SALAMANDER OLFACTORY EPITHELIUM (OE) AND BULB (OB): PHYSIOLOGICAL CORRELATES OF BEHAVIORAL OBSERVATIONS. J. White* and J.S. Kauer. Neuroscience Program, Tufts/NEMC, Boston, MA 02111.

Behavioral experiments suggest that tiger salamanders (*Ambystoma tigrinum*) discriminate between certain reagent grade odorants (butyl and propyl acetate; butyl and amyl alcohol; butyl acetate and butyl alcohol) but do not discriminate between others (butyl and amyl acetate; butyl and propyl alcohol) (Mason and Stevens, 1981, *Physiol. Behav.* 26:647). To determine whether these same odorants elicit similar and/or different physiological responses in the olfactory system, we have investigated their effects on salamander OB and OE by video imaging voltage-sensitive dye signals with high spatial (up to 256x240 pixels) and temporal resolution (16ms per frame). OE and OB were stained with the styryl dye Di-4-ANEPPS, which produced OB signals similar to those produced by the dye RH-414. In the ventral OE, each odorant elicited optical signals with time courses and spatial distributions similar to electro-olfactograms recorded in the same animal after the optical recordings. Propyl, butyl, and amyl acetate each elicited similar patterns of widespread activity with peaks anteriorly and posteriorly, while propyl, butyl, and amyl alcohol each elicited a much smaller area of activity with a peak anteriorly. In the OB, amyl, butyl, and propyl acetate each elicited long lasting depolarizations; however, the propyl acetate response tended to be smaller and at longer latency than the responses to the other two odorants. Propyl and butyl alcohol elicited large, long-lasting hyperpolarizations in the OB, while the amyl alcohol response was small and depolarizing. Thus, the differences in OB activity patterns evoked by these odors paralleled the behavioral responses of the animal, while the differences in OE activity were more subtle.

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502.8

OLFACTION IN RATS WITH REMOVAL OF 50-95% OF THE OLFACTORY BULB. X-C. M. Lu and B. M. Slotnick*. Department of Psychology, The American University, Washington, DC 20016.

Are odors processed at specific sites in the olfactory bulb? Available behavioral studies indicate that removal of odor activated bulbar sites identified with 2-DG or with electrophysiology does not produce deficits in detection of the target odor. We have examined the extent to which reduction of topographical organization of the olfactory bulb affects detection and discrimination of different odors in 21 experimental and 8 control rats.

Rats with unilateral bulbectomy plus removal of approximately 50%-97% of the remaining bulb (assessed by measuring total linear extent of remaining glomeruli) were tested for detection of amyl acetate, butanol, citral and proprionic acid vapor and then trained to discriminate these odors from cineole. Odor concentration was varied from 1-.01% of vapor saturation.

Some but not all rats with less than 5% of glomeruli remaining were anosmic. Those with approximately 25% of remaining glomeruli were able to perform all tasks as well or almost as well as unilateral bulbectomized controls. Experimental rats with 10-25% of glomeruli remaining failed on low odor concentrations but otherwise most performed nearly as well as controls. In general, deficits were related to the amount of remaining bulbar tissue but not to which segment (dorsal, lateral, medial or anterior) of the bulb was removed.

This study indicates that rats with up to 90% reduction of the olfactory bulb input to the forebrain retain significant olfactory abilities and may perform as well as controls on simple odor detection and discrimination tasks. The results provide no evidence that any one segment of the bulb is essential for detection of the odors tested.

502.9

***IN VITRO* SYNAPTIC ACTIVATION OF ADULT RAT OLFACTORY BULB MITRAL CELLS RECORDED BY CONVENTIONAL AND WHOLE CELL PATCH TECHNIQUES.** W. T. Nickell,* M. M. Behbehani, and M.T. Shipley. Univ. of Cincinnati Col. of Med., Cincinnati, Ohio 45267.

Responses of rat olfactory bulb mitral cells to stimulation of the olfactory nerve layer (ONL) were recorded *in vitro*. Olfactory bulbs were cut into 400 μm thick slices in the horizontal plane and submerged in a recording chamber. Patch clamp electrodes (1-2 μm tip diameter) were guided into the mitral cell layer under a dissecting microscope. A bipolar stimulating electrode was placed onto the ONL rostral to the recording electrode. Extracellular records of spontaneous and ONL evoked activity were obtained from 75 mitral cells. Most of these neurons responded to ONL stimulation. Some responded with one, or a short burst of spikes followed by a period of inhibition. In most cells, however, these initial events were followed by a long period (1-2 sec) of excitation. Intracellular whole-cell patch recordings were used to observe the synaptic events underlying this activity. ONL stimulation caused a prolonged depolarization accompanied by action potentials. Hyperpolarization blocked all but one spike, which preceded an EPSP. Under voltage clamp, ONL stimulation caused a long duration inward current. In some cells spontaneous EPSCs were present. High magnesium solution blocked these spontaneous currents. It is concluded: 1) There is a significant tonic excitatory synaptic input to olfactory bulb mitral cells. 2) The long period of excitation following ONL stimulation is caused by an unusually long duration depolarization. 3) ONL stimulation may generate spikes in the apical dendrites that are propagated to the soma with little decrement. *Supported by NIDCD DC00347 and DAMD17-91-C-1071.*

502.10

LAMINAR CONTRIBUTIONS TO INHIBITION DURING OLFACTORY BULB REPONSE TO ODOR: COMPUTER SIMULATION M. MEREDITH* Dept. Biological Science, Florida State University, Tallahassee, FL 32306

Previous work with a computer model based on olfactory bulb anatomy and physiology (Chem.Senses 16:556) showed complex spatial and temporal patterns of activity generated in response to relatively simply patterned olfactory input. Here the contribution of glomerular (superficial) and granule (deep) inhibitory circuits is examined in more detail. Lateral inhibition between glomeruli via PG cell axons is a potent contributor to spatial patterns of inhibition. ***Feedforward*** inhibition due to direct activation of PG cells by olfactory nerve fibers is especially important. When direct activation of PG cells is restricted, their effect is mainly by feedback inhibition and is, thus, self limiting. Intraglomerular inhibition between output cells of the ***same*** type (via PG dendrites) attenuates output with little effect on overall spatial patterns. However, spatial patterns can be created within the output cells of one lamina connected to one glomerulus. Intraglomerular inhibition ***between*** output cell types within one glomerulus, can produce different patterns of activation in the different laminae of output cells. Granule cells have feedback dendro-dendritic connections to output cells and thus have less potent afferent inhibitory action (although potentially more important in efferent inhibition). Their influence (via output cell secondary dendrites) is not restricted by glomerular boundaries. To the extent that they are divided into populations connecting selectively with one output cell type, granule cells also contribute little to the generation of different activity patterns in different output cell laminae. Output cell collaterals, however, could generate interlaminar differences in output by cross-connecting asymmetrically between output cells of one type and granule cells selectivly connected with another output cell type. The specificity of these connections is not yet known but the potential for complex pattern generation is considerable. Supported by NIH, DC00906 and NSF, BNS-8615159.

502.11

ORGANIZATION OF INHIBITION ON ASYMMETRIC TUFTED CELLS OF RAT OLFACTORY BULB. Patrick I. Ezeh and John W. Scott.* Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, Georgia 30322

Olfactory bulb tufted cells exist in several subtypes. Of those with basal dendrites, the most superficial cells extend their basal dendrites in the direction opposite that of the apical dendrite, and their basal dendrites do not mix with those of other mitral/tufted cells. We made intracellular recordings of 18 biocytin-labeled asymmetric, superficial tufted cells. These cells do not appear to share granule cells with mitral cells, because they respond with small IPSPs to stimuli that activate large IPSPs in mitral cells. The asymmetry of their basal dendrites offered a chance to study the contribution of the arrangement of dendritic shape to the organization of lateral inhibition in this system. This was investigated with localized olfactory nerve layer (ONL) stimulation. Although the effectiveness of stimulation at a particular ONL site decreased with distance from the tufted cell somata, the distribution of inhibitory influence was not determined solely by the direction of the basal dendrite. This observation, coupled with the fact that many granule cells are activated at short latencies by ONL stimulation, suggests that granule cells inhibiting superficial tufted cells may receive excitatory inputs from tufted cell axons.

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502.12

OLFACTORY BULB GRANULE CELLS DECREASE IN SIZE AND INCREASE IN DENSITY WITH EARLY OLFACTORY EXPERIENCE. J. F. McCollum*, C.C. Woo, and M. Leon. Department of Psychobiology, University of California, Irvine, CA USA 92717.

Young rats learn to approach an odor that had been experienced in the presence of tactile stimulation. Subsequent presentation of the familiar odor evokes an enhanced focal uptake of 2-deoxyglucose (2-DG) in the glomerular layer of the olfactory bulb. This enhanced uptake is associated with an increase in the focal glomerular-layer cell population (Woo and Leon, 1991). Since odors evoke a columnar response in the bulb (Guthrie, Anderson, Leon and Gall, 1991), we determined whether there were morphological changes in other bulb lamina. We investigated the size and density of mitral and granule cells found within columns both associated with the 2-DG foci and closely adjacent to these foci. We compared mitral and granule cell size and density in Nissl-stained sections from PND 19 odor-familiar pups with sections from controls. We found no difference between these groups in mitral cell nuclear area, perikaryal area, or density. Odor-familiar pups, however, had an increase in the density of superficial, but not deep granule cells within the foci-associated columns. These superficial granule cells also showed a decrease in soma size. The superficial granule cells within the foci-adjacent columns remained unchanged. A change in the density and size of superficial granule cells may contribute to the modification of olfactory bulb responses to familiar odors. These data suggest that early odor experience triggers restricted but multiple processes in the developing olfactory bulb.

502.13

THE ROLE OF THE LATERAL OLFACTORY TRACT (LOT) IN A TWO-ODOR DISCRIMINATION TASK. P.K.Thanos* and B.M.Slotnik. Psychology, The American University, Washington, D.C 20016.

It has been shown that odor memory in rats can be long lived as long as the animal has sufficient exposure to the odor. Little evidence however, exists as to the neuroanatomical stuctures. This study examined rat performance in a 2-odor discrimination task during short (10 sec.) and long (10 min.) inter-trial intervals (ITI). Each session consisted of a novel pair of odors presented over 30 trials. Trials began with the rat being placed at the start of a runway, at the end of which were two sample ports. Each port semi-randomly delivered one of the two odors. The trial terminated when the rat made a total of three lick responses to one port or 30 sec. had elapsed. If the rat responded to the port delivering the S+ odor, it was rewarded with 0.1 ml of water, and a correct response was recorded. A criterion of ten correct out of twelve consecutive trials was used as another measure of memory. The experimental group received unilateral lesioning of the LOT along with a contralateral bulbectomy, while controls received only a unilateral bulbectomy.

Preliminary results show LOT lesioned rats made more errors per session and it took longer to reach criterion. This effect was amplified in the 10 min. ITI.

502.14

SEROTONIN AND OLFACTORY LEARNING IN THE NEONATAL RAT. J.H. McLean[1], A. Darby-King[1], R.M. Sullivan[2] and S.R. King[1]. [1]Div. of Basic Med. Sci., Memorial Univ. of Newfoundland, St. John's, Nfld., Canada A1B 3V6 and [2] Dept. Psych., Univ. of Oklahoma, Norman, OK 73019.

There has been accumulating evidence that norepinephrine is necessary for the acquisition of learned olfactory behavior (Sullivan et al.1991; Sullivan et al.1989). The serotonergic input to the olfactory system has been given little attention in olfactory behavior although it densely innervates the olfactory bulb. In a previous paper we reported that serotonergic axons arrive in the olfactory bulb postnatally and densely innervate the glomeruli, the first site where olfactory information is integrated with circuitry of the olfactory system (McLean and Shipley, 1987). Thus, we hypothesize that serotonin could have important effects on olfactory learning.

In this study, the serotonergic input to the olfactory bulb of rats was depleted by bilateral injections of 5,7 dHT into the anterior olfactory nucleus on postnatal day 2 (birth=PND1). On PND 8, the depleted animals, shams or unoperated controls received one-trial conditioning to a peppermint odor (conditioned stimulus) paired with stroking (unconditioned stimulus). Half of the pups received no odor training. The next day the pups were tested to determine the amount of time spent over peppermint odor or normal bedding. The serotonin-depleted pups spent significantly less time over the peppermint odor than trained controls. The serotonin depletions of the olfactory bulb did not affect locomotion of the pups nor the noradrenergic input to the bulb. These results suggest that serotonergic input into the bulb is important for the acquisition of a learned olfactory behavior in the neonate. Supported by MRC of Canada.

502.15

DEVELOPMENT AND DECAY OF SELECTIVE LONG-TERM POTENTIATION IN THE PIRIFORM CORTEX AND OLFACTORY BULB. J. S. Stripling* and M. P. Galupo. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

High-frequency stimulation of cortical association fibers in the piriform cortex (PC) produces a selective long-term potentiation (LTP) of late components in potentials evoked in the PC and olfactory bulb (OB). The present study examined in detail the time course of the development and decay of selective LTP. Male Long-Evans rats were chronically implanted with a stimulating electrode in the association fiber system of the anterior PC and recording electrodes in the OB and a more caudal PC site. LTP was induced by 8 daily treatments of high-frequency stimulation (30 trains of 10 pulses at 100 Hz). The expression of LTP was monitored by continual stimulation at 0.1 Hz before, during, and after each LTP treatment. This testing revealed both a short-term potentiation that peaked during the daily LTP stimulation and decayed within 3 min, and a long-term potentiation that decayed very slowly and was still evident 24 hr later. The short-term component reached asymptotic levels well before the last LTP treatment, while the long-term component continued to grow in magnitude across the course of the experiment. Expression of the long-term component gradually diminished across an 8-day period following the last LTP treatment, but could be reinstated to a substantial degree by repeated stimulation at 0.1 Hz (latent potentiation). A single LTP treatment given on the eight day after the last daily LTP treatment completely restored LTP to its maximal level. Our results indicate that selective LTP is a very robust and long-lasting phenomenon that persists in latent form for a minimum of 8 days with little or no decay. These characteristics make selective LTP a suitable candidate for participation in long-lasting functional changes in the olfactory forebrain. Supported by NSF grant BNS 85-19700 and the Marie Wilson Howells Fund.

502.16

RELATIONSHIP BETWEEN SPONTANEOUS AND EVOKED POTENTIALS FOLLOWING SELECTIVE LTP IN THE PIRIFORM CORTEX AND OLFACTORY BULB. M. P. Galupo* and J. S. Stripling. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

High frequency stimulation that activates association fibers in the piriform cortex (PC) produces a selective long-term potentiation (LTP) of late components in potentials evoked in the PC and olfactory bulb (OB). This potentiation is accompanied by the appearance of spontaneous potentials that resemble potentiated components of the evoked potential (Brain Research 542; 107-122, 1991). The present study characterized these spontaneous potentials in detail and investigated their association with selective LTP. Male Long-Evans rats received LTP stimulation through chronically implanted electrodes in the anterior PC which produced either selective activation of PC association fibers or co-activation of PC association fibers and the lateral olfactory tract (LOT). LTP stimulation consisted of 30 10-pulse trains (100 Hz) administered on 4 consecutive days. Either type of stimulation reliably induced selective LTP. However, only stimulation that produced coactivation of PC association fibers and the LOT triggered the occurrence of prominent spontaneous potentials. Spontaneous potentials, when present, occurred in the first few minutes following LTP stimulation, coincided with the onset of selective LTP, and closely resembled potentiated components of the evoked potential. These results indicate that activation of PC association fibers is both necessary and sufficient for the induction of selective LTP, but that co-activation of LOT and association fibers is required to produce spontaneous potentials. Although these potentials may be a reflection of the same membrane conductances that underlie selective LTP, the dissociation between the two indicates that the induction of selective LTP does not require the generation of spontaneous potentials. Supported by NSF Grant BNS 85-19700 and the Marie Wilson Howells Fund.

502.17

CELL TYPES IN THE PRIMARY OLFACTORY CORTEX OF THE FROG. F. Scalia* and J.Y. Lettvin. Dept. of Anatomy and Cell Biology, SUNY Health Sci. Center at Brooklyn, Brooklyn, NY 11203 and Dept. of Biomedical Engineering, Rutgers, Piscataway, NJ 08855.

Rapid Golgi and modified Golgi-Kopsch preparations of adult R. pipiens reveal the presence of four distinct types of olfactory cortical neurons not previously distinguished in earlier studies on this amphibian. The first is a cell whose dendrites are covered densely with moderately short spines. A second type of cell has distinctly fewer and often longer spines, mixed with club-like processes. A third cell, is aspiny. Its dendrites are beaded at irregular intervals and varicose throughout their length. These three types of neuron have multiple apical dendrites that radiate obliquely toward the pia from a periventricular location. The dendrites of the aspiny cells often show a broader horizontal spread within the superficial neuropil. After crossing the superficial neuropil, the cortical dendrites may bend sharply beneath the pia to course parallel to the surface for as yet undetermined distances. The fourth cell is a small stellate neuron, which appears axonless. Its numerous primary dendrites branch repeatedly, and the branchlets are serially decorated with closely spaced small beads resembling axonal boutons. (Supported by PHS grant EY05284).

502.18

3-HYDROXY KYNURENINE RELEASE IN PREPYRIFORM CORTEX: A NEURAL RESPONSE TO AMINO ACID DEFICIENCY? **D.W. Gietzen*, B.L. Lee and M.P. Thomas. Dept Physiol Sci & Food Intake Lab, Sch of Vet Med, Univ Calif Davis, Davis, CA 95616.**

Recognition of amino acid (AA) deficiency induced by ingestion of AA imbalanced diets (IMB) has been localized to the prepyriform cortex (PPC), but the mechanisms underlying this recognition are not well understood. To determine what neuroactive substances were released in PPC after eating IMB, rats were implanted stereotaxically with bilateral guide cannulae (Plastics One, Roanoke VA) directed toward the PPC (coordinates: A: 9.8mm, L: 4.0mm and D: 5.5mm; perfusion needle extended 1mm). After prefeeding a low protein control diet for 7-10 days, rats were fed IMB and PPCs were perfused with artificial CSF (11 μl/min for 5 min) at varying times. Perfusates were analyzed by HPLC with electrochemical detection. Compounds were identified by peak conformation and retention time (RT); samples were spiked with external standards to verify RT. External standards included: norepinephrine (NE), dopamine, serotonin and the metabolites: DOMA, DOPAC, DOPEG, 5HIAA, HVA, L-DOPA, MHPG, 3-methoxytyramine, MOPET, normetanephrine, 3-OH-kynurenine (3HK), tyramine and VMA. NE was in the noise level in all chromatograms. 3HK was significantly increased at 1.5hr after introduction of IMB diet vs basal at 1.5hr IMB: 441±65 fg/μl vs control: 201±85 fg/μl ($p<0.05$, paired t, $N=8$). Previous results suggesting that NE was increased 1.5 hr after feeding IMB were based on HPLC conditions that did not allow separation of NE and 3HK. NE and 3HK were separated by 6 minutes in the present chromatograms. We conclude that 3HK may play a role in the recognition of essential AA deficiency in the rat. Parallel electrophysiological studies are in progress using the PPC slice preparation. **Supported by NIH: DK35747 (UCDavis Clin Nutr Res Unit) and USDA: CSRS 90 37200-5440.**

502.19

THE CELLS OF ORIGIN OF THE ENDOPIRIFORM-TECTAL PATHWAY IN THE RAT. W. Chen*[1] and N. Sahibzada[1,2]. [1]Dept. of Psychology, Univ. District of Columbia and [2] Dept. of Pharmacology, Georgetown Univ. Medical Center, Washington DC 20007.

In an animal such as the rat, olfactory stimuli play an important role in behavioral activities concerned with attention and orientation. A likely structure through which these activities may be expressed is the superior colliculus (SC). This structure, in a variety of species, has been shown to play a seminal part in the mediation of attention and orientation. There is some indication (Neasfey, et al., 1986) that a possible source of olfactory information, received by the SC, originates from Endopiriform nucleus (EN). Therefore, the purpose of this initial study was to identify the cells of origin of this pathway that projects to the SC.

Injections of Fluoro-Gold in the SC, besides labeling know collicular afferents, also label cells in the endopiriform nucleus (EN). Our preliminary results show that label in this nucleus appears to be related to the injection site in the colliculus. Thus, anterior collicular injections result in faint label in the anterior EN, whereas posterior SC injections result in dense label in the posterior EN.

On basis of our preliminary findings, we are in the process of verifying this topographical connection by injecting anterograde and retrograde tracers in the EN. These experiments should not only give us a map of the distribution of terminals in the SC, but will also determine if any reciprocal connections are present between the two structures.

502.20

DEVELOPMENTAL STUDY OF LECTIN BINDING TO THE VOMERONASAL AXONS IN THE RAT ACCESSORY OLFACTORY BULB. M. Ichikawa*[1], S. Takami[2], T. Osada[3] and P. P. C. Graziadei[2] [1]Dept. of Anat & Embryol., Tokyo Metropolitan Inst. for Neuroscience, Tokyo, [2]Dept. of Biol. Sci. The Florida State Univ, Tallahassee, Florida. and [3]Dept. of Biol. Sci, Fac. of Biosci & Biotechnol, Tokyo Inst. of Technol, Yokohama, Japan.

Bandeiraea simplicifolia lectin-I (BSL-I) and *Vicia villosa* aggulutinin (VVA) specifically bind to the vomeronasal (VN) axons (Ichikawa et al., 1992). The binding of these two lectins to VN axons was studied in the developing accessory olfactory bulb (AOB) of rat. Fetal (Embryonic day (ED) 16, 18, 20), postnatal (postnatal day (PD) 0, 3, 7, 14, 28), and adult (2 and 6 month old) rats were sacrificed and the binding of BSL-I and VVA to the vomeronasal nerve layer (VNL) and glomerular layer (GL) of the AOB was examined histochemically. In the adult, BSL-I bound to the whole VNL and GL, while VVA bound strongly to the posterior 2/3 of these layers but very weekly to the anterior 1/3 of them. During development the binding sites of VVA could be visualized earlier than those of BSL-I; the first day of VVA binding was at ED 18 and BSL-I was at PD 0. The staining pattern of two lectins found in adult AOB has been established by PD 14. These results suggest that the rat VN axons have two subpopulations in terms of binding sites to the two lectins and the binding sites are expressed differentially during development. (Supported by NIH Grants NS20699 and 1 R01DC01071-01, and a TMIN grant).

502.21

VOMERONASAL PATHWAYS ARE SELECTIVELY ACTIVATED DURING MATING BEHAVIOR IN MALE HAMSTERS. **G. Fernandez* and M. Meredith, Program in Neuroscience, Dept. Biol. Sci., Florida State Univ., Tallahassee, FL.**

Vomeronasal (VN) sensory input is important for male hamster mating behavior. The VN system projects via the accessory olfactory bulb (AOB) to the medial & posterior-medial cortical nuclei of the amygdala, the bed nucleus of the stria terminalis (BNST), and also to the medial preoptic area (MPOA), central structures shown to be important for reproductive behavior. In these experiments c-fos expression was used as a marker of neural activity to identify the pathways activated by mating behavior in intact animals, and in animals that had their vomeronasal organs removed (VNX). Sexually inexperienced male hamsters from each group were placed in clean boxes with fresh bedding and each exposed to a sexually receptive female for 45 mins. After an additional 45 mins they were perfused with 4% paraformaldehyde. Control animals from each group were put into clean boxes with fresh bedding and perfused 90 mins later. Fifty um vibratome sections were processed for immunocytochemistry using a polyclonal fos antibody. (Cambridge research). Results show a distinct difference in fos activation in stimulated animals compared to controls. Densely stained fos nuclei were evident in the AOB, medial amygdala, MPOA & BNST of stimulated animals. Unstimulated animals did not show this activation. VNX animals exposed to females did not mate, and had a dramatically reduced number of fos positive nuclei in all these areas. All animals (stimulated & unstimulated) showed activated nuclei in the main olfactory bulb and the paraventricular thalamus, suggesting that it is only the VN pathways and their central connections that are differentially activated as a result of mating. Ongoing studies involve double labelling for fos and LHRH to explore the participation of LHRH in the facilitation of mating behavior by VN sensory input. Supported by NIH Grant DC00906.

502.22

SEXUALLY DIMORPHIC DISTRIBUTION OF C-FOS IMMUNOREACTIVE CELLS IN THE MEDIAL NUCLEUS OF THE AMYGDALA AND MEDIAL PREOPTIC NUCLEUS MAGNOCELLULAR FOLLOWING EXPOSURE TO HAMSTER VAGINAL SECRETIONS. J.M. Fiber* and J.M. Swann, Institute of Animal Behavior and Dept. of Biol. Sciences, Rutgers Univ., Newark, NJ 07102.

Female hamster vaginal secretions (FHVS) strongly attract male hamsters, and stimulate copulatory behavior in males. FHVS do not have this effect on females. Using C-fos as a marker for cell stimulation, we previously reported stimulation in the bed nucleus of the stria terminalis (BNST), medial nucleus of the amygdala (M) and the medial preoptic area of male hamsters following exposure to FHVS. In this experiment we explored what regions within this mating behavior pathway of males differed in females in response to FHVS. Males, proestrus females and diestrus females were given a cotton swab with FHVS. Control animals received no stimulus.

Our results indicate that the BNST in both stimulated males (n=3) and proestrus females (n=3) showed similar distribution of C-fos immunoreactive cells. Medial preoptic nucleus magnocellular (MPN-Mgn) and M showed differential distribution of stimulated cells between males and proestrus females. Diestrus females (n=3) did not show stimulation in M or MPN-Mgn, and only one of the diestrus females showed some stimulation in the BNST. The differences between proestrus and diestrus females may be due to circulating steroid changes over the estrus cycle. Stimulated cells in M of the proestrus females are sparsely distributed throughout the nucleus whereas the stimulated cells in M of the males are clustered on the medial border of the posterodorsal part of this nucleus. Within MPN-Mgn of the female, stimulated cells are sparsely distributed within the ventromedial part of this nucleus. The MPN-Mgn of males has stimulated cells through the entire extent of the nucleus. In this experiment we have shown that MPN-Mgn and M are differentially stimulated in males and females in response to exposure to FHVS. These regions may regulate the sex difference in behavioral response to FHVS.

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502.23

SEX DIFFERENCES IN INDUCTION OF FOS IMMUNOREACTIVITY IN THE BRAINS OF PRAIRIE VOLES EXPOSED TO BEDDING SOILED BY CONSPECIFICS OF THE SAME AND OPPOSITE SEX. T.P. Goodness*, M.A. Novak, G.J. De Vries. Program of Neuroscience and Behavior and Dept. of Psychology, Univ of Massachusetts., Amherst, MA 01003.

Prairie voles show sex-specific responses to conspecific sexual odors. Female reproduction is stimulated by male odors and inhibited by female odors. Group-housed sexually experienced males show increased mounting behavior when exposed to odors of females in estrus. Little is known about which brain areas are stimulated when a vole is exposed to conspecific odors. We identified such brain areas with fos immunocytochemistry. Male and female prairie voles were exposed to male or female soiled, or clean bedding. Two hours following exposure, voles were sacrificed and their brains processed for fos immunocytochemistry. Fos immunoreactive (fos-ir) cell nuclei were counted in all mid- and forebrain areas that showed fos-ir nuclei in at least some vole brains. Significantly more fos-ir cells were found in the medial amygdala, antero- and posteromedial bed nucleus of the stria terminalis, and ventral premammillary nucleus of males and females exposed to bedding soiled by either sex. In the medial amygdala and antero medial bed nucleus, males and females showed more fos-ir cells when exposed to bedding soiled by opposite than by same sex. In the septohypothalamic nucleus, males but not females showed more fos-ir cells when exposed to bedding soiled by either sex. Although fos-ir cells were found in several thalamic areas, no significant differences were found between bedding conditions. These results indicate that there are sex differences in how brain areas respond to odors of the same or opposite sex. These sex differences may reflect the different responses of males and females to odprs of conspecifics.

503.1

OPPOSITE EFFECTS OF RESERPINE PRETREATMENT ON AMPHETAMINE-INDUCED AND COCAINE-INDUCED EXPRESSION OF ZIF268 AND C-FOS mRNAs IN RAT STRIATUM AND CORTEX. Moratalla, R.* and Graybiel, A.M. Massachusetts Institute of Technology, Department of Brain and Cognitive Sciences, Cambridge, Mass. 02139

In previous immunohistochemical experiments on the rat caudoputamen (CP), we found that amphetamine induces Fos-like immunoreactivity (FLI) in a striosome-predominant pattern in rostral CP, whereas cocaine induces FLI nearly equally in both compartments. We also found that induction of FLI by cocaine was nearly abolished by pretreatment with reserpine, whereas induction of FLI by amphetamine was not blocked and in fact was enhanced. Here we report dramatic differences in the effects of reserpine on induction of zif268 and cfos mRNA by amphetamine and cocaine in the rat striatum and cortex. Rats were treated with amphetamine (5 mg/kg) or cocaine (25 mg/kg) with or without pretreatment with reserpine (10 mg/kg, i.p., 18 hr before), and brains were processed 1 hr later for in situ hybridization. Induction of zif268 by cocaine was sharply reduced by reserpine pretreatment; zif268 mRNA levels appeared lower than in saline-treated controls, except in ventral striatum. Induction of zif268 by amphetamine was greatly elevated in reserpine-pretreated rats relative to rats treated with amphetamine alone. It is likely that the enhancement involved not only increased intensity of expression but also recruitment of new responsive cells especially in the matrix and lateral CP. Interestingly, the striosome-selective pattern of zif268 induction by amphetamine was scarcely visible after reserpine pretreatment. In situ hybridization experiments showed that cocaine induction of c-fos mRNA was also decreased, and amphetamine-induced expression of c-fos was also enhanced, by reserpine pretreatment. Supported by United Parkinson Foundation and NIH Javits RO1 NS25529.

503.2

COCAINE EXPOSURE THROUGH THE MATERNAL CIRCULATION SELECTIVELY INCREASES FOS-LIKE IMMUNOREACTIVITY IN CELLS OF DOPAMINE ISLANDS/STRIOSOMES OF EMBRYONIC STRIATUM. E. Fusco and A.M. Graybiel* Massachusetts Institute of Technology, Dept. of Brain and Cognitive Sciences, 45 Carleton St., Cambridge, MA 02139.

Cocaine, a monoamine uptake blocker with high selectivity for the dopamine transporter, acutely induces Fos-like immunoreactivity (FLI) in striatal neurons in adult and neonatal rats. In neonates, the response is selective for striatal cells in dopamine islands/striosomes (Johnson et al., 1992). We asked whether cocaine would induce FLI in embryonic striatum when given to pregnant mice and, if so, whether the induction would be selective for developing striosomes. We found that cocaine (50 mg/kg or 25 mg/kg, s.c.) induced intense expression of FLI in cells of proto-striosomes in E18/E19 mouse embryos exposed through the maternal circulation, and that a similar dopamine island/striosome selectivity of FLI induction occurred at E20/P0. FLI-positive patches were aligned with DARPP-32-positive patches. In embryos of saline-treated dams, weak FLI was also detectable in striosomes/dopamine islands. Interestingly, cocaine induced FLI in cells that were scattered through the E17/E18 embryonic caudoputamen. It has been proposed that future striosomal cells in rat embryos (identified by early projections to the substantia nigra by Fishell and van der Kooy, 1987) first are scattered (E18-E19) and later aggregate into patches corresponding to future striosomes. We propose that future striosomal cells may already have a functional phenotype (responsiveness to maternally transmitted monoamine stimulation) before they form patches, and that this phenotype persists through the period in which striosomal clusters are formed. (Supported by Monaise and Angus N. MacDonald 1946 Fund)

503.3

SELECTIVE MODULATION OF DYE-COUPLING IN RAT STRIATAL NEURONS *in vivo* BY D2 AGONIST ADMINISTRATION. S-P Onn*, T.W. Berger and A.A. Grace. Depts. of Behavioral Neuroscience & Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.

Using *in vivo* intracellular recording and Lucifer yellow staining, dye coupling in the striatum was observed in 17% (8/48) of the cells injected, which was twice that reported *in vitro* (9%; Cepeda et al., 1989). After i.v. apomorphine (0.1- 0.3 mg/Kg), we observed a significant increase in the incidence of dye-coupling (82%; 9/11) as well as an increase in the extent of coupling within sets of 3-7 cells often recovered after a single injection. It was unclear whether the apomorphine caused this enhancement of dye-coupling via a D1 and/or D2-specific action on striatal cells or by a presynaptic effect on dopamine (DA) terminals. Therefore, we examined the specificity of this response using the selective D1- or D2- specific DA agonists. Administration of quinpirole (2-3 mg/kg; i.v.) was found to increase the incidence of dye-coupling to 66% (6 out of 9 injections) and also resulted in dye-coupling among multiple cells in 3 cases, i.e., 3-5 cells were labelled. In contrast, administration of SKF 38393 (10 mg/kg; i.v.) did not significantly alter the incidence of dye-coupling from the control level (14%; 1/7). This apparent D2-specific enhancement of dye-coupling appeared to be mediated by a postsynaptic action of the drug instead of a decrease in D1 stimulation secondary to autoreceptor stimulation since a blockage of D1 receptors by administering the D1 specific antagonist SCH 23390 (10 mg/kg; i.v.) did not result in an increase in dye-coupling (0/5). Therefore, systemically applied dopamine agonists appear to exert different effects on dye-coupling within the striatum when compared to the results obtained by others *in vitro*, where DA depletions were reported to increase dye-coupling. It is unclear whether DA actions on other striatal afferent systems may have played a role in the response observed *in vivo*. (support by Tourette's Foundation grant, NS08288, NS19608, MH45156 & MH42217).

503.4

HETEROGENEOUS DISTRIBUTION OF 5-HT1C RECEPTOR mRNA IN STRIATUM AND SUBSTANTIA NIGRA. Z. Mikeladze and M-F Chesselet*. Dept of Pharmacology. University of Pennsylvania, Philadelphia, PA 19104.

The distribution of cells expressing the mRNA encoding the 5HT1C receptor was examined in the striatum and related areas by in situ hybridization histochemistry at the single-cell level. Sections from rat brain were hybridized with a ^{35}S-RNA probe complementary to the 3rd intracytoplasmic loop of the mRNA and processed for emulsion autoradiography as described (Chesselet et al. 1987). In the striatum, moderately labelled cells were observed in the ventral and ventrolateral part of the caudate-putamen and the rostral part of the nucleus accumbens. Labelled cells were less numerous in the caudal part of nucleus accumbens and virtually absent from dorsal caudate-putamen. Densely labelled cells were present in the subthalamic nucleus and the ventral pallidum, but not in globus pallidus. In the substantia nigra, 60% of neurons in both pars compacta and pars reticulata were densely labelled at caudal but not rostral levels. The data reveal a markedly heterogeneous distribution of 5HT1C receptor mRNA in striatum and substantia nigra, suggesting that this receptor subtype may be involved in regionally specific effects of serotonin in the basal ganglia. Supported by PHS grant MH48125. We thank D. Pritchett for the cDNA.

503.5

THE DEVELOPMENTAL MARKERS GAP-43 AND E-NCAM IN THE ADULT RAT STRIATUM: DISTRIBUTION AND EFFECTS OF CORTICAL LESIONS. F.G. Szele* and M.-F. Chesselet, Dept. of Pharmacology, U. of Pennsylvania, Philadelphia., PA 19104.

The expression of the Growth Associated Protein-43 (GAP-43) and the embryonic (polysialilated) form of the Neuronal Cell Adhesion Molecule (E-NCAM) is high during neuronal development but is limited to some areas capable of plasticity in the adult brain. The distribution of GAP-43 and E-NCAM was investigated with immunohistochemistry in the normal adult rat striatum and 5 days after thermocoagulatory lesions of the somatosensory cortex. Immunoreactivities to GAP-43 and E-NCAM were undetectable in most of the normal striatum except for its dorsomedial and medial parts where both proteins were found in topographically overlapping patterns. Cortical lesions did not result in changes in E-NCAM immunoreactivity but GAP-43 immunoreactivity was increased in the dorsolateral striatum, the area which receives inputs from the lesioned cortex. The data show that a discrete region of the striatum retains the markers of developing neurons GAP-43 and E-NCAM in adult rats. Lesions of cortical neurons projecting to a different striatal area result in increases in GAP-43 but not E-NCAM 5 days after surgery. Supp. by PHS grant NS 29230. We thank L. Benowitz and G. Rougon for the gift of antibodies to GAP-43 and E-NCAM, respectively.

503.6

EXPRESSION OF GAD (Mr 67,000) AND ITS mRNA IN BASAL GANGLIA AND CEREBRAL CORTEX AFTER UNILATERAL CORTICAL LESIONS IN RATS. P. Salin* and M-F Chesselet. Dept of Pharmacol., U. of Pennsylvania, Phila, PA 19104.

Glutamic acid decarboxylase (GAD) is present in most efferent neurons of the striatum and in interneurons both in the striatum and the cerebral cortex. We have examined the effects of lesions of the sensory-motor cortex by thermocoagulation of pial vessels in adult rats. Levels of GAD were measured in the striatum and its target areas with radioimmunohistochemistry and an antibody specific for GAD (Mr 67,000) (Kaufman et al. J. Neurochem.'91); levels of GAD mRNA were measured at the single-cell level by in situ hybridization histochemistry with a ^{35}S-RNA probe complementary to the corresponding mRNA (Kaufman et al. Science, '86). Five days after surgery, GAD immunoreactivity was markedly increased in striatal target areas on the side of the lesion. In the striatum, increases in immunoreactivity were small at 5 days, larger at 21 days, and accompanied by an increase in mRNA levels lasting up to 3 months after surgery. In contrast, in frontal cortex contralateral to the lesion, levels of GAD mRNA per neuron were decreased 21 and 90 days after surgery. The results suggest that local ischemic lesions of cerebral cortex in adult rats lead to prolonged and opposite alterations in GAD synthesis in basal ganglia and contralateral cortex. We thank A.J. Tobin UCLA for the gift of antibody and cDNA. Supp. by PHS grant NS 29230.

503.7

EFFECT OF EXCITATORY AMINO ACIDS ON DOPAMINE AND SEROTONIN RELEASE IN THE RAT STRIATUM. T. Nakazato[1],* and A. Akiyama[2], [1]Dept. Physiol., Juntendo Univ. School of Med., Tokyo, [2]Dept. Electrochem., Tokyo Inst. of Tech., Yokohama, Japan.

This study was designed to investigate whether dopamine (DA) and serotonin (5-HT) are released in the rat striatum by excitatory amino acid using microcomputer-controlled in vivo voltammetry. Glutamate (Glu), NMDA, quisqualate (Quis) and kainate (KA) were administered intrastriatally, extracellular releases of DA were measured every 3 min in freely-moving rats. Glu (10^{-3} M, 6 µl, 24 min) and NMDA (10^{-5} M, 6 µl, 24 min) were intrastriatally injected, DA was released soon after the start of the injection. However, 5-HT release was not found. NMDA-mediated DA release was significantly suppressed after the administration of APV (10^{-5} M). Quis (10^{-5} M) was also administered, DA release increased in almost the same time course as in NMDA. CNQX suppressed Quis-mediated DA release. KA was injected, DA release was small, although in 2 of the 5 cases releases of DA and 5-HT were much increased after the injection. This suggests that 10^{-5} M of KA sometimes induces striatal lesion. In conclusion, DA release in the striatum is increased by drugs in the following order: NMDA = Quis > KA.

503.8

SUBSTANCE P INCREASES EXTRACELLULAR ACETYLCHOLINE IN RAT STRIATUM. J.J. Anderson*, T.M. Engber, and T.N. Chase, Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

Neurons projecting from striatum to substantia nigra contain large amounts of the tachykinin substance P. These substance P-containing striatonigral neurons also possess axon collaterals which branch within the striatum. Recent evidence suggests that receptors for substance P (neurokinin-1) are selectively expressed in cholinergic neurons in the striatum (Brain Research 556:165, 1991). The relationship between substance P and extracelluar acetylcholine in striatum was examined using microdialysis in awake behaving rats. Rats were implanted with chronic guide cannulae and after at least a three day surgical recovery period, microdialysis probes were inserted through the guide and into striatum. The acetylcholinesterase inhibitor neostigmine (10 uM) was included in the perfusion solution to increase the recovery of acetylcholine. Following a baseline stabilization period and collection of control dialysates, 100 uM substance P was perfused through the probe. The concentrations of acetylcholine and choline in collected dialysates were analyzed by HPLC and electrochemical detection. Substance P significantly increased acetylcholine concentrations from baseline levels in striatal dialysates. In contrast, choline levels were not elevated relative to control concentrations. These results suggest that substance P stimulates release of acetylcholine in the striatum and support anatomical evidence suggesting the presence of substance P receptors on cholinergic neurons in the striatum.

503.9

NBQX AND MK-801 DIFFERENTIALLY MODIFY REGIONAL CEREBRAL METABOLIC RESPONSES TO L-DOPA. T.M. Engber*, J.J. Anderson, R.C. Boldry, S.M. Papa, S. Kuo and T.N. Chase. Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

Excitatory amino acid receptor (EAA) antagonists have been proposed as potential antiparkinsonian agents, either alone or in combination with L-Dopa. We used the 2-deoxyglucose autoradiographic technique to examine the neural substrates for the interaction between L-Dopa and antagonists of either the AMPA or NMDA type of EAA receptor. Thus, we compared the effects of the AMPA antagonist NBQX (10 mg/kg, i.v.) and the NMDA antagonist dizocilpine (MK-801; 0.1 mg/kg, i.v.) on regional cerebral metabolic responses to L-Dopa (25 mg/kg, i.v. with 12.5 mg/kg benserazide) in rats with a unilateral 6-hydroxydopamine lesion of the nigrostriatal pathway. L-Dopa increased glucose utilization in substantia nigra pars reticulata and entopeduncular nucleus and decreased metabolic rate in lateral habenula; NBQX and MK-801 by themselves did not affect glucose utilization in these regions. Pretreatment with NBQX reduced the effect of L-Dopa in substantia nigra pars reticulata but not in entopeduncular nucleus, while MK-801 attenuated the effect of L-Dopa in both of these striatal output regions. Neither NBQX nor MK-801 altered the effect of L-Dopa in lateral habenula. These findings indicate that AMPA and NMDA antagonists differentially modify dopamine receptor-mediated striatal output. AMPA receptor blockade appears to reduce dopaminergic stimulation of the striatonigral but not the striatoentopeduncular pathway, while NMDA blockade appears to reduce dopaminergic stimulation of both of these striatal output pathways.

503.10

POTENTIATION OF SKF 38393-INDUCED ROTATIONAL BEHAVIOR BY MK-801 IS DEPENDENT UPON PREVIOUS DRUG EXPOSURE. R.C. Boldry*, T.N. Chase, and T.M. Engber Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

Directly acting dopaminergic agonists produce contralateral rotations in rats with a unilateral 6-hydroxydopamine lesion of the nigrostriatal pathway. Previous studies have shown that the noncompetitive NMDA antagonist dizocilpine (MK-801, 0.1 mg/kg i.p.) can potentiate the stimulation of rotational behavior produced by the dopaminergic D-1 agonist SKF 38393 (1.5 mg/kg s.c.), and inhibit the stimulation of rotation produced by the D-2 agonist quinpirole (0.1 mg/kg i.p.) (Morelli et al. JPET 260:402-408, 1992). We have confirmed that MK-801 decreases the stimulation of rotation produced by quinpirole regardless of previous drug exposure and increases the rotational response to SKF 38393 when given three days after screening with a single high dose of L-DOPA (50 mg/kg with 30 mg/kg benserazide, i.p.). However when MK-801 is co-administered with SKF 38393 one week after screening with a single low dose of apomorphine (0.05 mg/kg s.c.), no potentiation of the rotational response is observed. This negative finding at one week after apomorphine is independent of the dose or route of administration of MK-801. Since rats which have been treated 3 days previously with L-DOPA display significantly less rotational behavior than those treated 1 week earlier with apomorphine, we have concluded that MK-801 can reverse the suppression of rotational behavior caused by recent exposure to L-DOPA.

503.11

MK-801 DIFFERENTIALLY MODIFIES REGIONAL CEREBRAL METABOLIC RESPONSES TO D1 AND D2 DOPAMINE AGONISTS. S.M. Papa*, J.J. Anderson, R.C. Boldry, S.Kuo, T.N. Chase and T.M. Engber, ETB, NINDS, NIH, Bethesda, MD 20892.

Dopamine and excitatory amino acids play important roles in the basal ganglia control of motor behavior; elucidating the manner in which these transmitters interact may provide new therapeutic approaches to the treatment of Parkinson's disease. We used the 2-deoxyglucose (2-DG) autoradiographic technique to examine the effect of the NMDA antagonist dizocilpine (MK-801) on regional cerebral metabolic responses to D1 and D2 dopamine agonists in rats with a unilateral 6-hydroxydopamine lesion of the nigrostriatal pathway. The D1 agonist SKF 38393 (5 mg/kg, i.v.) increased glucose utilization in substantia nigra pars reticulata and entopeduncular nucleus. The D2 agonist quinpirole (1 mg/kg, i.v.) was without effect in these regions, but decreased 2-DG uptake in the nucleus accumbens. Pretreatment with MK-801 (0.1 mg/kg, i.v.), which had little effect on cerebral metabolism by itself, reduced the effect of SKF 38393 in both substantia nigra pars reticulata and entopeduncular nucleus and prevented the effect of quinpirole in the nucleus accumbens. Both SKF 38393 and quinpirole decreased glucose utilization in the lateral habenula. MK-801 pretreatment did not alter the effect of SKF 38393 in the lateral habenula but substantially reduced the effect of quinpirole in this structure. These results indicate that D1 and D2 receptor-regulated brain mechanisms are differentially influenced by NMDA receptor stimulation. D2-mediated cerebral metabolic responses appear to require concurrent NMDA receptor stimulation, while D1 receptor-regulated neuronal pathways exhibit varying degrees of sensitivity to NMDA receptor blockade.

503.12

IMMUNOCYTOCHEMICAL STUDY OF NONPHOSPHORYLATED NEUROFILAMENT PROTEIN DISTRIBUTION IN MONKEY BASAL GANGLIA. M.J. Campbell*, Mt. Sinai, NY, NY 10029

SMI-32 (Sternberger Monoclonal Inc.), is a monoclonal antibody that avidly recognizes the KSP segment of NF-H and weakly recognizes the KSP2 segment of NF-M in their nonphosphorylated state (J. Neurosci. Res. 30:47). Unlike some NFP antibodies it lacks cross-reactivity to the microtubule associated proteins (PNAS 85: 1998, & PNAS 84: 3410). In studies of primate neocortex, SMI-32 labels primarily the soma and dendrites of a subpopulation of pyramidal neurons whose distribution differs markedly across cortical areas (eg., J. Comp. Neurol. 282:191). This examination of the basal ganglia reveals striking differences between the striatal and pallidal compartments. The caudate and putamen are relatively unique brain regions in that there is an absence of immunoreactive neurons. Rather, there is a dense staining of the neuropil which exhibits some regional variation (generally, the dorsal striatum is more densely stained than the ventral striatum), but importantly it exhibits variations in intensity that correspond to the patch/striosome and matrix organization of the striatum as revealed by AchE or calbindin immunoreactivity for example (PNAS 75:5723 & PNAS 82:8780). In contrast in pallidal segments, a large subpopulation of neurons and their extensive dendrites are immunoreactive. These observations suggest that one or more of the terminal projections delineating a subcompartmentalization of the striatum also requires this form of NFP. Whereas, in the pallidum, as in the neocortex, it is a cytoskeletal specialization in the soma and dendrites of a large subpopulation of projection neurons, despite differences in neurotransmitter used by neurons in these two regions (GABA versus excitatory amino acids).

503.13

5'-NUCLEOTIDASE ACTIVITY IN THE DEVELOPING CAUDOPUTAMEN: ASSOCIATION WITH DOPAMINE ISLANDS AND STRIOSOMES IN RAT, BUT EXTRASTRIOSOMAL MATRIX IN MOUSE. S.W. Schoen* and A.M. Graybiel, Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139

In the striatum, patchy compartments (the striosomes and their developmental forerunners, the dopamine islands) can be distinguished from the surrounding tissue (the extrastriosomal matrix) by their differential content of numerous neurochemical compounds. The adenosine-producing ectoenzyme 5'-nucleotidase (5'N) is a new marker for striosomes in the mature rat caudoputamen, revealed with a histochemical lead technique (Schoen and Graybiel, Soc. Neurosci. Abstr. 17, 452). On the basis of serial-section comparisons with the distributions of tyrosine hydroxylase and calbindin-D_{28k} immunoreactivity (marking dopamine islands and matrix), we show here that 5'N is selectively enhanced in the neuropil of dopamine islands/striosomes of the rat caudoputamen from postnatal day 1 to adulthood. This holds for all but the caudal caudoputamen. In mouse, by contrast, 5'N activity is associated with extrastriosomal matrix of the anterior and middle caudoputamen from embryonic day 18 until postnatal day 21; rarely, zones of enhanced 5'N reaction product overlap with dorsally located dopamine islands. In adult mice, most of the caudoputamen is filled with dense histochemical reaction product, but rostral striosomes remain visible by low 5'N activity. These results indicate a converse enzyme architecture of the caudoputamen in rat and mouse. As 5'N is associated with glia and malleable synapses, this enzyme could reflect specific glial compartmentalizations or sites of synaptic plasticity within striatum. Roles of 5'N in purinergic neuromodulation and cellular contact-formation may contribute to activity-dependent or adhesive mechanisms underlying the differentiation and maintenance of the striosome and matrix compartments. Supported by NIH (RO1 NS25529) and DFG.

503.14

GRADIENTS IN THE DOPAMINE-REGULATED SYNTHESIS OF NEUROPEPTIDES AND DOPAMINE RECEPTORS IN RAT NUCLEUS ACCUMBENS. P.Voorn* and G.J. Docter. Dept. of Anatomy and Embryology, Vrije Univ., Amsterdam, the Netherlands.

Quantitative in situ hybridization histochemistry was used to study the effects of unilateral 6-hydroxydopamine lesions of the ascending dopaminergic fibers on the synthesis of enkephalin (Enk), dynorphin (Dyn), substance P (SP) and the dopamine D-1 and D-2 receptors in projection neurons in subregions of the nucleus accumbens (Acc) and in the caudate-putamen (CP). In CP of control animals a systematic increase of mRNA along the rostrocaudal axis was found for Dyn and D-1, whereas in Acc a decrease in mRNA was noted in the rostral to caudal direction for all three neuropeptides and the two dopamine receptors. Two weeks after the lesion an increase was found in Enk and D-2 mRNA, both in CP (Enk +130%, D-2 +36%) and in Acc (Enk +73%, D-2 +13%), in the lesioned side compared to the non-lesioned side. A decrease was observed for Dyn, SP and D-1, which was the same in CP and Acc for D-1 (-15%) and Dyn (-58%), and slightly higher for SP in CP (-44%) than in Acc (-39%). The adaptive changes in mRNA levels appeared to be proportional along the rostrocaudal axis for all neuropeptides and receptors except for Enk. For Enk mRNA the increase in Acc was rostrally higher than caudally, indicating regional differences in the effects of blockade of the dopaminergic neurotransmission.

503.15

CHARACTERIZATION OF A NEW BRAIN-SPECIFIC PROTEIN TYROSINE PHOSPHATASE IN RAT STRIATUM AND CEREBRAL CORTEX. J. R. Naegele*, M. Lerner. and P. J. Lombroso. Dept of Biology, Wesleyan University, Middletown, CT 06457 and Child Study Center and the Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510.

Protein tyrosine phosphatases (PTPs) act in opposition to tyrosine kinases to regulate phosphorylation of tyrosine residues. These two classes of enzymes are implicated in cell differentiation but may have additional functions in mature cells. We have explored the possibility that different PTPs might be present within functionally distinct regions of the brain. A novel intracellular PTP was cloned from a rat striatal cDNA library (Lombroso et al. '91, PNAS 88: 7242-7246). Northern analyses of brain revealed a 3 kb and a 4.4 kb mRNA. The 3 kb mRNA was highly enriched in the striatum relative to other brain regions and was termed STriatal Enriched Phosphatase (STEP). An amino acid consensus sequence found in all protein tyrosine phosphatases and tyrosine phosphatase activity identified STEP as a PTP. Rabbit polyclonal antisera against STEP recognized a major band of 46 kDa and minor bands of 37 and 33 kDa on Western blots of adult striatum. In sections of fixed rat brain, STEP antiserum stained striatal neurons and neuropil. In cerebral cortex, a subset of neurons were stained in layers 2+3, 5 and 6. Staining was also found within the neuronal subsets in hippocampus and lateral septal nucleus. These findings suggest the existence of other brain specific PTPs showing regional heterogeneity.

503.16

CHOLINERGIC REGULATION OF SUBSTANCE-P/NEUROKININ A GENE EXPRESSION IN RAT STRIATUM L.R. Lucas* and R. E. Harlan. Neuroscience Training Program, Tulane Univ. Sch of Med., New Orleans, LA 70112.

Cholinergic interneurons and substance P/neurokinin A medium spiny projection neurons comprise 5% and 40%, respectively, of the total number of neurons in the rat caudate-putamen (CPu). Since the influence of cholinergic interneurons on neuropeptidergic projection systems in the striatum is poorly understood, this study explores the relationship between cholinergic receptor activation or inhibition on tachykinin gene expression.

Adult male Sprague-Dawley rats were treated chronically either with a cholinergic agonist (physostigmine: 0.5mg/kg/3Xday), a muscarinic antagonist (scopolamine HCl: 0.4mg/kg/3Xday), or vehicle (PBS: 0.1ml/100g) administered for 6 days (s. c.). Rats were perfused with 3% paraformaldehyde and coronal cryostat sections (20μm) were cut and mounted on Vectabond (Vector) coated slides. *In situ* hybridization was performed with a full-length (560nt) ribonucleotide probe directed against ß-ppt (a transcript containing substance P, neurokinin A, and other tachykinins). Physostigmine administration resulted in a small decrease in tachykinin expression in the CPu and olfactory tubercle, while scopolamine treatment resulted in an increase in expression in both regions, as compared to vehicle treated animals. The increases after scopolamine treatment appeared to be greatest ventrolaterally in the CPu, paralleling the distribution of ChAT-mRNA positive cells. The results suggest that acetylcholine in the striatum may regulate levels of tachykinin expression in a manner opposite to that observed with dopamine. Supported by NS24148.

503.17

STRIATAL c-*fos* EXPRESSION AND D1/D2 SYNERGISM. G.J. LaHoste*[1,3], Jen Yu[1,3] & J.F. Marshall[2]. Depts. of [1]Phys. Med. & Rehab. and [2]Psychobiology, University of California, Irvine, CA 92717, and [3]State Developmental Research Institutes.

Dopamine (DA) agonists have been shown to induce c-*fos* gene expression in the caudate-putamen (CPu) of rats. To test for a synergistic interaction between D1 and D2 receptors in DA-mediated c-*fos* expression, we injected neurologically intact rats with either the D1 agonist SKF 38393 (20 mg/kg), the D2 agonist quinpirole (3 mg/kg), the combination of both SKF 38393 and quinpirole, or saline. Two hours later, rats were perfused (4% paraformaldehyde) and the brains prepared for immunohistochemical visualization of Fos, the protein product of the c-*fos* gene using Cambridge Research Biochemicals antibody OA-11-823 (1:1000). Striatal Fos immunoreactivity (Fos-ir) was virtually undetectable in rats injected with saline, SKF 38393, or quinpirole. In rats given the combination of D1 and D2 agonists, however, patches of nuclear Fos-ir were readily detectable, particularly in the posterior CPu. In a second experiment, rats were given daily injections of vehicle or reserpine (1 mg/kg), a treatment that results in a breakdown in D1/D2 synergism. On the fifth day, they were injected with either SKF 38393 (20 mg/kg) or saline and perfused 2 hours later. SKF 38393 alone elicited pronounced Fos expression homogeneously throughout the CPu of rats pre-treated with reserpine, but not vehicle. The 5-day regimen of reserpine treatment did not itself elicit Fos expression. These data suggest that DA-mediated c-*fos* expression is a histochemical indicator of the state of D1/D2 synergism.

504.1

DIFFERING CLIMBING FIBER LENGTH IMPLIES VARIABLE CONDUCTION VELOCITY TO ESTABLISH ISOCHRONICITY OF CLIMBING FIBER CONDUCTION TIMES. I. Sugihara*, E. J. Lang, C. I. de Zeeuw and R. Llinás. Dept. of Physiology & Biophysics, New York University Medical Center, 550 First Avenue, New York, N.Y. 10016.

Previously we demonstrated that the climbing fiber conduction times are tuned closely to 4.0 ms in lobule crus 2a regardless of path length from the inferior olive (IO) to the cerebellar cortex, and that the rostro-caudal banding pattern of synchronous spontaneous complex spike (CS) activity in Purkinje cells (PCs) extends down the side of the cerebellar folia (Soc. Neurosc. Abs., 1990). We investigated this isochronicity further in ketamine-anesthetized adult rats. Conduction times for the other hemispheric and vermal lobules were also found to be about 4.0 ms (4.0 ± 0.4, mean $\pm$ SD, n=660 cells). Although CS activity in the vermal and hemispheric PCs are not strongly correlated in control condition, systemic application of picrotoxin can increase synchronicity of their CS activity by increasing the electrotonic coupling between olivary cells. In that situation, the CS activity of vermal and hemispheric PCs, recorded simultaneously using a multi-electrode technique, are synchronous to within 1 msec as determined from the peak of the cross-correlograms. To investigate the basis of CS isochronicity, PHA-L injections into the IO were made to visualize the climbing fibers to the vermis and hemisphere. The coordinates of the center of the stained fiber bundles in 90 µm thick coronal sections were measured and reconstructed in three-dimensional space. The length of the fiber bundles to the vermis (zone A) ranged from 9.5 to 14.0 mm while the bundles to the hemisphere (zone D) were shorter, 7.0 to 11.0 mm. These results further confirm the finding that the conduction velocity of a climbing fiber is correlated to its length so as to establish isochronic conduction times. Supported by NIH grant NS13742.

504.2

BISTABLE DISCHARGE PROPERTIES OF PURKINJE CELLS IN THE *IN VITRO* TURTLE BRAIN. R.R. Carter*, Y. Kwon, S.B. Arnold, R.R. Matsumoto, and J.C. Houk. Department of Physiology, Northwestern University School of Medicine, Chicago, IL 60611.

Purkinje cells of the cerebellar cortex were recorded extracellularly in the *in vitro* turtle brain preparation. The spontaneous activity of these cells was examined for evidence of bistable states of discharge. One group of Purkinje cells demonstrated sudden onsets and offsets of bursts of action potential discharge interspersed between periods of silence. Both the ON periods and OFF periods were typically 2-30 s. When in the ON state a given cell's inter-spike-interval varied as much as 20% with the mean rate between 10-60 imp/s. However, for each Purkinje cell the mean ON discharge rate was typically repeatable to within 10% for successive bursts. Thus the Purkinje cells functioned as bistable units. In another group of Purkinje cells the mean discharge rate in the ON state began at 60-80 imp/s and increased to about 100 imp/s at which point the ON period was punctuated by a series of several short bursts and pauses.

Two series of experiments suggest that the presence of two stable states of discharge and the cycling between them are due to intrinsic Purkinje cell properties. First, we recorded both groups of Purkinje cells in the isolated cerebellum and thus in the absence of influence from other brain regions or peripheral feedback. Second, when synaptic transmission was blocked by altering the bathing solution to contain high Mg^{2+} and low Ca^{2+} concentrations, we again recorded both groups of Purkinje cells although in some cases the timing of the cycling was altered.

It is possible, however, for external influences to take advantage of the intrinsic properties of the Purkinje cells. Experiments have indicated that single pulses of electrical stimulation delivered to the spinal cord often caused a unit in the OFF state to switch to the ON state, and, occasionally, a unit in the ON state to switch to the OFF state. These results are consistent with theories suggesting that Purkinje cells may contribute to the stable, controlled expression of motor programs and that peripheral feedback may modulate a motor program through influence of Purkinje cells.

504.3

QUALITATIVE DYNAMICAL MODEL OF BISTABILITY IN PURKINJE CELL DENDRITES. G. L. F. Yuen*, P. E. Hockberger, L. E. Massone and J. C. Houk. Dept. of Physiology, Northwestern University Medical Center, 303 E. Chicago Ave., Chicago, IL 60611-3008.

Previous computational and electrophysiological studies suggest that cerebellar Purkinje cells may function as bistable elements with hysteresis in the context of movement control (see Carter et al, this meeting). To develop more realistic models of this bistability, a phase-plane analysis was carried out using recent whole cell and single channel data. In particular, combinations of calcium and potassium channels were introduced into a dendritic compartment [with reasonable impedance loads] and the possibility of generating bistability was investigated.

Preliminary results from the use of high-threshold calcium and delayed rectifier potassium channels, which have been described in these neurons, suggest that they can support bistability in a dendritic compartment. In this model, the dynamic state variables are membrane potential and potassium channel activation. Calcium activation is assumed to be instantaneous and a sigmoidal function of membrane potential. The nullclines of the state variables have three intersections or equilibrium points, of which two are stable (representing the excited and the rest state) and one is unstable. State transitions can be induced by either short or long current pulses. For example, a depolarizing current pulse can send the system into the excited state (at a higher membrane potential) where it will remain stably until a hyperpolarizing pulse returns it to the resting state. A larger stimulus was needed to effect the {excited->rest} transition compared to excitation {rest->excited}. Thus the voltage-current relationship exhibited both bistability and hysteresis in that different stimulus thresholds were observed for excitation and recovery. These properties may allow Purkinje cells to participate in delayed feedback pathways without causing system instability.

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504.4

CEREBELLAR CONTROL OF CONDITONED EYEBLINK TIMING. G.T. Bartha* and R.F. Thompson. Neural, Informational, and Behavioral Sciences Program, University of Southern California, Los Angeles, CA 90089.

It is of general significance for sensorimotor control that rabbits can learn to delay peak nictitating membrane/eyeblink responses from 100 ms to over one second from the time of conditioned stimulus (CS) onset. This delay is adaptive in that the peak eyeblink amplitude occurs near the time of the unconditioned stimulus (US) onset used in training. Our objective is to develop a new model of conditioned eyeblink timing that is consistent with the extensive data on the synaptic organization and physiology of the underlying neural circuitry.

We propose that the cerebellum is the major contributor to adaptive timing of eyeblink responses for CS-US intervals less than 500 ms. The primary mechanism subserving the delay is prolonged feedback inhibition of granule cells by Golgi cells. This depresses the firing of the Purkinje and basket cells they tonically activate. Purkinje cells inhibited by these basket cells, but activated by another population of granule cells, increase their activity. Appropriate integration of depressed and excited Purkinje cells by interpositus neurons yields adaptively delayed responses. Our hypothesis implicates Purkinje, interpositus, and basket cells as sites of plasticity.

Computer simulations demonstrate that our model accounts for the single neuron activities observed experimentally in trained rabbits. The simulated cerebellar circuitry is constructed using conductance based, single compartment neuron models.

Our model is among the most biologically plausible accounts of eyeblink timing. The model is also a rare example of how a single neuron property beyond simple leaky integrator models can be crucial for behavior. The timing mechanism could apply to all conditioned somatic motor responses and may be a general cerebellar function.

Supported by ONR grant N00014-88-K-0112 to R.F. Thompson.

504.5

SIMULATION OF CEREBELLAR-VESTIBULAR INTERACTIONS DURING VOR ADAPTATION. K.J. Quinn*, J.F. Baker and B.W. Peterson. Northwestern Univ., Chicago, IL 60611

Both brainstem and floccular sites have been proposed as principal locations for plasticity resulting in vestibulo-ocular reflex (VOR) adaptation. In each case, specific predictions have been made concerning the nature of the error signal required for the adaptation process. Here we explore the computational characteristics of simple neural network models simulating these locations and conditions. We constructed a network based on the original formulation of Miles and Lisberger (Ann.Rev. Neurosci.,1981,4,273) and tested its ability to generate normal and adapted eye velocity signals in response to a variety of head velocity step inputs. Tuning the network using an unphysiological global optimization method indicated that the most accurate adapted response occurs when synaptic weights are allowed to change at both sites.

Turning to more physiological learning rules, we found that using output of gaze-velocity purkinje cells isn't effective as a teacher signal for driving plasticity at brainstem locations. Rather, it leads to wildly oscillating behavior because this output is part of a positive feedback loop. Optimal adaptation occurred when retinal slip velocity, representing output of the accessory optic system, was used to directly instruct changes in vestibular input weights at brainstem and cerebellar sites using a Widrow-Hoff learning rule. Unsupervised Hebbian rules are inadequate and cause the system to adapt to inappropriate gains. This finding on learning rule efficacy confirms for a specific instance the general observations of Sutton and Barto (Psych.Rev., 1981,88,135). Supported by EY06485, EY07342, DC01559.

504.6

A NEW TREATMENT OF SPATIO-TEMPORAL STRUCTURE IN ARRAY RECORDING OF THE RAT INFERIOR OLIVE.

A. Sivaramakrishnan, E. J. Lang, I. Sugihara, S. Sivaramakrishnan*† & R. Llinás, Department of Physiology and Biophysics, New York University Medical Center, 550 First Avenue, New York NY 10016 & † Biology Department, 139-74, California Institute of Technology, Pasadena CA 91125.

Statistical tools developed for data with continuous or many-valued data are often inappropriate for binary data that may be the best representation of neural on/off states. We present an approach that incorporates a non-linear prefiltering of data from microelectrode array recordings of Purkinje cell complex spikes from the surface of the rat cerebellar cortex, followed by calculation of a correlation-type statistic that is tailored to binary data. This statistical approach appears to be better in many ways than the traditional forms of correlation. Data from such recordings are known to show a 10Hz oscillation and rostrocaudal banded patterns of Purkinje cell climbing fiber activation that seem to form the "grammar" of neuronal organization of the inferior olivary nucleus, the site of origin of the climbing fiber system. This statistical approach is robust in the sense that it is not affected by the considerable variation in the corresponding 100ms "periods" between spike trains. The method shows structure in the data that is often washed out by the usual approaches of autocorrelation and cross-correlation. (NINCDS-13742)

504.7

MICROCIRCUIT ASSOCIATIVE MEMORY MODEL OF THE CEREBELLAR CORTEX. Coe F. Miles and H. B. Nudelman*, Stuttering Center & Speech Motor Control Laboratory, Department. of Neurology, The Methodist Hospital/Baylor College of Medicine, Houston, Texas, 77030.

In this work, information and analytical techniques from the fields of biology, mathematics, computer science, and engineering are used to model the information processing characteristics of the mammalian cerebellar cortex. While previous studies have provided a foundation upon which to model and analyze the functional characteristics of the cerebellum, they do not account for many recently discovered physiological and structural aspects of the cerebellum. This work extends these earlier efforts by formalizing putative computational operations using mathematics.

By viewing anatomically different neurons as representing network elements whose input-output functions are different, a mechanism for distributing information throughout the memory is proposed. The functional circuitry developed here to implement this feature is called the MicroCircuit. The MicroCircuit provides a means of combining sensory input signals from different modalities. Unique to the MicroCircuit construct is its use of interneuron arborization patterns to establish intermodel communication links and a proposed functional role for climbing fiber activation during memory recall.

Interconnected MicroCircuits form the MicroCircuit Associative Memory (μAM) architecture, which can be viewed as a distributed associative memory or as a sparsely interconnected feed forward neural network. Key features of the μAM include 1) its ability to manipulate very large input patterns, 2) its distributed storage of input data patterns, and 3) its statistical reconstruction of stored patterns during memory read operations.

Analytic results describe the μAM's read and write operations, pattern fidelity, capacity and immunity to noise. Computer simulations, using a 4096 processor Connection Machine, are used to verify our theoretical predictions.

504.8

REALISTIC COMPUTER SIMULATIONS OF MEDIAL VESTIBULAR AND DEEP CEREBELLAR NUCLEI NEURONS. R. Quadroni, J. Simonet and Knöpfel, T.*. Federal Institute of Technology, Dep. of Physics, CH-8093 Zürich and Brain Research Institute, University of Zürich, CH-8029 Zürich.

We have developed a user-friendly program running on UNIX workstations for computer simulations of single nerve cells, involving an X-window-based editor permitting design and variation of morphological and membrane parameters. An integration routine based on an implicit second order algorithm allowed fast and stable integration of coupled differential equations describing the kinetics of ion-channels as well as concentrations, diffusion and buffering of Ca^{2+}.

We have designed compartmental models of Type A and B guinea pig medial vestibular nuclei neurons (Serafin et al., Exp. Brain Res. 84:417-433) and of rat deep cerebellar nuclei neurons. Compartments comprise up to eight active ionic conductances (g_{Na}, $g_{Ca(LVA)}$, $g_{Ca(HVA)}$, $g_{K(DR)}$, $g_{K(A)}$, $g_{K(AHP)}$, $g_{K(C)}$ and g_Q) whose kinetics were obtained from voltage-clamp studies in a variety of preparations. Synaptic inputs of the AMPA-, NMDA- and $GABA_A$-type were simulated by appropriate changes in conductance. Some kinetic parameters as well as distribution and density of ion-channels were modified to faithfully reproduce the responses of living neurons as revealed in current-clamp experiments.

HUMAN COGNITION II

505.1

HAPTIC INFORMATION TRANSFER DEFICITS ASSOCIATED WITH CLOSED HEAD INJURY: DOES CALLOSAL DYSFUNCTION DISCONNECT ACCESS TO THE MEMORY OF A TACTILE STIMULUS? C.M.Fernandez-Carol* and B.D.Fantie, Human Neuropsychology Laboratory, The American University, Washington, DC.

Neuropathological studies have consistently indicated there is disproportionately severe damage to the corpus callosum in closed head injury (CHI) and that this can occur when the trauma seems relatively minor. To determine if we could detect callosal dysfunction in the interhemispheric processing of tactile manual stimuli, we employed a haptic information transfer test that required subjects to match small patterns of pinheads utilizing only their sense of touch. Each person used either the same hand (Right-Right and Left-Left) or opposite hands (Right-Left and Left-Right) to examine a model stimulus and find its matches from within an array of distractors. One phase of the experiment (Simultaneous) allowed participants to leave one hand on the model while searching with the other, or, in the 1-hand condition, return to the model as often as desired. Another phase required that the subject search for matches from memory only (Successive).

In the Simultaneous condition, the performance of the CHI group (n=30, mean age=27.4 years) did not differ significantly from that of Controls (n=20, mean age=24.8 years) although there appeared to be a trend for the CHI group to make more errors than Controls in the 2-hand condition. In the more memory-dependent Successive condition, the CHI group was significantly slower and made more errors than Controls when searching for targets with the right hand. We propose that this pattern of results is compatible with a retrieval deficit resulting from a disconnection between the left hemisphere's motor control of the right hand and transcallosal access to the representation of the target stimulus presumably encoded spatially in the right hemisphere.

505.2

SPECIFICITY OF INTERHEMISPHERIC TRANSFER FOLLOWING A PARTIAL LESION OF THE CORPUS CALLOSUM. K. Baynes, M.J. Tramo,† R. Fendrich,*†† A. G. Reeves, and M.S. Gazzaniga,††
Program in Cognitive Neuroscience, Dartmouth Medical Schoool, Hanover, NH 03755, † Dept. of Neurobiology, Harvard Medical School, Boston MA , and ††Center for Neurbiology, UC Davis, Davis, CA.

Evidence is presented to demonstrate partial sparing of somesthetic transfer with relatively intact visual transfer following ischemic infarction of the central four fifths of the corpus callosum, most of the right calcarine cortex, right hippocampal region, and right posteriomedial parietal lobe. Intermanual judgments of weight and texture were accurate, but those concerning the location of tactile stimulations were not. The right hemisphere was able to build representations of objects via the tactile modality that could be used for tactile matching, but could not be accessed consistently for naming or auditory comprehension. Palpated objects could be matched to pictures with a left hand pointing response. These findings indicate that 1) somesthetic information within the right hemisphere could be used to perform unimodal and crossmodal intra- and inter-hemispheric recognition tasks, 2) transfer of somesthetic information within the right hemisphere to left hemisphere language areas was disrupted, and 3) different regions of the callosum mediate the transfer of somesthetic information about texture vs. tactile location.

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505.3

SELECTIVE IMPAIRMENT OF CONCEPT RETRIEVAL THROUGH A TACTILE CHANNEL. H. Damasio,* D. Tranel, U. Bellugi, A.R. Damasio and J.P. Brandt. Div. of Behav. Neurol. & Cogn. Neurosci., Univ. Iowa Col. of Med., Iowa City IA 52242.

In a recognition task in which 215 objects from varied conceptual categories were presented visually, a patient with a lesion in right parietal cortices (areas 5 and 7) performed at the same level as controls (98%). However, when the patient was blindfolded and 100 such stimuli were presented in the tactile mode, to the left and right hands independently, there was a severe entity-selective impairment. When the stimuli were grouped into those that are manipulable and those that are not, we found that right- and left-hand recognition of manipulable items was 94% and 92%, respectively (comparable to controls), while the performance on nonmanipulable items was only 57% and 47%, respectively (severely defective). Thus, the patient could nearly always retrieve the concept behind a manipulable entity, but was often unable to retrieve the concepts behind nonmanipulable entities. We propose that the lesion prevents signals from left and right somatosensory cortices from accessing right inferotemporal systems that are critical for mapping the conceptual structure of *visually ambiguous, nonmanipulable entities*. However, such signals can still access the motor system through a frontal route and thus activate part of the conceptual structure for manipulable entities.

505.4

LAUGHTER PUNCTUATES SPEECH: LINGUISTIC AND SOCIAL CONTEXTS OF LAUGHTER IN CONVERSATION. R. R. Provine*, L. A. Greisman and C. N. Runyan. Dept. of Psychology, UMBC, Baltimore, MD 21228.

The stereotypic, species-typical character of laughter facilitates the analysis of the neurobehavioral mechanisms for the production, perception and evolution of human auditory signals of which speech is a special case (Ethology, 89 (1991) 115-124). Laughter and speech are seldom considered in the same context, a tradition that limits our understanding of both behaviors. This study describes the position of naturally-occurring laughter in the speech stream of anonymous, mostly adult subjects, observed in public places. Laughter of both speaker and audience occurred during pauses at the end of sentences in over 99% of the sample of 1200 episodes of laughter, indicating that speech has priority access to the single vocalization channel and that a lawful neurobehavioral process governs the placement of laughter in the speech stream. Laughter by speaker or audience rarely interrupted the phrase structure of speech. Because laughter followed both statements (84.3%) and questions (15.7%), it was not restricted to a specific type of preceding sentence. Contrary to experience with professional comedians, most laughter was not preceded by jokes or material that seemed humorous outside of the conversational context. Another counterintuitive finding was that speakers laughed more than audiences. Speakers, especially females, laughed more than their audiences, but the relative amount of speaker and audience laughter depended on the gender composition of a group. Audiences of both males and females laughed more to male than female speakers. This may be why most professional comedians are male. These baseline data define the variables for future studies of laughter in neuro- and psychopathology. Laughter may provide novel insights into the neurobehavioral mechanisms of normal and abnormal emotional communication in humans.

505.5

ELEMENTS OF ATTENTION: PERFORMANCE OF HEALTHY ADULTS AND NEUROPSYCHIATRIC PATIENTS ON THE NIMH-LPP ATTENTION BATTERY. J.E. Tatman†, B.D. Fantie*†‡ and A.F. Mirsky‡. †Human Neuropsychology Laboratory, The American University, Washington, DC and ‡Laboratory of Psychology and Psychopathology, NIMH, Bethesda, MD.

Healthy adults (n=103), aged 18 to 90 years, performed the NIMH-LPP Attention Battery; a set of neuropsychological tests tapping a broad range of attentional abilities. Extending and refining the four elements of attention that Mirsky et al. (1991) identified in the battery (i.e., 1-Focus/Execute, 2-Shift, 3-Sustain, and 4-Encode), a new five-component solution best resolved the variance in these data. We derived the newly-identified factor from variance and error scores on the Continuous Performance Task (i.e., 5-Reliability of Performance) and renamed the original 4 factors 1-Scan/Focus Speed, 2-Flexibility/Shift of Set, 3-Arousal/Effort, and 4-Encode/Retain, respectively. A reanalysis of a subset of Mirsky et al.'s data resulted in a comparable 5-component solution among 52 neuropsychiatric patients (i.e., patients with petit mal and complex partial seizure disorders, anorexia nervosa and bulimia nervosa, affective disorders, and closed head injuries), aged 20 to 63. Despite interesting differences, we argue that the underlying components reflect congruent cognitive processes in each group. We also propose that the disordered attention in these clinical subjects may be primarily attributable to poor executive regulation of attentional shifts, i.e., an impaired flexibility of cognitive set. In summary, we obtained general support for the reliability of Mirsky et al.'s original four elements while the new 5-component solutions cast several new lights on the performance of both healthy and neuropsychiatric subjects on tests of attention.

505.6

EXTINCTION OF TRACE CONDITIONED EYE-BLINK RESPONSES IS IMPAIRED IN ANTEROGRADE AMNESIA. R. A. Deyo* and M. J. Jacobson. Department of Psychology, Winona State University, Winona, MN 55987.

Lesion studies involving rabbits have shown that the cerebellum is required for acquisition of delay conditioned eye-blink responses, while lesions to the hippocampus block acquisition of trace conditioned responses (Moyer, et al., 1990; *Behav. Neurosci. 104*: 241-250). These data suggest that different classical conditioning tasks may require different combinations of neural systems. The question of whether a similar dissociation applies in human learning has not been addressed. Thus, the purpose of the present study was to examine the retention and extinction of trace conditioned eye-blink responses in a subject with anterograde amnesia.

A 32 year old female with anterograde amnesia (following an automobile accident) served as the amnesic in the present study. Five females (controls) were selected to match the age and intelligence of the amnesic.

The apparatus consisted of a microcomputer programmed to record behavioral responses and to control the presentation of stimuli. Each acquisition trial consisted of a tone presented for 100 ms followed 500 ms later by a 100 ms corneal air puff. Extinction trials consisted of the tone presented alone.

Two weeks prior to the accident, the amnesic (D01) had received 60 acquisition trials, as a participant in an earlier study. Sixteen months after the accident D01 received an additional 50 acquisition trials followed by 20 extinction trials. Controls received the same number acquisition and extinction trials at the appropriate conditioning intervals.

Statistical analyses indicated that there were no pre-injury differences between D01 and controls. Analysis of performance 16 months after the injury indicated that while retention of the conditioned eye-blink response was not altered, D01 did show a much higher resilience to extinction relative to the matched controls.

505.7

AUDITORY STREAMING REDUCES REACTION TIMES TO INFREQUENT TARGETS. C. Alain* and D. L. Woods. Clinical Neurophysiology Laboratory, Dept of Neurology, UC Davis, VA Medical Center, Martinez, CA 94553, USA.

When a sequence of different tones is presented rapidly, the sequence may split into two or more perceptually concurrent streams. The tones that are similar in frequency will tend to be perceived as a group. This phenomena is called "auditory stream segregation" or "streaming" and is sensitive to stimulus rate and tonal separation. In two experiments we examined whether streaming would affect reaction times as well as other measures of auditory perception. Stimulus sequences consisted of tones (40 ms, 78 db SPL) of three different pitches presented in random order. Tones were delivered in blocks at either fixed (180 ms) or at variable interstimulus intervals (ISI, 140 to 220 ms). In the ***BASELINE*** condition, the three pitches were evenly spaced in frequency (by six semitones, 1048, 1482, 2096 Hz). Subjects responded to infrequent (2.5%) longer duration tones (65 ms, Exp. 1) or infrequent louder tones (85 db SPL, Exp. 2) of a designated extreme pitch (1048 or 2096 Hz). In the ***GROUPING*** condition, the middle pitch was unchanged (1482 Hz) whereas the low or high pitched tones were made more similar (by one semitone) to tones of the middle pitch. It was hypothesized that the increased proximity of the irrelevant pitches would segregate them into a separate stream, thereby facilitating the monitoring of the target belonging to the relevant pitch. In both experiments, tonal grouping increased the number of correct responses and decreased RTs compared to the ungrouped condition. This grouping effect on RT was similar at a fixed or variable ISI. This result suggest that stream segregation is a general phenomenon that influences the speed as well as the accuracy of auditory identification.

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505.8

LATERALIZED PROCESSING OF CENTRALLY PRESENTED VERBAL AND SPATIAL TASKS. F.G.Freeman‡ G.Pearson and D. Evans. Psychol. Dept., Old Dominion Univ., Norfolk, Va. 23529-0267.

In three separate experiments, subjects were required to attend to either their right or left ear in a dichotic listening, target ID task. In Experiment one, subjects simultaneously were required to perform a verbal task in which word pairs were centrally presented on a monitor. The words were to be identified as either antonyms or unrelated. In Experiment two, subjects simultaneously had to perform a mental rotation task in which two histograms were presented successively and had to be identified as being identical or mirror images. In Experiment three, subjects simultaneously performed a two dimensional tracking task in which two cursors had to be kept in the center of the video screen.

Results of Experiment one indicated that, for male subjects, attending to the right ear interfered with performance of the verbal task more than attending to the left ear. Ear attention did not differentially effect female performance. For Experiments two and three, for females, left ear attention interfered with performance on the spatial tasks more than right ear attention. For males attention did not effect spatial tasks.

505.9

NONTRIVIAL SEX DIFFERENCES IN BRAIN WEIGHT AND CRANIAL CAPACITY CONTROLLING FOR BODY SIZE. J.P. Rushton*. Department of Psychology, University of Western Ontario, London, Ontario, N6A 5C2, Canada.

Although it has long been known that human females have absolutely smaller brains (mass or volume) than do human males, it is widely thought that after correcting for body size the differences disappear. Two large data sets show that after covariance adjustment for body size, women's brains average 100 g lighter and 110 cm^3 smaller than men's.

First, Ankney (in press, Intelligence) reanalyzed autopsy data published in 1980 on 1,261 subjects between the ages of 25 and 80. Second, Rushton (in press, Intelligence) analyzed external head measurements (length, width and height) collected on a stratified random sample of 6,325 U.S. Army personnel in 1988. The sex difference in brain size was replicated across Black and White samples in the first study and across Asian, White, and Black samples in the second. These differences may be related to human evolution and those intellectual abilities at which males excel.

505.10

DEFECTIVE ORGANISATION OF KNOWLEDGE IN NON-DEMENT PARKINSONIANS: EVIDENCE FROM A SCRIPT PRODUCTION TASK. L. Godbout and J. Doyon*. Univ. Laval, Ecole de psychologie, Quebec, QC., Canada, G1L 7P4.

Shallice (1982,1988) and Grafman (1989) proposed that the frontal lobes are involved in organizing the representation of large-scale conceptual units, called scripts (Schank, 1975). Scripts refer to rehearsed sequences of events that have a typical temporal and semantic structure (eg. going to a restaurant). The purpose of this study was to examine whether the organisation of scripts would be affected in patients with Parkinson's disease. 16 Parkinson's patients and 16 normal control subjects were required to produce verbally scripts of common activities (eg. going to see a movie, shopping for grocery) in a forward and reversed order. According to Grafman, frontal-lobe dysfunction should affect both forward and reverse scripts. In contrast, Shallice predicted that frontal-lobe dysfunction should affect the production of non-familiar (reverse) scripts, resulting in sequencing errors. Basal ganglia dysfunction, on the other hand, should impair production of well-rehearsed (forward) scripts by the intrusion of non pertinent elements. Sequencing errors and alterations in the semantic structure of the script (eg. adding or deleting an element) were found in Parkinson's patients, but not intrusions. The semantic and sequencing errors found in both the forward and reverse condition were correlated with performance on others frontal-sensitive tests such as verbal and non-verbal fluency tests. The results indicate that the type of error made by the Parkinson's patients is the same as the one found in a group of patients with circumscribed frontal-lobe lesions (Godbout & Doyon, 1992), and are thus consistent with Grafman's, but not Shallice's hypothesis.

505.11

HABITUATION AND SENSITIZATION FOLLOWING BILATERAL ANTERIOR CINGULOTOMY. M.E. Meadows, R.A Cohen*, R.F. Kaplan, and H. Wilkinson. Dept. of Neurology, UMMC, Worcester, MA 01655

We studied habituation and sensitization of the orienting response (OR) following bilateral anterior cingulotomy. Skin conductance response (SCR) was measured during an orienting response paradigm in 4 pre- and 10 post-cingulotomy, and 10 age-matched control subjects. Pre-surgery, no patient exhibited impaired OR, habituation or sensitization. Post-surgery, patients exhibited abnormalities of habituation maintenance. Paradoxically, the initial rate of habituation after cingulotomy was more rapid compared to normal controls, yet slower relative to the criterion of complete habituation. Extended habituation training did not produce different effects on spontaneous recovery for patients and control subjects, nor was sensitization to new stimuli increased post-cingulotomy. A greater frequency of spontaneous activation and greater SCR variability over trials occurred post-cingulotomy. These results suggest that the cingulate cortex influences maintenance rather than rate of habituation.

505.12

DISRUPTION OF SHORT-DURATION TIMING FOLLOWING DAMAGE TO THE HUMAN SUPRACHIASMATIC REGION (SCN). R.A. Cohen and H.J. Barnes. Dept. Neurol., Univ. Mass. Med. Sch., Worcester, MA 01655, and H.E. Albers*. Dept. Biol, Georgia State Univ., Atlanta, GA 30303.

We previously demonstrated impairment of sleep-wake and temperature rhythms, and temporal attention dynamics in a human (AH) following SCN region destruction. We have since studied AH's short-duration timing. Severe disruptions of motor timing and time estimation compared to a age-matched controls was found. A tapping continuation paradigm was employed. The Wing & Kristofferson model was used to derive clock and motor-delay variance estimates. AH exhibited greater clock variances than previously reported after cerebellar lesions. AH's clock variances increased dramatically on a divided attention task, indicating that short-duration timing was greatly influenced by attentional biases. These results indicate that other neural systems, in addition to the cerebellum, may influence short-duration timing and also suggest a hierarchical role for the SCN in such timing.

505.13

DICHOTIC AND MONAURAL LATERALITY EFFECTS IN CALLOSAL AGENESIS. C. Paquette, I. Peretz* and M. Lassonde. Dept. of Psychology, Université de Montréal, C.P. 6128, Succ. A, Montréal, Qué. H3C 3J7, Canada.

In a recent study (Lassonde et al., B&L 1991), we reported that language functions, as assessed by dichotic listening performance, are more strongly lateralized in callosal agenesis subjects than in IQ-matched normal controls. However, the task used (CV detection) was so demanding that it produced a performance at chance in some of the subjects. The present study was thus designed to verify whether the stronger lateralization effect found in acallosals could be observed regardless of task difficulty. Four adult acallosal Ss and four IQ-matched controls were tested on two simple dichotic tests. The tasks consisted of word and musical timbre detection and were devised such that they would yield both laterality effects and high accuracy levels. The two tests were also presented monaurally since this easier procedure has occasionnally been reported to produce laterality effects. In terms of accuracy, the two groups performed at ceiling under both monaural and dichotic presentations (average performance: 96% correct). Analysis of response times, however, revealed different patterns for the two groups in the dichotic and the monaural conditions. In the dichotic condition, controls as well as acallosals, showed reliable laterality effects. In the monaural condition, controls did not demonstrate any ear advantage while acallosals still exhibited ear advantages of the same magnitude as in the dichotic condition. These findings suggest that callosal absence may weaken, rather than reinforce, the influence of ipsilateral auditory pathways. The results further suggest that the ipsilateral suppression normally observed under dichotic presentation is not related to callosal inhibition.

505.14

LOSS OF VISUAL IMAGERY BUT NOT SPATIAL LOCALIZATION IN A CASE OF VISUAL AGNOSIA IN CHILDHOOD. M. Lassonde*, A. Schiavetto, J. Flessas and G. Geoffroy. Dept. of Psychology, Université de Montréal, C.P. 6128, Succ. A, Montréal, Qué. H3C 3J7, Canada and Hôpital Ste-Justine, Montréal, Canada.

A.R. contracted a viral encephalitis at age 9. She was evaluated over a period of 4 years. CT scan revealed a right temporal hypodensity affecting gray and white matter and hyperdense sub-cortical zones in the left temporal and right parieto-occipital areas. A.R. showed evidence of associative visual agnosia, prosopagnosia and color agnosia. At first, she was unable to name objects from visual inspection but could do so by verbal description, tactual and auditory cues. Moreover, she could not recognize familiar faces or identify colors. With time, A.R. has shown limited improvement. She can now recognize familiar objects although occasionnally making intra-categorical mistakes. She can also name colors but cannot associate them to objects. Her prosopagnosia has not resolved. In fact, while she can match and copy visual representations of objects, she cannot do so in the case of faces. A clear dissociation between object and spatial representations is present in this patient. A.R. is unable to draw an object from memory but she can draw a map of familiar routes. She cannot correctly reproduce shapes that she has manipulated on a board but can accurately reproduce their localization. A.R.'s inability to recognize objects is interpreted as reflecting a deficit in the inferior temporal ventral pathway specialized for object perception, whereas the posterior parietal dorsal pathway specialized for spatial perception remains functional. Finally, the little recovery displayed by A.R. indicates limits in cerebral plasticity for visual agnosia.

505.15

A REACTION TIME BASED CONTINUOUS PERFORMANCE TASK FOR MEASUREMENT OF LATENT INHIBITION IN HUMAN SUBJECTS. L. A. Dunn*. Dept. of Psychiatry, Duke Univ. Med. Ctr., Durham, NC 27710.

Latent inhibition (LI) occurs when repeated exposure to a non-reinforced stimulus inhibits later learning of associations to that stimulus. LI is an indirect measure of selective attention. Interest in the measurement of LI in humans has increased because of the finding that LI is reduced in acutely psychotic patients, and that LI in rats is sensitive to drugs that are dopamine agonists and antagonists. LI paradigms are limited in that they measure across groups of subjects and are difficult to repeat because of the learning effects of the preexposures and the subsequent associations. A continuous performance task for LI measurement that overcomes some of these limitations has been developed through a series of pilot studies. The task is divided into four phases. Subjects are instructed to watch a monitor and press a button each time a target X appears. Stimuli appear for 1 second with a 0.25 second inter-stimulus interval. During phases 1 and 3 20% of the stimuli are targets, 60% are letters, and 20% are a non-letter preexposure stimulus. During phase 2 a novel symbol always cues the target. The subject learns this through repetition and reaction times decrease. Phase 2 continues until the computer records 3 reaction times that are lower than those recorded in phase 1 or the subject has responded 99 times. During phase 4 the preexposed stimulus cues the target. Reaction times decrease as in phase 2. Reaction times are averaged for each phase. A subject demonstrates LI when the reaction time in phase 4 is slower than in phase 2. Pilot work shows that normal controls show LI in aggregate ($p<.0001$), and individually in 60% of normal controls at the 0.95 significance level. LI is robust after 3 weekly repetitions but learning effects appear to confound the test on the fourth repetition. (Supported by MH47503)

505.16

PERCEPTION OF HAND MOVEMENTS AFTER LEFT-HEMISPHERE DAMAGE. Doreen Kimura*. Psychology, Univ. Western Ont., London Canada N6A 5C2.

Left-hemisphere pathology often results in manual apraxia, a difficulty in selecting movements, to command or imitation. The author has proposed that the deficit relates to control of movements within personal space, with the body as framework. The question arises whether perception of movements is adequate in such cases. Since the processing of movements which can be reproduced might employ the same defective selection mechanism which impairs their production, the question is better asked outside the movement-generating framework.

Patients with left- or right-hemisphere damage (N= 77, 59) were scored on two tests: 1. Copying of meaningless hand movements, reliably sensitive to manual apraxia. 2. A test requiring them to point to which one of 4 unfamiliar symmetrical shapes was outlined in the air by E, using both hands. As before, left-damaged patients were significantly poorer at copying movements; but not in identifying hand-generated shapes. Manual apraxia is thus not a result of a deficit in processing hand movements in extra-personal visual space.

505.17

HOMOSEXUALITY, COGNITIVE ABILITIES AND THE ORGANIZATIONAL HYPOTHESIS. J. A. Hall* and D. Kimura. Dept. of Psychology, Univ. of Western Ontario, London, Canada, N6A 5C2.

Previous research has suggested that, due to potential differences in pre and/or perinatal levels of sex hormones, homosexual males may possess patterns of brain organization and cognitive abilities which fall intermediate between those of heterosexual males and females. The performance of 17 homosexual male undergraduates was compared to a heterosexual male control group (N=69) on spatial and verbal tasks which reliably show sex differences. Unlike previous studies, homosexual males showed no significant reduction on a task of spatial rotation; however, they were significantly outperformed by heterosexual males on a practical throw-to-target task. Additionally, a significant gay male advantage on an ideational fluency task and a significant non-gay advantage on a test of mathematical reasoning were found. Testosterone levels, assayed from saliva, showed no difference between gay and non-gay males. These data do not support the idea that homosexual males have ability profiles which are simply intermediate between heterosexual male and female extremes.

505.18

NICOTINE EFFECTS INTERHEMISPHERIC PROCESSING IN SIMPLE RECOGNITION VERSUS ARITHMETIC RELATED TASKS IN A SEXUALLY DIMORPHIC PATTERN. S.Demirgören*, M.Pehlivan, H.Aydın, Y.Çete, Ş.Pöğün. Dept. of Physiology, Ege University School of Medicine, 35100 İzmir, Turkey

Cerebral hemispheric specialization differs between sexes and the improving action of nicotine on cognitive functions may show sexual dimorphism. The aim of this study was to study the effects of cigarette smoking on interhemispheric processing. Male(M) and female(F) smokers and non-smokers were tested using a computer-based program where numbers were projected randomly to different visual fields. In one set of trials, the subjects had to recognize matching pairs; in the other set, they had to perform a simple calculation. For all groups, the trials were repeated with a 15 min. interval during which the smokers, deprived for 12 hrs, smoked a standard cigarette. Reaction times and the number of correct responses were evaluated(ANOVA). In simple recognition tasks(SRT), F smokers performed better in tasks designed to test right hemisphere function ($p<0.05$) with regard to reaction time. In arithmetic related tasks(ART), however,F smokers had more correct responses in tasks designed to test left hemisphere function($p<0.005$). In SRT, smoking shortened reaction times during the second trial more prominantly in Fs ($p<0.005$). In ART however, M smokers benefited more from nicotine regarding reaction times($p<0.05$). M smokers were affected more from nicotine deprivation in ART($p<0.05$)and F smokers more in SRT($p<0.05$). Our results imply that nicotine improves interhemispheric processing of SRT and ART in a sexually dimorphic pattern.

505.19

TYROSINE REVERSES A COLD-STRESS-INDUCED MEMORY DEFICIT IN HUMANS. D. Shurtleff*, J.R. Thomas, J. Schrot, K. Kowalski, R. Harford, M.O. Thornton, P.A. Shea and M. Malik. Naval Medical Research Institute, Bethesda, MD 20889-5055.

Eight male subjects performed a delayed matching-to-sample (DMTS) task at an ambient temperature of 4°C (cold) or 22°C two hours after ingesting the catecholamine precursor tyrosine (150 mg/kg) or placebo, administered double-blind. The DMTS task required a correct choice to one of two simultaneously presented matrices, one of which had been presented as a sample matrix 2, 8 or 16 sec before. Each matrix was composed of 32 red and 32 green squares, randomly distributed. After ingesting placebo at 22°C, subjects demonstrated a characteristic delay gradient in which accuracy declined as the delay interval increased between sample and comparison stimuli from 2 (Mean percent correct=83.76% ± SEM=1.38) to 8 (80.21% ± 1.66) to 16 (69.31% ± 2.60) sec. A one hour cold exposure following placebo ingestion significantly reduced matching accuracy at the 16-sec delay interval (50.41% ± 2.06), which is attributed to cold's effect on short-term, or working, memory. Administration of tyrosine significantly improved matching accuracy at the delay interval affected by cold exposure (16-sec), such that mean matching accuracy was 65.76% ± 2.8, equal to that at 22°C following placebo ingestion. Plasma norepinephrine, epinephrine and tyrosine levels during cold and 22°C exposure were also measured and will be presented. These results indicate that tyrosine was effective in ameliorating cold stress effects on DMTS performance, possibly by preventing a cold-stress-induced reduction in brain catecholamine levels.

505.20

DYNAMICS OF MODULATED NOISY BIOLOGICAL SYSTEMS. D.R. Chialvo, C.J. Hodge* and A.V. Apkarian. Computational Neuroscience Program, Dept. of Neurosurgery, SUNY Health Science Center, Syracuse, New York 13210.

The output signal from noisy bistable systems can be modulated in time by applying a weak external periodic forcing. Such systems when driven with noise exhibit Poisson distributed intervals (ISIH). When a sine wave is added to the input noise, the ISIH is multipeaked at multiples of the sine wave period with an exponential envelope. For certain amplitudes of noise and signal, bistable systems exhibit an increase in signal-to-noise ratio with an increase in input noise (stochastic resonance). Here we present: 1. numerical simulations of a neuron, 2. single unit recording experiments, and 3. a visual perception task, to show that the theory of bistable systems can be applied to information processing in the brain.

1. A two dimensional difference equation model of excitable tissue driven with white noise results in spike trains with a Poisson ISIH. For a fixed noise amplitude, increasing the amplitude of an additional sine wave input results in spike trains with multipeaked ISIHs which become single peaked at higher sinusoidal amplitudes.

2. In anesthetized cats, single unit responses were studied in the spinal cord and the somatosensory cortex. Rapidly adapting type neurons showed multipeaked ISIHs during sinusoidal vibrotactile stimulation of the receptive field. The envelope of these ISIHs had an exponential decay which increased with stimulus amplitude.

3. A cognitive visual task was developed to study hysteresis in viewing ambigous figures. Subjects were familiarized with and tested to rank images consisting of a smooth transiton from the face of a man to the body of a woman. The input/output map for ranking random sequences of these images showed hysteresis and was modeled as a 1-D piecewise linear map. When the images were presented iteratively with underlying noise and periodic modulation, increases in noise amplitude increased the perceptual signal-to-noise ratio, exhibiting bistable stochastic resonance. This paradigm can be used to study short term memory.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS—MODELS

506.1

NEUROPOPULATIONAL MECHANISM OF PARALLEL CORTICAL PROCESSING IN THE MILLISECOND RANGE. K. Nakamura*. Grad. School of Sci. & Eng., Tokyo Inst. of Tech., 4259 Nagatsuta, Yokohama 227, Japan.

The cerebral cortex is capable of processing sensory signals to work the motor system in a few hundred milliseconds, though single neurons relatively slow to respond (several milliseconds). A mathematical model of the cortical processing is presented to show the processing is performed in an optimal parallel style. The cortex is represented by a sequence of areas consisting of cortical columns. Each column includes two populations of pyramidal cells and interneurons inhibiting the cells. Membrane characteristics of the model neurons is represented by the Hodgkin-Huxley electric circuit. Analysis of the model shows (1) the columns are capable of detecting several milliseconds with ratios of firing cells in the populations, even though firing of single cells fluctuates, (2) the areas perform *competitive parallel computation* using the temporal resolution, where only the first activated columns are allowed to fire out of the columns activated in parallel, and (3) synaptic plasticity regulated by the hypothalamic reward system reinforces association connections between the areas so that the competitive computation may lead to rewarding move of muscles. (1) and (2) indicate processing of each area completes in several milliseconds. This and (3) suggest repetitive rewards reinforce the whole cortex to respond to sensory stimuli within a few milliseconds.

506.2

EXPECTATION LEARNING IN THE BRAIN USING DIFFUSE ASCENDING PROJECTIONS

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Diffuse projections originating in subcortical nuclei are known to influence activity-dependent cortical plasticity and learning both during and after development. These signals may report to the cortex important events in the world as well as which activity patterns in the cortex result from actions taken by an organism e.g. proprioceptive signals associated with a movement. Although a number of physiological actions have been attributed to the neurotransmitters used by these pathways (acetylcholine, norepinephrine, serotonin, etc.), their precise functional effects on learning within a large network of neurons are unknown. We explore here a theory in which the derivative of the activity of the ascending pathway drives learning at cortical synapses. This learning is gated locally by a rapid diffusible signal produced by glutamate transmission in a local volume of tissue. We call this effect *volume learning*. This scheme forces the cortical networks to *learn to predict the future changes in activity in the ascending pathway*. Moreover, a powerful influence driving cortical learning obtains when one part of the cortex captures control over the mid brain structures that release the neuromodulators. This occurs by allowing for modifiable NMDA synapses on the path from cortex to the midbrain nuclei. Using a large scale computational model we demonstrate the theory and test it in a variety of tasks including visual recognition. This scheme may result in impoverished cortical representations in the absence of some way by which cortical patterns can be combined, recoded, and redistributed to the cortex. We show how the hippocampus can play a natural role in restructuring representations to make this prediction possible.

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506.3

HOW PLACE CELLS CONNECTED BY HEBBIAN SYNAPSES CAN SOLVE SPATIAL PROBLEMS. R. Saypoff, R.U. Muller* and J.L. Kubie. Depts of Anatomy and Physiology, SUNY, Brooklyn. Brooklyn, N.Y. 11203

If place cells are connected by Hebbian (eg, LTP-modifiable) synapses, the strength of the synapse between a pair of cells will come to encode the distance between the firing fields of the twocells. A synapse between cells with overlapping fields will strengthen because the two cells will fire together at intervals short enough for LTP to occur. In contrast, synapses between cells with separated firing fields will remain weak because the two cells never fire in close temporal order. In this preliminary approach, such synapses do not affect the firing of the post-synaptic cells; they only register the degree to which the two cells fire together in time.

If strength/distance encoding is added to a network that models the recurrent connections of CA3 (Traub and Miles, 1991), the result is an environmental representation that has the properties of a weighted graph. If a starting point and an end point in real space are specified, a searching algorithm can be used on the graph to find the shortest path in reciprocal synaptic weight space. The sequence of place cells on this path corresponds to a path through real space. The length of the path in real space is very long if the network is small or sparsely connect. As the network becomes richer, however, the algorithmically computed path length converges on the Euclidian distance between the starting and end points. The size and connectivity of adequate networks compare reasonably to the real thing. We conclude that graph-like networks of the proposed type contain enough information to make it possible to compute the shortest distance between any pair of points in the environment. They therefore fulfill many of the requirements for the hippocampal mapping system first proposed by O'Keefe and his colleagues.

506.4

INFORMATION PROCESSING IN EXCITABLE DENDRITES. B. W. Mel*, Computation and Neural Systems Program, Caltech, 216-76, Pasadena, CA, 91125.

Compartmental modeling studies of the input-output behavior of NMDA-rich neocortical pyramidal cells have previously shown that their dendritic trees were "cluster-sensitive", i.e., gave rise to larger cell responses when synapses were activated in clusters, rather than diffusely scattered about the dendritic arbor. This work has been extended to cells containing fast sodium and slow calcium spiking mechanisms in various distributions throughout the dendritic tree. Results of simulations show that strong cluster sensitivity can result when the dendrites contain 1) only NMDA channels, 2) only fast spiking channels, 3) only slow-spiking channels, or 4) combinations of any two or three of these voltage-dependent mechanisms in a variety of spatial distributions.

A model neuron called a "clusteron" was used to abstract the cluster-sensitive behavior of a dendritic tree containing excitatory membrane mechanisms, and to explore a family of Hebb-type synaptic learning rules capable of manipulating the ordering of afferent synaptic connections onto the dendritic arbor. It is shown that the dendritic tree of a single pyramidal cell, if richly endowed with excitatory voltage-dependent nonlinearities, is capable of reliably discriminating thousands of learned complex patterns from unfamiliar controls. It is also shown that a cluster-sensitive dendritic tree can approximatively implement a cross-correlation operation, relevant to biologically significant nonlinear sensory processes such as binocular disparity selectivity.

506.5

SIMULATIONS OF ADAPTIVE INTERACTIONS BETWEEN LIMBIC AND NEOCORTICAL STRUCTURES. S. D. Murphy and E. W. Kairiss*, Department of Psychology and The Interdepartmental Neuroscience Program, Yale University, Box 11A Yale Station, New Haven, CT 06520.

Current views of long-term memory storage in mammals emphasize the importance of dynamic interplay between limbic structures (amygdala and hippocampus) and cortical areas (e.g. L.R. Squire & S. Zola-Morgan, *Science*, 253:1380, 1991). The goals of this study were to construct a computational model that incorporates selected features of temporal lobe physiology and anatomy, and to examine the dynamical behavior of the adaptive processes that might support memory formation.

Hippocampal subfields (fascia dentata, CA3, CA1) were represented as networks of compartmental neurons, whose physiological properties, statistical connectivity and principal neuron:interneuron ratio were abstracted from physiological studies. A simplified cortical region was constructed as a multilayer network of cortical pyramidal and non-pyramidal neurons; this network was reciprocally connected with the hippocampal networks. Input patterns were applied to the cortical and hippocampal structures in tandem, and stable cortical activity patterns (cell assemblies) were used as measures of information storage. Our studies focussed on two properties thought to be important for mnemonic function: use-dependent synaptic plasticity (particularly at the hippocampo-cortical connections) and the role of theta rhythm in synchronizing connected systems. The spatio-temporal interactions between these phenomena were found to be important for the emergence of dynamic assemblies in the cortical network.

These computational studies will provide a framework for future analytical and experimental investigations of the dynamic processes underlying distributed memory storage in cortical systems.

506.6

IS FIELD CA3 A REVERBERATING SHORT TERM MEMORY SYSTEM? M. Taketani, J. Ambros-Ingerson, R. Myers, R. Granger and G. Lynch*. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717.

Given its placement on the olfactory-hippocampal circuit and unique anatomical features, it has been hypothesized that field CA3 of hippocampus maintains cue-specific activity after the cue is gone (and behavior continues) by generating recurrent and specific patterns of activity set in motion by momentary perforant path input, thus providing a short term memory system that can be used to form associations between cues that are separated in time and space.

To explore this hypothesis, we have constructed a computer model that incorporates many salient anatomical and physiological features of this region. CA3 has a large population of pyramidal cells and is unique in its extremely dense recurrent associational system which comprises ~70% of the synaptic population (ipsilateral and contralateral). A smaller population of interneurons mediate fast and slow inhibitory currents. The model consists of 300 pyramidal cells, mossy fiber inputs, and a collection of interneurons. Total connectivity is less than 10% and is divided into global (random across the network) and local (random but spatially restricted) patterns for the various kinds of cells. Cells are modeled as single compartments (1) incorporating driving force effects, multiple synaptic currents overlapping in time, axonal delays and frequency facilitation. Baseline theta activity (5Hz) is generated by extrinsic input and on its own does not reliably trigger pyramidal cells.

Preliminary results indicate that recurrent patterns of activity can be generated in this network. Perforant path stimulation that resulted in activation of a few (~1%) pyramidal cells can generate recurrent activation, specific to the initial input, that can last for ~100 ms. Further experimentation with the model and network scaling is expected to improve performance. Thus, in principle, salient anatomical and physiological features of CA3 can be integrated to form a reverberating short term memory system. (supported by grants from ONR and Matsushita Electric Industrial Co., Ltd.)

(1) P. Anton, R. Granger and G. Lynch. Temporal Information Processing in Synapses, Cells and Circuits. in *Single Neuron Computation*, T. McKenna, J. Davis and S. Zornetzer, Eds. (Academic Press, NY, 1991)

506.7

SEQUENTIAL CONFIGURATION MODEL AND TRACE DISRUPTION IN LOCAL NEURAL NETWORKS. K.A. Flach[1], R.J. MacGregor[1], G.A. Gerhardt*[2]. [1]Dept. Of Aerospace Engineering, University of Colorado, Boulder, Colorado, 80309, [2]Dept. of Psychiatry, Denver, University of Colorado Health Sciences Center, Denver, CO 80262.

The sequential configuration model is an explicit model of coordinated multi-unit firing patterns for representing information in neural populations. A sequential configuration is an ordered sequence of sets of cells whose temporal firing relationships define its pattern. This work applies the sequential configuration model to represent memory storage and retrieval in two interconnected and recurrently connected populations of cells, one excitatory and one inhibitory, representative of cortical modules. Individual traces can be selectively recalled by extrinsic stimulation of the network. The recurrent connections inherent in the trace produce internally sustained recalls of the sequential configurations. A theoretical derivation of memory capacity has shown the level of cross talk, produced when cells participate in more than one trace, is a random variable with mean and variance functions of network size. Calculations of storage capacity show that the variance of the cross talk becomes small for cortical module size nets (30,000 cells). Random noise reduces the storage capacity to 1/3 the value from cross talk alone. Results from computer simulations are accurately predicted by this theory. Detailed simulations have shown how cross talk can limit the number of traces that can be successfully recalled. The cross talk raises the average cell potential, producing a burst of activity. This burst causes many cells in the trace to be refractory at the same time, and produces simultaneous activation of a large population of inhibitory cells. This renders the trace nonrecallable. MacGregor, R.J. Sequential configuration model for firing patterns in local neural networks Biol. Cybern. 65, 339-349, 1991.

506.8

A MODEL OF FRAGMENTED SPATIAL MEMORY. Catherine Thinus-Blanc* & Marielle Krimm Cognitive Neuroscience Laboratory, CNRS, 31, ch. J. Aiguier 13402 Marseille Cedex 9 France. Tel: 91 16 40 88

Although the old opposition between behaviorists and cognitivists is now obsolete, the dual conception of two extreme and sometimes mutually exclusive forms of spatial memory is still alive. We propose another conception based on several inter-related levels of processing of a common "material", namely frontal local views whereby any terrestrial vertebrate, including human, has visual access to the environment. Some of these local views (or topographical maps), stored in long-term memory, are the building-blocks of different forms of spatial knowledge.

This conception calls for several remarks. First vision is endowed with a definite spatial function supported by data from studies of unsighted adult subjects.

Second, since topographical maps are assumed to be processed in different ways by the same individual, their storage and handling should be executed by distinct brain structures. In line with recent hypotheses, the topographical maps would be stored in visual areas whereas associative structures such as the hippocampus would be the repository of their "combinatorial" code.

This model leads to several hypotheses and predictions which will be evoked.

506.9

PHYSIOLOGICAL MEASUREMENTS PREDICT THE LIFETIME FOR HUMAN AUDITORY MEMORY OF A TONE

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Using the Magnetic Source Imaging (MSI) technique, we found that responses to tone stimuli observed in the primary and association areas of human auditory cortex provide evidence that the neuronal activation trace established by a stimulus decays exponentially with time, and the lifetime in association cortex is significantly longer than that in primary cortex in individual subjects. The strength of the activation trace at a time t following a stimulus was defined as the difference between the averaged response amplitude for a very long interstimulus interval and the averaged response amplitude for a given interval t. The difference was found to decrease exponentially according to $e^{-(t-t_0)/\tau}$, where τ is identified as the lifetime of the cortical activation trace. Behavioral measurements on the same subjects reveal that the short-term loss of auditory memory shows a central tendancy, namely, the internal representation of the loudness of a sound evolves as a decaying exponential toward the mean of all the stimuli experienced in the recent past. The time course is well predicted from the MSI measurements of the lifetime of the activation trace in human primary auditory cortex. This close agreement suggests that primary auditory cortex plays a significant role in echoic memory. This application of MSI and behavior studies opens new opportunities to relate human physiology and cognition.

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LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS—NEUROPSYCHOLOGY II

507.1

HUMANS, LIKE RATS, HAVE SPATIAL WORKING MEMORY CAPACITY GREATER THAN SHORT-TERM MEMORY CAPACITY OF 7±2.

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Although humans show short-term memory capacity of 7±2 items for a wide variety of tasks (Miller, Psychol. Rev., 1956; recent review: Baddeley, Science, 1992), rats have a working memory capacity of ≥12 in the radial maze (Olton, Collison, & Werz, Learn. Motiv., 1977). Is this a species or situational difference? Using a 17-arm radial maze analog drawn on paper, human subjects attempted to lift in random order each of 17 cardboard flaps arranged radially around a center. Fifteen undergraduates, each tested on 14 trials (each trial limited to 17 choices) averaged 15.4 correct responses. This suggests memory capacity comparable to rats. Miller's (1956) discussion of information load in absolute judgments implies a reason for greater spatial working memory capacity, in the two-dimensionality of the radial maze by comparison with the "linearity" of verbal sequences. Our finding presents a difficulty for the hypothesis that STM is a neurocognitive constant, based on widespread characteristics of mammalian cortex (Glassman, Neurosci. Abs., 1991).

507.2

ATTENTIONAL DEFICITS IN KORSAKOFF'S DISEASE.

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Response times were measured for Korsakoff and alcoholic control subjects to execute key-press responses following presentation of a visual target preceded by a cue. Korsakoff patients responded more slowly in all conditions, however, their performances were significantly more impaired when cue and target occurred in different locations and when endogenous processing was required to interpret a cue. Comparable results were obtained when cues were not presented, but were expected in a location different from the target. We argue that the Korsakoff patients are impaired in their ability to disengage attention and shift it to a spatially distinct location.

507.3

THE ROLE OF THE PREFRONTAL CORTEX IN HUMAN WORKING MEMORY: rCBF ACTIVATION DURING A SPATIAL DELAYED RESPONSE TASK.

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Numerous animal studies have suggested a role for the prefrontal cortex in working memory processes. We developed a computer-administered human analogue of a spatial delayed response (SDR) task in order to study patterns of blood flow activation associated with the cognitive demands of this task. Subjects were required to remember the locations of 4 identical stimuli within an array of 20 possible locations, over a delay of 7 seconds. Patterns of regional cerebral blood flow (rCBF) during this task were compared to rCBF during a no-delay control task. Fourteen normal volunteers were studied using a PET $H_2{}^{15}O$ water technique. The results of this study support the hypothesis that the prefrontal cortex is involved in the cross-temporal maintenance of response-relevant information.

507.4

DISSOCIABLE COMPONENTS OF SENSORY-MOTOR SKILL LEARNING IN PATIENTS WITH HUNTINGTON'S DISEASE.

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Sensory-motor skill learning has been dissociated from explicit memory in global amnesia and from repetition priming in Alzheimer's disease (AD). Patients with Huntington's disease (HD) learn poorly on some sensory-motor skill learning tasks, and these results suggest a critical role for the frontal-striatal system in these tasks. The present study examined whether two different kinds of sensory-motor skill learning, rotary pursuit and mirror tracing, are neurologically dissociable from one another. The subjects were 6 early-stage HD patients and 6 normal control (NC) subjects. For the rotary pursuit task, speed of rotation was adjusted for all subjects to equalize initial levels of performance, and subjects performed 4 20-second trials in each of 6 blocks. The main measure of learning was time on target. For the mirror tracing task, subjects traced a star seen in a mirror for 5 trials. The measures of learning were time to trace the star and the number of departures from the star. The HD patients showed impaired learning on the rotary pursuit task, but not on the mirror tracing task. These results suggest that there are at least two dissociable components of sensory-motor skill learning, and that one component, critical for rotary pursuit learning, is mediated by the frontal-striatal system. Preliminary data from two patients with cerebellar lesions suggest that another component, critical for mirror tracing, is mediated by a cerebellar system. Supported by a grant from the McDonnell-Pew Cognitive Neuroscience Program.

507.5

IMPAIRED WORKING MEMORY IN UNMEDICATED ADULTS WITH GILLES DE LA TOURETTE'S SYNDROME. G.T. Stebbins*, J. Singh, J.D.E. Gabrieli, C.L. Comella, and C.G. Goetz. Department of Neurological Sciences, Rush-Presbyterian-St.Luke's Medical Center, Chicago, IL 60612 and Department of Psychology, Stanford University, Stanford, CA 94305

We assessed memory performance (including working memory (WM), immediate and long-term memory, and motor skill learning) in a sample of 13 unmedicated adult GTS patients, and 10 healthy controls (NC) matched for sex, mean age and education. WM was assessed using a verbal working memory task (Salthouse & Babcock, 1990) which required the subject to answer questions about orally presented sentences, while simultaneously remembering the last word in the sentences. Immediate memory was assessed with forward and backward word span tasks. Long-term memory was assessed with free-recall and forced-choice recognition of word lists, and vocabulary knowledge (WAIS-R Vocabulary subtest). Motor skill learning was assessed with the rotary pursuit task. GTS patients were impaired on working memory ($p<0.01$), word span backwards ($p<0.005$), long-term free recall ($p<0.05$), and rotary pursuit learning ($p<0.005$). In contrast, GTS and NC performed comparably on long-term recognition memory (forced-choice recognition), long-term semantic memory (WAIS-R Vocabulary subtest), and one test assessing immediate memory (word span forward). This pattern of memory performance is similar to that seen in diseases of the basal-ganglia (Parkinson's disease and Huntington's chorea), and suggests that the memory deficits in GTS may be due to selective frontal-striatal dysfunction.

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507.6

PREFRONTAL CORTEX INVOLVEMENT IN WORKING MEMORY. L. Nielsen-Bohlman* and R. T. Knight; Neurology Dept., UC Davis, VAMC, 150 Muir Rd, Martinez CA 94553

We examined prefrontal contributions to immediate and sustained components of working memory by recording long latency ERPs in patients with lesions in dorsolateral frontal cortex (n=10, mean age=64 years; controls n=10, mean age 71 years) in a recognition memory paradigm. Stimuli were line drawings of familiar objects presented in quadrafields (duration 800 msec, ISI 1200 msec). Eighty percent of the images were presented twice. The second presentation occurred at 1.2 sec with no intervening images (immediate), or at delays of 4-158 sec with a mean of 40 intervening images (delayed). Subjects indicated whether an image had been previously presented by pressing a 'yes' or 'no' button. Control subjects showed enhanced frontal positivity to all stimuli (at Fpz 15.9uV) which was reduced in the frontal patients at all intervals (7.8uV, $p<0.025$). Stimulus identification was impaired for the immediate repetition, but was normal for delayed repetition. Long latency parietal activation (P600) was normal at all delays while an earlier latency P400 component generated by immediate stimulus repetition was selectively reduced by prefrontal lesions. The data support prefrontal activation during working memory and indicate that prefrontal lesions result in selective behavioral and electrophysiological deficit during immediate recognition.

507.7

INFLUENCE OF FRONTAL LOBE LESIONS ON SUBJECTIVE ORGANIZATION IN VERBAL LEARNING. P.J. Eslinger* & L.M. Grattan. Depts. of Neurology, Penn State Univ. College of Medicine, Hershey, PA 17033, & Univ. of Maryland Medical School, Baltimore, MD 21201.

Subjective organization (SO) refers to the extent to which subjects spontaneously impose a sequential structure on the free recall of unrelated words. It is well established that SO is important to efficient learning and memory in normals; however, the neuroanatomic underpinnings of this process are minimally known. In light of recent findings implicating the frontal lobes in spatial-temporal processing, we hypothesized that frontal lobe lesions may cause a disproportionate deficit in subject-imposed sequential organization. To investigate this possibility, we compared the SO of 30 patients with stable, focal lesions to frontal and non-frontal cerebral regions. Using a free recall paradigm, each subject was administered a 15 word list for 5 learning trials. We tabulated the extent to which each subject imposed a second-order sequential organization among recalled words, using the standardized formula derived by Tulving (1962). Results indicated: 1) A diversity in SO performance amongst the frontal lesion group depending upon specific lesion site. Subjects with dorsal lateral frontal lesions obtained lower SO scores than the orbital frontal lesion group; and 2) Subjects with dorsolateral frontal lesions also obtained lower SO scores than non-frontal lesion subjects, but the SO of the orbital frontal and non-frontal lesion groups did not differ. We propose that the dorsal lateral frontal lobe plays an important role in the subjective organization of unrelated words in free recall, but the orbital sector does not.

507.8

EVIDENCE FOR A DOUBLE DISSOCIATION AMONG LEARNING PARADIGMS IN PATIENTS WITH FRONTAL AND NON-FRONTAL CEREBRAL LESIONS. L.M. Grattan,[1] *P.J. Eslinger[2] & K.E. Mattson.[1] Depts. of Neurology, [1]Univ. of Maryland Medical School, Baltimore, MD 21201 & [2]Penn State Univ. College of Medicine, Hershey, PA 17033.

A variety of experimental procedures have been identified for investigating the conditions under which learning occurs. Studies have commonly employed three major learning paradigms: free recall, paired associate learning, and serial learning. Factor analytic study of normals has demonstrated that serial learning may operate independently from paired associate and free recall learning, clustering with executive measures on a "frontal lobe" factor (Eslinger, Grattan, Benton, 1988). This difference raises the possibility that these learning paradigms are subserved by different neural systems. To test this hypothesis, we examined 30 patients with CT/MR verified frontal and non-frontal lobe lesions with standardized measures of verbal free recall, paired associate learning and serial digit learning. Results indicated a dissociation amongst the 3 learning paradigms. The frontal lobe group demonstrated significantly greater impairment on the serial learning task while the non-frontal lobe group had more deficiencies on associate learning and free recall tasks. The results support the hypotheses that 1.) serial learning may involve distinctive processes from other learning paradigms and 2.) the frontal lobes may have a specialized role in establishing the stable temporal-spatial positions necessary for serial learning.

507.9

CASE-STUDY EVIDENCE FOR A CRITICAL AND SPECIFIC RIGHT OCCIPITAL-LOBE CONTRIBUTION TO PERCEPTUAL IDENTIFICATION REPETITION PRIMING. D.A. Grosse*, J.D.E. Gabrieli, and S.L. Reminger. Rush Medical College, Chicago, IL. 60612.

Two case studies are reported that suggest right occipital mediation of one form of visuoperceptual implicit memory but no critical role for that same brain region in conceptual implicit memory or in explicit memory. Subjects were 2 patients with unilateral left or right occipital resections. The performance of the left occipital patient was compared to 3 matched controls on a perceptual implicit test of word perceptual identification (PI), with a matched yes/no explicit memory recognition test, and a conceptual implicit memory test of category exemplar generation (CE), with a matched cued recall explicit memory test. For the PI and recognition study, subjects read aloud 24 words each presented twice. For the PI test, subjects identified briefly presented and masked words (24 repeated, 24 baseline). Perceptual priming was measured as the advantage in identifying repeated versus baseline words. For the recognition test, subjects judged whether 48 words (24 repeated, 24 foils) were seen in the study phase. For the CE and cued recall study, subjects made semantic or surface-level judgments about 48 low-typical exemplars in 6 semantic categories. For the CE test, subjects were given 12 category names and asked to provide 8 exemplars; 6 categories provided a baseline measure. Conceptual priming was measured as the number of study-list exemplars generated relative to baseline. For the recall test, category names were used as cues to recall study list words. The right occipital patient showed normal levels of conceptual priming, yes/no recognition accuracy, and cued recall accuracy, but no PI priming. The left occipital patient showed normal magnitudes of priming and normal levels of recall and recognition accuracy. These results (1) show a dissociation between the neural substrates supporting perceptual and conceptual implicit memory; (2)suggest that right occipital cortex mediates at least one component of perceptual implicit memory; and (3) provide a reverse dissociation to that seen in global amnesia, with impaired implicit memory on the PI test despite intact explicit memory on the recognition test. Supported by McDonnell-Pew Cognitive Neuroscience Program.

507.10

VERBAL IMPLICIT RECALL OF SUBJECT PERFORMED TASKS BUT NOT VERBAL TASKS IN ALCOHOLIC KORSAKOFF'S AMNESICS. T.W. PARKER*, Department of Psychology, Augustana University, Camrose, Alberta, T4V 4A2

Implicit recall of subject performed tasks (SPT's) versus verbal tasks (VT's) was tested for alcoholic Korsakoff patients with dense amnesia and for normals. Tasks involved having subjects either perform (SPT) or describe (VT) an unusual manipulation of pairs of common objects. Implicit recall tests, which made no mention of the earlier study phase, were then conducted after 10 minutes, 24 hours and 30 days for each subject. No feedback was given during the tests.

An overall ANOVA for repeated measures showed significant main effects for tests and for task type, and a significant test by task interaction. These results indicated that for both groups implicit recall of SPT's was markedly superior to that of VT's and that more decay over tests occurred for VT recall. This decay was more pronounced for Korsakoff subjects. However overall Korsakoff performance did not differ from normals, especially in recall of SPT's.

These results are seen as supporting the proposal that SPT's involve implicit memory components (Nilsson & Backmann, 1989) and hence are recalled normally by amnesics, in contrast to verbally mediated tasks. On the other hand, better recall of VT's by the normals suggests they may be using explicit memories to aid their recall.

507.11

SEPARATING DECLARATIVE MEMORY AND PROCEDURAL LEARNING IN ALCOHOLIC AMNESICS. A.G.M.Canavan, V. Hömberg and G.E. Stelmach* Neurological Therapy Centre, University of Düsseldorf, Federal Republic of Germany, W4000 Düsseldorf 13.

Abstinent alcoholic amnesics (n=10), as defined by performance on a battery of verbal and visuospatial declarative memory tasks (e.g., memory for stories, memory for abstract designs), were also severely impaired in the Sternberg memory scanning paradigm compared to alcoholic non-amnesics (n=8) and healthy controls (n=9). Standard tests of short term memory (e.g., digit span, block tapping span) yielded mixed results.

Procedural learning was intact in the amnesics. Learning to draw with only a mirror image as visual guidance improved normally within and between two consecutive daily sessions, with the amnesics eventually achieving performances identical to those of controls, despite the fact that they were initially much poorer.

On a new computerised version of the fragmented pictures task, administered over three consecutive days, the amnesics were unable to say (free recall) which famous faces they had seen the day before, but they were able to identify the masked pictures sooner (i.e., with a higher degree of masking) if they had seen them the day before than if the pictures were novel. Visual scanning of the facial stimuli, as assessed with an infrared eye-movement recording system (ASL EM-Monitor 210) was not abnormal in either alcoholic group, providing yet another dissociation between procedural and declarative elements of task performance, and ruling out an attentional deficit as underlying impaired recall.

507.12

SPEECH ONSET IN BOY AGED 10 FOLLOWING LEFT HEMISPHERECTOMY AND WITHDRAWAL OF ANTICONVULSANTS. F. Vargha-Khadem and E. Isaacs. Institute of Child Health and Hospital for Sick Children, London, England. (SPON: European Brain and Behaviour Society)

Patient PO, born with port-wine stain characteristic of Sturge-Weber Disease, developed seizures neonatally that later turned intractable. By six months of age, PO exhibited right hemiplegia and hemianopia. By age 8½, though PET and MRI-scans revealed a normal right hemisphere, PO's developmental age was less than 2, and his only spoken word was "mama". Left hemispherectomy, performed when PO was nearly 9, arrested all seizures, but anticonvulsants were continued as prophylaxis for one year. Follow-up at this time showed virtually no change in cognitive development, at which point anticonvulsants were withdrawn. One month later, PO began speaking, initially in single words but then progressing rapidly to phrases and long sentences. Now age 11, his mental age is 6 years, receptive vocabulary 5.11 years, productive grammar 6.6 years, and mean length of utterance nearly 7 years. PO's rapid and constant improvement to date indicates that further progress is to be expected. This surprising outcome challenges the traditional view pertaining to critical periods for the development of language and speech.

507.13

SLOW CORTICAL POTENTIALS IN CLASSICAL CONDITIONING. H.Flor, N.Birbaumer, W. Lutzenberger, and T.Elbert*. Inst. of Medical Psychology, Univ. of Tübingen, 7400 Tübingen, FRG.

The repeated occurrence of events with high probabilistic associaton elicits a distinct brain reponse, the contingent negative variation (CNV), which may be used as a tool in the description of the associative process during conditioning. It was assumed that the early component of the CNV (initial CNV, iCNV) might represent the amount of anticipatory arousal with respect to the US that is elicited by the CS and would thus be especially sensitive to changes in meaning of the CS over time. The late component of the CNV (terminal CNV, tCNV) should indicate preparation for the CR.

Experimental evidence from a differential conditioning study in humans that used slides with varying emotional content as CS, electric shock as US, and autonomic and motor responses as UR/CR, indicated a clear lateralization of the tCNV on the side of the CR. A significant differentiation between the CS^+ and CS^- could be observed. The quality of conditioning was reflected in the height of the tCNV.

The data from this experiment suggest that the CNV may be regarded as a valid measure of the associative strength during classical conditioning.

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507.14

ELECTROPHYSIOLOGICAL INDICANTS OF READING DISABILITY OBTAINED IN THE FIRST AND THIRD GRADE. S.L. Miller*. Dept. of Psychology, University of North Carolina at Greensboro, Greensboro, NC 27412.

Fifteen reading disabled (RD) and fifteen non-reading disabled (NRD) subjects, selected as being "at-risk" for later reading problems, were compared on event-related potentials (ERPs) and behavioral measures. ERPs were recorded during a black-white discrimination task of visually presented letter and non-letter stimuli when the subjects were an average age of 6.7 years and again two years later. The RD, as compared to the NRD group, showed longitudinally stable reductions in neural activity (a) present within the initial 100-140 msec after stimulus presentation and (b) greater for letter than non-letter stimuli and larger over the left than right hemisphere at 180-240 (N2) and 460-600 (P3) msec after stimulus presentation. Behaviorally, individuals with a reading disability demonstrated slower and less accurate task performance. Both groups, however, did demonstrate a high level of task accuracy and showed faster performance during the black-white discrimination of letters as compared to non-letter patterns. These reductions in ERP amplitude are considered to reflect a reduction in selective neural processing for the reading disabled group during the task. The data suggest further that these deficits involve multiple levels of neural processing in the geniculate-striate visual processing system and are frequently greater over the left than right hemisphere. Supported by NIH Grant RO1 NS19413-08

507.15

COORDINATE FRAME FOR IMAGE DESCRIPTION STORED IN MEMORY. M.B. Pavlovskaya* and I.A. Vol. Dept. Physiol. and Pharmacol., Sackler Sch. Med., Tel Aviv Univ., Tel Aviv, 69978, Israel.

We have recently found that an appropriate description of the object seems to be magnitude of its spectral description in a frame of reference whose origin point is chosen for each object separately. We have now carried out psychophysiological experiments to test the hypothesis if the performance is better with attention allocation to this point. Observers were presented under time-pressure condition with one of five pre-learned stimuli. Stimulus presentation was preceded by a precue signal that was relevant for one of the stimuli, in that it coincided with the origin point for this stimulus. The results show that percentage of correct identification for all stimuli is significantly higher for relevant as compared to other precuing. We calculated the Euclidean distances between the relevant stimulus description assumed to be stored in memory and the Fourier spectrum with the actual precue. A good fit of experimental to theoretical confusion matrices generated on the basis of these distances is manifested by the high values of correlation coefficients. We conclude that attention allocation by precuing the origin point of the object's reference frame improves significantly the recognition process.

508.1

ANALYSIS OF SINGLE UNIT RECORDINGS FROM CEREBELLAR CORTEX OF CLASSICALLY CONDITIONED RABBITS. M.R. Foy*, D.J. Krupa, J.Tracy and R.F. Thompson. *Dept. of Psychology, Loyola Marymount University, Los Angeles, CA 90045 and Neurosciences Program, Univ. So. Calif., Los Angeles, CA 90089.

The cerebellum forms an essential portion of the neural circuitry necessary for the development and expression of the associative elements which mediate rabbit eyeblink conditioning. An analysis of extracellular single unit activity recorded primarily from hemispheral lobule VI (HVI), but also from portions of ansiform cortex (Crus I and II) and the paramedian lobule (PM) of cerebellar cortex in classically conditioned rabbits was done to characterize neural responses to the various components of the stimulus events (tone CS and airpuff US) and the behavior exhibited on the trial (CR and UR). Through a raster analysis of unit activity sorted by onset of behavior in the well-trained rabbit, we have found units that display either a decrease in rate of simple spike discharge that preceded and modeled the learned behavioral eyelid response, along with a smaller population of cells which displayed an increase in the rate of simple spike firing that preceded and modeled the CR. A third population of units displayed neither an increase or decrease in their rate of firing to the stimuli events. These analyses reveal several classes of neural responses correlated with behavior in well-trained rabbits which may play important modulatory roles in the activity of "deep" cerebellar nuclei to which they project. Supported by LMU Institution Faculty Research Grants (MRF), ONR N00014-19-J-1392, NSF BNS-8718300 and The McKnight Foundation (RFT).

508.2

ASSOCIATIVE LONG-TERM DEPRESSION REVEALED BY FIELD POTENTIAL RECORDING IN RAT CEREBELLAR SLICE. C. Chen* and R. F. Thompson. Neurosciences Program., University of Southern California., Los Angeles, CA 90089-2520.

Field potential recording in the brain slice is essential for understanding long-term plasticity because it reflects population changes and it is stable. The difficulty in obtaining well-defined field potentials in cerebellar slice has been a major obstacle in studying long-term depression (LTD) (Ito, *Ann. Rev. Neurosci.*, 1989). We have reliably recorded the field potential of Purkinje cell (PC) dendrites evoked by parallel fiber (PF) stimulation in young adult rat cerebellar slice (sagital section of 400 um), and have identified TTX-sensitive action potentials and AMPA receptor-mediated EPSP (blocked by 10 uM CNQX). The response evoked by activating the climbing fiber (CF) through the white matter stimulation can also be blocked by CNQX. By using the field potential recording in the absence of GABA blockers, consistent LTD (30±12% decrease from baseline EPSP amplitude, 60 min post pairing, n=9) of PF-PC synapses can be induced by 600 pairings (1 Hz) of a PF stimulus (0.1 ms) followed 250 ms later by a CF stimulus. The PF-EPSP in the nonpaired pathway recorded 300 um away from the paired one shows a small increase (18+15%, n=9), indicating that the LTD is localized in the paired pathway and the slice is healthy over the course of study. This new interstimulus interval for LTD induction may have some relevance to behavioral motor learning.

508.3

CHANGES IN THE BINDING PROPERTIES OF GLUTAMATE RECEPTORS FOLLOWING LONG-TERM ENHANCEMENT (LTE) OF PERFORANT PATH SYNAPTIC TRANSMISSION IN AWAKE RATS. G. Rao*[1], S. Maren, G. Tocco, M. Baudry, R. F. Thompson, B. L. McNaughton[1], and C. A. Barnes[1]. Neurosciences Program, Univ. So. Cal., Los Angeles, CA 90089, and [1]ARL Div. Neural Systems, Memory and Aging, Univ. Arizona, Tucson, AZ 85724.

The mechanism of long-term synaptic enhancement (LTE) is still vigorously debated, and there is evidence for both pre- and postsynaptic changes. We have examined the binding properties of glutamate receptors following induction of LTE at perforant path-granule cell synapses of awake rats. Anesthetized adult male Fisher-344 rats were implanted bilaterally with a monopolar stimulating electrode in the perforant path and a recording electrode in the hilus of the dentate gyrus. Following recovery, 8 rats received 11 high-frequency perforant path stimulation sessions (10 25 msec 400 Hz bursts separated by 10 sec per session) over 3 days, and 6 rats received 10 high-frequency sessions over 6 days. An equal number of stimuli were delivered to the opposite hemisphere at low frequency (1 Hz) to yield an internal control. Either 4 hours (8 rats) or 24 hours (6 rats) following the final stimulation session, rats were sacrificed and their brains rapidly dissected and frozen. Quantitative autoradiography of [^{3}H]-AMPA and [^{3}H]-TCP binding to frozen brain sections was used to examine the AMPA and NMDA subclasses of glutamate receptors, respectively. LTE induction resulted in a significant increase in AMPA binding in the dentate gyrus (stratum moleculare) as compared to the hemisphere receiving low-frequency stimulation. No significant changes in [^{3}H]-TCP binding were observed in any treatment group. These results suggest that the expression of hippocampal LTE involves a persistent modification in some properties of postsynaptic AMPA receptors. [Supported by AG05142 and the McKnight Foundation to RFT, NSF to MB, AG03376 to CAB, and ONR to BLM].

508.4

SYNAPTIC ACTIVATION OF TRANSCRIPTION FACTORS: DISSOCIATION OF STIMULUS REPETITION AND LTE IN HIPPOCAMPUS OF AWAKE BEHAVING RATS. P.F. Worley[1], R.V. Bhat[1], J.M. Baraban[1], G.D. Stevenson*, B.L. McNaughton, G. Rao and C.A. Barnes. [1]Dept. Neuroscience, Johns Hopkins University, Baltimore, MD 21205, and ARL Div. Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

High-frequency synaptic activation of hippocampal granule cells induces a rapid increase in mRNA of specific transcription factors, suggesting a role in long-term synaptic enhancement (LTE or LTP; Cole et al., Nature 340:474, 1989). This genomic response appears to include *zif*268, c-*fos*, and *jun*-B; however, the reproducibility and magnitude of these responses has been variable and may be altered by anesthesia, injury and the stimulation protocol. To evaluate the relationship between LTE and genomic responses further, we have used chronically implanted, unanesthetized animals that receive a stimulation protocol sufficient to induce LTE lasting about 3 days (10, 25 msec trains at 400 Hz, separated by 10 sec). In each of 18 preparations, *zif*268 mRNA was induced to levels identical to those induced by maximal electroconvulsive seizures (MECS) 30 min after the stimulus. By contrast c-*fos* and c-*jun* were not detectably increased in any of the high frequency preparations but were strongly induced by MECS. *Jun*-B mRNA was weakly induced, relative to MECS in all preparations. With more repetitions of high frequency stimulation at a shorter interval (i.e., 50, 20 msec trains at 400 Hz, separated by 20 sec), there was no further LTE induction; however a weak c-*fos* mRNA response was observed. Our results indicate a differential threshold for synaptic activation of these transcription factors and suggest a special role for *zif*268 in LTE. [Supported by AG09219 and ONR]

508.5

NITRIC OXIDE SYNTHASE INHIBITION *IN VIVO* HAS NO EFFECT ON HIPPOCAMPAL SYNAPTIC ENHANCEMENT OR SPATIAL MEMORY C.A. Barnes*[1], B.L. McNaughton[1], D.S. Bredt, C.D. Ferris and S.H. Snyder [1]ARL Div. Neural Systems, Memory & Aging, U. Arizona, Tucson, AZ 85724, and Dept. Neuroscience, Johns Hopkins Sch. Med., Baltimore, MD 21205.

In vitro studies suggest that nitric oxide translates induction events into expression of long term enhancement (LTP/LTE) of hippocampal synapses (Bohme et al., 1991; Shuman & Madison, 1991; O'Dell et al., 1991). NO synthesis is induced post-synaptically by an NMDA receptor dependent mechanism, but appears to increase transmitter release from terminals near the site of NO release; however, such a presynaptic mechanism for LTE has been the subject of controversy (Malinow and Tsien, 1990; Bekkers and Stevens, 1990; Foster and McNaughton, 1991; Menabe and Nicoll, 1992). We have studied the effects of *in vivo* treatment with L-N^G-nitroarginine (NO_2ARG), an irreversible inhibitor of NOS (Dywer et al., 1991). Rats were implanted bilaterally with electrodes for stimulation and recording of perforant path synaptic potentials in fascia dentata. Responses were collected daily over a 14 day period. On day 6, daily i.p. injections of NO_2ARG (20-50 mg/kg) [n=7 rats] or vehicle [n=6 rats] were begun. On days 10 and 11, spatial memory was tested using the Morris water task. On day 12 and 13, unilateral high frequency stimulation (HFS) was given twice at a 2 hr interval. Fractional enhancement of the EPSP and population spike 24 hours after HFS were compared with the control hemispheres between groups. Enzymatic assay indicated at least 90% inhibition of NOS. There was no effect of NOS inhibition on either control responses or the enhancement of the EPSP or population spike, and no effect on spatial learning. We conclude that either there is an additional source of NOS activity in hippocampus, or that the form of LTP blocked *in vitro* in CA1 by NOS inhibition does not reflect the same process as is induced *in vivo* in FD. [Supported by NIMH].

508.6

LACK OF AN EFFECT OF TEMPERATURE ON TETANUS INDUCED ENHANCEMENT OF SYNAPTIC FIELD POTENTIALS IN CA1. J. Shen*, G. Rao, C.A. Barnes and B.L. McNaughton. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Previous studies (Foster & McNaughton, 1991) suggest that low temperature (22°C) results in a substantial increase (compared to 32°C) in the magnitude of synaptic enhancement induced by pairing 2 Hz stimulation of single or small numbers of presumed Schaffer collateral axons with intracellular depolarizing current pulses. The excess EPSP enhancement was associated with an apparent increase in quantal content that was not seen at 32°C, where only changes in quantal size were observed. Curiously, although quantal size changes were apparently blocked by APV or the omission of the depolarizing pulses during 2 Hz stimulation, the apparent increase in quantal content was not. Because reduced temperature in some systems leads to a very marked prolongation of PTP (Schlapfer et al., 1975), we have investigated the effects of temperature on tetanus (100 Hz, 1 sec x 2) induced potentiation and enhancement of field EPSPs in CA1. Low temperature led to an increase in the initial elevation of the field EPSP (PTP), but there was little effect on its apparent decay rate, and no effect on the magnitude of the persistent phase of enhancement (about 40%). Apart from the possibility that the previous results reflect statistical error (i.e., false positive results), we see two possible explanations. There could be important differences between pairing induced synaptic enhancement and the response to synchronous high frequency stimulation. Alternatively, it is quite possible that there are persistent, temperature dependent effects of repetitive stimulation on axonal excitability. If there were fewer conduction failures following 2 Hz stimulation at 22°, this would appear as an apparent increase in quantal content. These possibilities are under investigation. [Supported by ONR].

508.7

COMPLEMENTARY ROLES OF HIPPOCAMPUS AND NEOCORTEX IN LEARNING AND MEMORY. J.L.McClelland, B.L.McNaughton*, R. O'Reilly, & L.Nadel. Dept. Psych., Carnegie-Mellon U., Pittsburgh PA 15213 & ARL Div. Neural Systems, Memory & Aging, U. Arizona, Tucson, AZ 85724.

We think of experiences as samples drawn from a probabilistic environment, and suggest that the neocortex adjusts its synaptic strengths to maximize its ability to respond appropriately to future inputs drawn from the same environment. Finding satisfactory connection strengths requires gradual adjustment to allow the overall direction of weight changes to be guided by an adequate sample of the environment. In simulations, such gradual learning leads to powerful cognitive representations, but a key requirement is that new information must be accommodated slowly. Weight changes large enough to store arbitrary aspects of individual events derail the sampling, producing 'catastrophic interference'. We suggest hippocampus is specialized for rapid storage of just such information. Such information is at the same time treated like any other sample by the cortex and if the information recurs in the mix of other experiences, it gradually becomes integrated into the cortex. One way the information may recur is via recall from hippocampus; in such cases hippocampus acts as teacher to the cortex. Because cortical learning must be gradual, memories initially dependent on hippocampus can only gradually lose this dependence. Thus, graded retrograde amnesia is seen as a reflection of a key design characteristic of the two-part memory system. A further consequence is that the connections in the pathways between the cortex and the hippocampus must themselves be stable. This notion has led us to consider a new possible function for area CA1, for its relatively point-to-point reciprocal connections (Tamamaki pers. com.) with entorhinal cortex, for the low density of NMDA receptors found in stratum lacunosum, and for the ability of direct EC inputs to drive CA1 pyramidal cells (Yeckel & Berger, 1991). [Supported by grants MH47566 & MH00385 to JLM and MH46823 to BLM]

508.8

LARGE SCALE PARALLEL RECORDING OF MULTIPLE SINGLE UNIT ACTIVITY IN THE HIPPOCAMPUS AND PARIETAL CORTEX OF THE BEHAVING RAT. M.A. Wilson*, B.L. McNaughton, K. Stengel. ARL Div. of Neural Systems, Memory and Aging, Univ. of Arizona, Tucson, AZ 85724.

A microdrive array capable of independent vertical adjustment of 12 recording electrodes ("tetrodes") each containing 4 recording sites and separated by 250 μm was used to simultaneously isolate multiple single unit activity from the somatosensory regions of parietal cortex and hippocampal formation of the freely moving rat. Spike and EEG data were preprocessed using a custom built, computer controlled, 56 channel amplifier/filter array, and collected using seven synchronized 80486 computers. Animals were trained to perform a forced-choice alternation task on a two-armed maze for food reward. Animal position and head direction were recorded by video camera while single unit activity was monitored. Activity has been successfully recorded from both cortical and hippocampal regions; however, only the cortical data have been analyzed to date. During a single recording session, 61 cells in sensory-motor cortex were simultaneously monitored while the animals either performed the task or were subjected to somatosensory stimulation. Of these cells, 30% showed various degrees of turn biased firing, 20% had preferred responses during linear running behavior, and 50% had non-specific or non-movement related responses. A preliminary analysis of the ensemble activity was performed by constructing population vectors composed of firing rates for each cell over 100 msec to 10 sec time bins. The correlations between vectors at different times over the course of a single maze trial were compared and repeating vector patterns which encoded turning direction were identified. Ongoing work involves extending the analyses of ensemble encoding of behavioral state and spatial information. [Supported by NSF, NS20331 and MH46823].

508.9

QUANTIFICATION OF WHAT IT IS THAT HIPPOCAMPAL CELL FIRING ENCODES. W. E. Skaggs*, B. L. McNaughton, ARL Div. Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

We describe an information theoretic approach to determining what it is that neural firing encodes, obtaining a measure of the amount of information (in bits) that each spike conveys about any particular measured variable(s). The technique treats the cell as a communication channel whose input is the variable(s) and whose output is the spike train. Using data recorded from hippocampal cells in freely moving rats, this *information* measure is compared to several other possible measures of position-dependency of activity, including *sparsity* (the mean square spatial firing rate divided by the square of the mean), *coherence* (the correlation of firing rate with the firing rate at neighboring locations), *dispersion* (the RMS distance of location from the centroid of the firing-rate distribution, weighted by firing rate), and *existence of a place field*, determined using a method described by Muller et al. (1987). As a control, each measure is also computed 100 times with the spike data randomly time-shifted relative to the animal's position and behavior data, and a Z-score is obtained from which the significance can be determined. The information measure is the most sensitive detector of place-dependency, with sparsity following closely. Dispersion is only useful when the cell has a single strong place field. Coherence is less sensitive and depends on selecting the right spatial scale. The existence measure is easy to understand, but difficult to treat statistically, and depends on selecting good thresholds for firing rate and field size. We discuss several issues, including: how to bin data to obtain the most meaningful measures; what to use as the *a priori* probability distribution for the information measure; and the critical importance of an appropriate control. Finally, we use the information measure to disentangle the contributions of place, head direction, and velocity to a cell's activity, and compare the results with the outcome of a multiple regression analysis. [Supported by MH46823]

508.10

PLACE FIELD SPECIFICITY DEPENDS ON PROXIMITY OF VISUAL CUES. K.M. Gothard*, W.E. Skaggs, B.L. McNaughton, C.A. Barnes and S.P. Youngs. ARL Division of Neural Systems, Memory, and Aging, University of Arizona, Tucson, AZ 85724.

Gaese et al. (1991) showed that the "place fields" of hippocampal complex spike cells are more specific when visual cues are proximally situated. Using a quantitative measure of place field specificity (see preceding abstract), we investigated how this difference reflects the animal's previous experience and the information provided by the visual world. Place cells were recorded with the stereotrode technique consecutively in an opaque cylinder with cue cards on the walls of the cylinder (proximal cues), and in a transparent plexiglass cylinder with a visually similar array of cues on the walls of the recording room (distal cues). The order of the two environments was alternated. Between trials, the animals were allowed to experience the whole room. Using either multiple regression or factorial ANOVA to account for contributions of mean running speed, mean firing rate, trial order, and environment, we found that place cells (89) showed significantly ($p < .005$) higher specificity in the opaque cylinder. We also found that the overall distribution of place cell mean firing rates is 25% more sparse in the transparent cylinder. These results suggest that the distal-cue environment is represented by a smaller population of cells with less specific place fields. This outcome supports the theory that place cell activity is partially determined by the "local view" seen by the animal, because in the distal-cue environment there is less parallax of visual cues as the animal moves from place to place. Order also had a significant ($p < .01$) effect on specificity, with place fields more specific in the second of the two consecutive sessions. As the second session was always preceded by exploration of the entire room, this may reflect the short-term effect of the intertrial experience. [Supported by NS 20331].

508.11

SPATIAL FIRING CHARACTERISTICS OF DENTATE GYRUS GRANULE CELLS. M.W. Jung*, B.L. McNaughton and T.W. Abel. ARL Division of Neural Systems, Memory and Aging, Univ. of Arizona, Tucson, AZ 85724.

Theoretical arguments concerning the role of the dentate gyrus in pattern separation and associative memory (Marr, 1971; McNaughton and Nadel, 1990) suggest that granule cells should exhibit sparse coding, i.e., they should fire only rarely at high rates, and should have tight 'place' fields. In contrast, several previous attempts to characterize granule cells *in vivo* have concluded that they fire at high rates, have no obvious place fields, and are similar to 'theta' cell interneurons found throughout the hippocampus; however, Mizumori et al. (1989) found, in anaesthetized animals, that the high rate and low rate cells in the granular layer could be separated on the basis of the failure of high rate cells to be inhibited during paired-pulse inhibition of the population spike, and the ability to drive high rate cells (but not low rate ones) below population spike threshold. They concluded that granule cells fire at low rates. We have studied the spatial and temporal firing properties of cells located (on the basis of histology and field potential profiles) in or very near the granular layer. Using stereotrode recording and real-time cluster plotting of spike parameters that facilitates the detection of very low rate cells, we find that most (38/44) cells in the layer have low rates (0.01 - 0.5 Hz), cannot be driven orthodromically below population spike threshold, and exhibit spatial selectivity equal to or better than the average CA3 pyramidal cell. Firing fields are highly directional. A number of these cells exhibit unusual spatial firing distributions consisting of three or four very tight fields distributed over the radial maze surface. Although the issue of the identity of low and high rate cells is by no means definitively resolved, the weight of evidence currently supports the conclusion that granule cells exhibit temporally and spatially sparse encoding. [Supported by NS20331].

508.12

DECREASE IN THE INFORMATION CONTENT OF HIPPOCAMPAL CA1 CELL SPATIAL FIRING PATTERNS IN THE DARK. E.I. Markus*, C.A. Barnes, B.L. McNaughton, V. Gladden, T.W. Abel and W.E. Skaggs. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

In adult rats some hippocampal cells exhibit similar "place fields" in a given environment both under light and dark conditions as long as the light condition precedes the dark (McNaughton et al., 1989; Quirk et al., 1990). We were interested in quantifying the relationships between spatial firing in the light and dark and comparing young and old rats.

Young adults (9 month) and old (22 month) F-344 rats were trained to perform a forced choice task on an eight arm radial maze in a soundproof room. The rat was placed on the center of the maze in an illuminated room and subsequently ran alternate trails with the room either illuminated or darkened. Cells were recorded in the CA1 pyramidal cell layer using stereotrode recording methods.

Place fields were less specific and less reliable in the old rats and both age groups exhibited reduced place field specificity and reliability in the dark. There was no significant interaction between age and light condition. [Supported by AG03376 and NS 20331].

508.13

EXPLORATION-INDUCED CHANGES IN SYNAPTIC STRENGTH IN HIPPOCAMPUS CAN PREDICT SPATIAL MEMORY IN THE MORRIS WATER TASK. C.A. Erickson*, D.L. Korol, C.A. Barnes & B.L. McNaughton. ARL Div. Neural Systems, Memory and Aging, U. Arizona, Tucson, AZ 85724.

Exploratory behavior results in increased perforant path evoked EPSPs and decreased population spike area and onset latency. These changes, collectively called short-term exploratory modulation (STEM), can be predicted from the amount of exploratory behavior (Green, et al., 1990; Sharp, et al., 1989). It is unknown whether STEM reflects information storage *per se* or some less specific process. We compared the magnitude of STEM with the amount of exploration during recording sessions in a cue-filled environment and with spatial learning ability in the Morris water task in young and old Fisher-344 rats. There was a strong relationship between the amount of STEM and spatial learning ability: EPSP growth was significantly correlated with spatial learning, measured by latency to escape to a hidden platform ($r^2 = 0.308$, $p = 0.002$) and percent of time spent in the target quadrant during a free-swim probe trial ($r^2 = 0.434$, $p = 0.011$). There was also a correlation between exploration and probe trial quadrant search ($r^2 = 0.562$, $p = 0.003$). When changes in the EPSP (STEM), age, and exploratory behavior were entered into a stepwise regression analysis, synaptic change (STEM) emerged as the best predictor of the variability in spatial learning. The relative spike attenuation in a recording session was significantly correlated with the amount of exploratory behavior ($r^2 = 0.170$, $p = 0.025$), but not to spatial ability. These data provide support for the hypothesis that STEM may be involved in spatial memory. It remains to be determined, however, whether this relationship reflects the actual storage of spatial information via specific synaptic alteration, or whether STEM reflects an important, but non-specific modulator of information transmission through the hippocampus. [Supported by AG03376 and ONR].

508.14

THE ROLE OF NORADRENERGIC INPUT IN THE GENERATION OF SHORT-TERM SYNAPTIC PLASTICITY IN THE DENTATE GYRUS. S. Davis*, G.L. Wenk, A. Sage, C.A. Barnes and B.L. McNaughton. ARL Div. Neural Systems, Memory and Aging, Univ. of Arizona, Tucson, AZ 85724.

Short-term exploratory modulation (STEM) is a form of naturally occurring synaptic plasticity that has been observed in the rat dentate gyrus (Sharp et al., 1989). It occurs during exploration, learning of a task or simply if the rat is placed in a novel environment. STEM shares some, but not all properties of LTE induced by high frequency stimulation (Erickson et al., 1991; McNaughton et al., 1991). Typically, when using only low frequency stimulation of the perforant path, the population EPSP increases and the population spike (PS) decreases during exploration. These effects persist for many minutes. Norepinephrine (NE) has been shown to modulate the induction of LTE (Bliss et al., 1983) and also produces lasting effects of its own (LaCaille & Harley, 1985; Stanton & Sarvey, 1985). We tested the effect of DSP4, a neurotoxin that depletes forebrain NE, on the generation of STEM. Rats with chronically implanted electrodes (N=10) were trained to run a forced choice (FC) task on the radial arm maze. The perforant path evoked response was measured before and after behavioural testing to assess the amount of STEM generated, both before and after DSP4 treatment. This treatment had no overt effect on the behavior or the baseline EPSP and PS. It did, however, reduce the magnitude of the increase in the EPSP by approximately 50% and the decrease in the PS by approximately 50%. Systemic administration of either α or β adrenergic antagonists resulted in dose-dependent reductions in STEM. These results indicate that NE is necessary for the full expression of STEM; however, whether NE produces STEM directly or whether it modulates the neuronal activity required for STEM is not as yet known. [Supported by ONR, NSF & McDonnell-Pew Fdn.]

508.15

DOES SATURATION OF LONG-TERM ENHANCEMENT OF PERFORANT PATH SYNAPSES IMPAIR SPATIAL LEARNING IN THE MORRIS WATER TASK? A FAILURE TO REPLICATE. D.L. Korol*, T.W. Abel, L.T. Church, C.A. Barnes and B.L. McNaughton. ARL Div. Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Electrically induced hippocampal synaptic enhancement (LTE/LTP) is believed to reflect activation of a physiological memory mechanism. One of the cornerstones of this theory relies upon the observation that experimental saturation of LTE at a substantial fraction of perforant path terminals leads to an impairment in the acquisition of different spatial memory tasks. Two of these tasks include the Barnes circular platform task, in which animals seek to escape from a brightly illuminated uniform surface to a dark tunnel located under one of 18 peripherally located holes (McNaughton et al., J. Neurosci., 1986), and the Morris water task, in which rats are required to learn the location of an escape platform located just below the surface of a pool of water (Castro et al., Nature, 1990). Recently, several groups of investigators have attempted, without success, to replicate the results of Castro et al. (Morris et al., pers. com.; Cain et al., pers. com.; Sutherland et al., pers. com.). In the belief that these failures might reflect strain differences in the animal subjects used, we have attempted to replicate the Castro et al. study using both (albino) F-344 and (hooded) Long-Evans rats. We found no effects of LTE induction on probe-trial quadrant search time, regardless of whether the animals were tested immediately (14 rats total) or 24 hours (21 rats total) following the last stimulation session. There were also no strain differences. At present, we have no good explanation for the discrepancies, apart from the smaller number of rats used by Castro et al.; however, there are several variables under investigation including pool size and previous experiential history of the animals. Any further results will be reported. [Supported by AG03376, AG05540, and ONR]

508.16

SHORT-TERM EXPLORATORY MODULATION (STEM) OF DENTATE GYRUS EVOKED POTENTIALS IN SIMPLE AND COMPLEX ENVIRONMENTS. S.D. Croll* and E. Bostock, Dept. of Psychology, Queens College - CUNY, Flushing, NY 11367.

Dentate gyrus evoked responses of rats transferred into different environments show rapid increases in EPSPs and decreases in population spikes termed short-term exploratory modulation (STEM). Rats exposed to complex environments show increases in EPSPs and spikes. We studied STEM in complex environments. Rats were chronically prepared for stimulating in the perforant path and recording from the dentate gyrus. Rats were transferred into simple or complex environments for 7 daily 20 min sessions. Complex environment rats received either novel or the same daily stimuli. Simple environment rats were in either dim or normal lighting. During all sessions comparable STEM occurred in all groups, confirming that STEM occurs after any environmental transfer, including to a complex environment. EPSP enhancements decreased across daily sessions for all groups, suggesting that STEM may decrease in magnitude with repeated exposure to similar environments. Further analyses will examine whether EPSP and spike values change between days independent of STEM.

508.17

THE SHORT-TERM EXPLORATORY MODULATION (STEM) OF PERFORANT PATH SYNAPSES REFLECTS INCREASED BRAIN TEMPERATURE. E.I.Moser*, I.Mathiesen & P.Andersen, Inst. of Neurophysiology., Univ. of Oslo, Box 1104 Blindern, 0317 Oslo, Norway.

During free exploration, the field synaptic potential (epsp) in the rat dentate gyrus gradually increases whereas the spike amplitude and latency is reduced (Sharp et al.,1989, *Psychobiol.,17*, 257-69; Green et al.,1990, *J.Neurosci.,10*, 1455-71). These effects (STEM) outlast the exploration and have been interpreted as altered synaptic efficiency associated with temporary memory for recent events.

We asked whether STEM is related to increased brain temperature, which gives similar changes of potentials. Rats were chronically implanted with a stimulation electrode in the perforant path, a recording electrode in the dentate granular layer and a thermistor at the same depth in the contralateral hemisphere. Field potentials, brain temperature and exploratory activity were recorded simultaneously. Exploration in a novel environment produced both STEM and enhanced brain temperature (<2.0°C) which increased and decayed with identical time courses. In spite of vigorous exploratory activity, STEM was prevented by keeping the rat at a constant brain temperature using radiant heating. This temperature was equal to the maximal value observed in the same rat during control exploration. In other experiments, the changes in field potentials were larger the lower the brain temperature at the start of exploration. Walking on a motorized treadmill produced both STEM and increased temperature.

Taken together, our results imply that STEM is due to increased brain temperature and also question any relation to memory processes.

509.1

A Ca^{2+}-DEPENDENT PROCESS MEDIATES 1S,3R-ACPD-INDUCED POTENTIATION OF NMDA RESPONSES IN RAT HIPPOCAMPUS.
J. Harvey and G.L. Collingridge. (SPON: Brain Research Association). Dept. of Pharmacology, The Medical School, Univ. of Birmingham, Birmingham, UK.

We have reported previously that 1S,3R-ACPD selectively potentiates responses to NMDA but not AMPA in hippocampal slices (Harvey et al 1991, Br. J. Pharmacol. 104, c79) and these findings have been confirmed in single cells by Anikszteljn et al (Eur. J. Pharmacol. 1991, 205, 327). We have gone on to investigate the mechanisms of this potentiation, using a grease gap method for hippocampal slices. Experiments were performed on slices obtained from female rats (5-8 weeks old) and perfused with a standard Mg^{2+}-containing solution at 28 °C. Staurosporine (1 μM), a PKC inhibitor, thapsigargin (10 μM), which depletes intracellular Ca^{2+} stores, and bromophenacyl bromide (50 μM), a PLA_2 inhibitor, did not affect this potentiation. However these compounds were active as they prevented full expression of long-term potentiation, as reported previously (Matthies et al 1991, Neurosci. Lett. 121, 259; Harvey et al 1992, Neurosci. Lett. in press; Massicotte et al 1990, Brain Res. 537,49).

In a further 5 slices perfused with Ca^{2+}-free medium there was no evidence of this 1S,3R-ACPD-induced potentiation of responses to NMDA. These findings suggest, therefore, the involvement of a Ca^{2+}-dependent process in this potentiation, although the actual mediator has yet to be identified.

509.2

EFFECT OF OLFACTORY LEARNING ON AMPA BINDING IN THE HIPPOCAMPUS AND PIRIFORM CORTEX.
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Modifications of glutamatergic synapses are likely to be involved in learning associated plasticity. We were interested in evaluating a possible change in binding properties of glutamate receptors in regions showing plasticity following an olfactory discrimination task, i. e. the hippocampus and piriform cortex. Radiolabelled AMPA and CNQX on one hand and TCP on another hand were used to label the AMPA and NMDA receptors respectively.

Rats were trained to associate a natural odor with a water reward while an other odor was associated with a light. Forty eight hours before the first training session, the rats were water-deprived. Training consisted in approaching a water delivering spout when one of two odors was delivered. Approach response to the second odor resulted in a 10 sec presentation of a non aversive light. Rats reached 80% of correct responses after 4 training sessions and were overtrained during a 5th session. Animals were sacrificed 3-4 hours after the last session, their brains rapidly removed and frozen in -20 °C isopenthane. Ten μm thick sections were cut in a cryostat and subjected to quantitative ligand binding autoradiography.

Olfactory training in rats produced a significant increase in the binding of [^{3}H]-AMPA in hippocampal regions, dentate gyrus as well as in the piriform cortex,.

These results suggest that an increased responsiveness of the AMPA receptors might be a possible substrate for the synaptic plasticity observed in hippocampus and piriform cortex after an olfactory discrimination learning.

509.3

QUANTITATIVE AUTORADIOGRAPHY OF ENHANCED [^{3}H]MK-801 BINDING IN TRACE CONDITIONED HIPPOCAMPUS.
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NMDA receptors may be critical in the neuronal plasticity underlying associative learning, and are abundant in dendritic fields of hippocampal CA1 and dentate neurons. In rabbits, agonists of the glycine site on the NMDA receptor facilitated acquisition of hippocampally-dependent trace conditioning, while a non-competitive antagonist blocked acquisition (Disterhoft *et al.*, 1990; Thompson *et al.*, 1991). Preliminary work indicated delay conditioning enhanced [^{3}H]MK-801 binding in whole hippocampal homogenates (Thompson *et al.*, 1990). The present study used quantitative autoradiography to determine if learning-dependent changes in MK-801 binding are localized to discrete hippocampal neuronal populations after hippocampally-dependent 500 msec trace conditioning.

Brain sections (20 μm) from trace conditioned, pseudoconditioned, or naive rabbits were labeled with [^{3}H]MK-801 in 30 mM HEPES buffer (pH 7.4) containing 100 μM glutamate, 100 μM glycine, and 1 mM EDTA for 2.5 hr at 23°C (Subramanian & McGonigle, 1990). Nonspecific binding was determined in the presence of 200 μM ketamine. Autoradiographs were quantitated with a high resolution Macintosh™ imaging system and NIMH software (Image 1.44). Hippocampal [^{3}H]MK-801 binding (20 nM) exhibited no dorsoventral or left-right gradients in controls.

Trace conditioning significantly enhanced whole hippocampal [^{3}H]MK-801 specific binding in saturation studies (1—40 nM). Small enhancements were seen in the dentate gyrus, but much larger increases in binding were observed in CA1. This effect was lateralized, with somewhat greater increases in binding seen in the CA1 region contralateral to the conditioned eye. These results suggest that alterations in NMDA receptor number (or in functional receptor number) may underlie some of the CA1-specific learning-dependent changes reported earlier (deJonge *et al.*, 1990). SUPPORTED BY 1 R01 DA07633.

509.4

EFFECT OF MK801 ON LATENT INHIBITION OF THE CLASSICALLY CONDITIONED RABBIT NICTITATING MEMBRANE RESPONSE.
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This study examined the effect of the NMDA antagonist MK801 on latent inhibition (LI), a paradigm wherein nonreinforced pre-exposure to a conditioned stimulus (CS) typically retards subsequent learning to that CS. New Zealand rabbits (2-2.5 Kg), deeply anesthetized with halothane, had a headgear assembly (to hold an airjet and potentiometer) attached to the skull and a nylon loop sutured in the NM. Following a 2 week recovery period, rabbits received either saline or MK801 (0.10 mg/kg; sc) 75 mins prior to each of 4 days of CS pre-exposure (108 nonreinforced presentations of a 1 KHz, 400 ms, 82 dB tone). The intertrial interval averaged 60 s. A third group received saline but no CS pre-exposure and served as controls for LI effects. Conditioning began the day after the fourth CS pre-exposure session. Each conditioning session consisted of 12 blocks of 9 trails each; 8 of the trials were reinforced with a corneal airpuff US (5 psi, 100 ms) that coterminated with the CS and 1 trial served as a nonreinforced test-trial.

As a consequence of nonreinforced CS preexposure, both MK801 and saline treated rabbits showed marked retardation of learning during the early stages of acquisition. Thus, NMDA receptor-mediated plasticity does not appear to be essential for animals to learn to ignore an irrelevant stimulus. This finding is compatible with recent speculation that LI is mediated by nucleus accumbens/medial raphe input to hippocampus (Weiner, 1990). Supported by NSERC.

509.5

NMDA ANTAGONIST MK-801 IMPAIRS ACQUISITION OF LATENT INHIBITION FOR A PREEXPOSED FLAVOR STIMULUS.
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Previous studies have shown that simple exposure to a stimulus will retard later conditioning of that stimulus (latent inhibition), and that septohippocampal circuits play a critical role in mediating the effect. The present study investigated whether the NMDA antagonist MK-801, which blocks induction of LTP in the hippocampus, would also impair acquisition of latent inhibition.

Rats were initially adapted to restricted water access (20 min/day). Separate groups then received injections of saline or MK-801 (0.08 mg/kg, sc) 35 min prior to exposure to a .2% (w/v) saccharin solution or to tap water. A fifth group of rats received MK-801 immediately after preexposure to saccharin. Two days later, all groups received a conditioning trial in which saccharin was paired with delayed illness (0.3M LiCl, 63.5 mg/kg, ip). Subsequent tests showed that rats preexposed to saccharin after receiving saline injections acquired significantly weaker aversions than did non-preexposed rats (latent inhibition). Rats that received MK-801 before or immediately after saccharin preexposure, however, displayed saccharin aversions like those seen in non-preexposed rats (no latent inhibition).

Various aspects of the data suggest that MK-801 disrupted latent inhibition because of its effects on learning, and not because of other possible effects of the drug (e.g., sensorimotor deficits). These results therefore support the idea that NMDA receptors in limbic system circuits play a critical role in certain forms of learning and memory.

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509.6

NMDA ANTAGONIST MK-801 PRODUCES A DOSE-DEPENDENT IMPAIRMENT ON SPATIAL REVERSAL LEARNING IN THE RADIAL ARM MAZE.
L.H. White*, K.B. Austin, and M.L. Shapiro. Dept. of Psychology, McGill Univ., Montreal, Quebec, Canada, H3A 1B1.

Many experiments have shown that MK-801 blocks or retards spatial learning in an unfamiliar environment. The present experiment investigated the effects of MK-801 on learning a new subset of spatial relationships in a *familiar* environment. Rats were trained on a version of the radial arm maze task which requires the rat to enter only four of eight arms to retrieve a food reward (4/8 RAM). Upon reaching criterion performance, they were assigned to one of four groups; a saline control, and either 62.5 ng/kg, 100 ng/kg, or 125 ng/kg of MK-801 (n=9 per group). Injections were given I.P. 30 minutes prior to training. Performance was assessed for four days, and MK-801 had no effect on working and reference memory (WM and RM). Each rat was then tested on a RM reversal task by baiting the set of arms opposite to those used for the initial acquisition. No significant differences were found between the groups on blocked trials of RM or WM errors throughout training. However, the 125 ng/kg MK-801 group took significantly longer than the SAL group to learn the RM reversal task compared to acquisition (trials to criterion reversal minus trial to criterion acquisition). The 62.5 ng/kg, 100 ng/kg, and SAL groups did not differ significantly on this difference score. The delayed acquisition of the RM reversal task for the 125 ng/kg MK-801 group is consistent with one interpretation that MK-801 retards the acquisition of a new subset of spatial relationships in a familiar room by inhibiting the ability to suppress previously learned information.

509.7

NMDA ANTAGONIST MK-801 RETARDS REVERSAL PERFORMANCE IN THE RADIAL ARM MAZE TASK. K.B. Austin*L.H. White and M.L. Shapiro, Department of Psychology, McGill University, Montreal, CANADA.

Preliminary data from our laboratory has shown that MK-801 (62.5 ng/Kg, IP), administered to Long-Evans rats immediately after the first exposure to a spatial environment, alters the hippocampal place cell firing. To test the effects of MK-801 on spatial learning, we used a version of the radial maze task which requires the rat to enter only 4 of 8 arms to retrieve a food reward (4/8 RAM). 30 male Long-Evans rats were assigned to either a control (SAL), a pre-MK (given MK-801 30 min before each trial), or a post-MK (given MK-801 immediately after each trial) group. Rats were trained for 27 days and reached a criterion performance of less than 2 errors per day for 4 consecutive days. Analysis of reference memory (RM) errors, working memory (WM) errors and trials to criterion (TC) showed no group differences. Retention tests performed 15 days after training showed that there was no forgetting. During the reversal phase all of the previously baited arms were not baited and vice-versa. The TC difference scores for each animal (TC for acquisition minus TC for reversal) showed that both saline and post-MK groups reached criterion performance in fewer trials during reversal than during initial acquisition (5.5 and 5.8 trials sooner, respectively). In contrast, the pre-MK group took slightly longer to reach criterion in the reversal task (1.7 trials later). Thus, rats trained and tested under the influence of MK-801 did not show the same savings on reversal as rats receiving saline or MK-801 post-trial. The male Long-Evans rats given 62.5 ng/Kg of MK-801 did not show *any* overt behavioral side effects. Here, the deficit caused by MK-801 was relatively subtle compared to other RAM studies using higher doses of MK-801 and/or rats of different strain and sex. Which effects of MK-801 are the direct result of NMDA blockade as it relates to LTP and which effects may be attributed to other PCP receptor-mediated events remain unknown.

509.8

MK801 IMPAIRS MEMORY FORMATION IN THE 2 DAY-OLD CHICK. D.R. Smith, S.C. Fromont, M.R. Rosenzweig and E.L. Bennett*. Department of Psychology, University of California, Berkeley, CA 94720.

The amnesic effect of MK801, a NMDA receptor antagonist, was determined using 2 day-old chicks trained on a 1-trial passive avoidance task. Groups of chicks were given bilateral injections into the intermediate medial hyperstriatum ventrale 45, 30, or 15 min pretraining, using saline or 0.0015, 0.015, 0.15, 1.5 mM MK801, and tested at 24 h. 1.5 mM and 0.15 mM MK801 produced significant amnesia when injected 15 min or 30 min pretraining when compared to saline controls ($p<.01$). No other dose of MK801 produced amnesia. These results indicate that NMDA receptor activation is important for learning and memory formation in the chick. The appearance of amnesia after injections at 15 min or 30 min pretraining is consistent with the findings of other researchers, in that MK801 has been shown to have maximal effect at about 30 min. Further experiments are necessary to determine if activation of NMDA receptors is important for the acquisition or retrieval of a task.
Supported by NSF grant BNS-88-10528.

509.9

EFFECTS OF L-GLUTAMATE AND N-METHYL-D- ASPARTIC ACID(NMDA) ANTAGONISTS ON ACTIVE AVOIDANCE TASK IN MICE.F.Motamedi* and M. Eslami ,Dept . of physiology , shaheed Beheshti Univ.of Med .Sci. P.O.Box 19835-181 , Tehran. Iran.

Systemic and central administration of glutamate agonists and antagonists are involved in memory in a variety of tasks . In the present study the effects of L-Glutamte(L-GLU) and NMDA antagonists 2-Amino -7- phosphonoheptanoic Acid (AP7)AND MK-801 on active avoidance task in mice were examined.Intracerebroventricular injection of L-GLU(5ug/Brain) before and after training sessions improved performance in a retention test 24 hour later .No such effect were seen at lower doses of L-GLU. pre - training administration of AP7 a competitive antagonist of NMDA (30mg/kg,i.p.) and MK-801 a non competitive antagonist of NMDA(0.2mg/kg. i .P)30 min prior to training sessions , significantly impaired memory , but immediately after post-training injection of these two antagonists no significant change in memory was observed.

Our results show that while NMDA receptors are involved in memory formation, the enhacement of memory seen after post-training administration of L- GLu is probably due to a non- NMDA receptor

509.10

DIFFERENTIAL REDUCTION OF FEAR CONDITIONING TO CONTEXTUAL AND AUDITORY CONDITIONAL STIMULI BY ICV ADMINISTRATION OF AN NMDA ANTAGONIST (APV). M. S. Fanselow*, J. J. Kim & J. Yipp. Dept. of Psychology, UCLA, LA, CA 90024.

Rats with cannula implanted in the lateral ventricle received infusions of DL-2-amino-5-phosphonovaleric acid (APV, 0, .625, 1.25, 2.5, or 5.0 µg/rat) prior to pairings of a tone with shock. The rats were later tested without drug, shock or tone for conditional fear of the original training context by scoring freezing. APV dose dependently reduced freezing with the 2 highest doses virtually eliminating the response. The next day the rats were placed in a different chamber and no freezing was observed until the tone was presented. The tone produced freezing in all animals. APV did not have a statistically reliable effect on freezing to the tone, although responding was reduced by 50% at the highest doses. A second study gave rats tone shock pairings or shock alone. The tone elicited freezing only in the paired animals. APV (5 µg) completely eliminated responding to the training context but only reduced responding to the tone by 50%. Contextual conditioning appears to be more susceptible to reversal by ICV administration of NMDA antagonists than does conditioning to discrete auditory cues. There appears to be some degree of divergence between the circuitry mediating the learning of fear to contextual and discrete auditory conditional stimuli.

509.11

NMDA-MEDIATED MECHANISMS IN AUDITORY FILIAL IMPRINTING IN CHICKS

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In the imprinting-relevant forebrain area medial neostriatum/hyperstriatum (MNH) the induction of LTP can be blocked by the NMDA-antagonist 2-amino-5-phosphonovaleric acid (APV) (Wang et al Proc. 20. Göttingen Neurobiol Conf, 1992). To test the behavioral influence of APV on auditory filial imprinting, newly hatched chicks received bilateral injections of 1μl APV (1μM-50μM) into MNH or into a reciprocally connected area, the caudal neostriatum (Nc). They were then exposed to rhythmic 400Hz tone pulses (imprinting session). During the following days, approach to different sound sources was tested (approach and discrimination test). Compared to the control groups the imprinting rate of APV-injected chicks decreased in a dose-dpendent manner to 0% with APV-concentrations >12.5μM for both, MNH- and Nc-injections. APV-treatment does not cause irreversible changes in these areas, since after termination of the APV-injections after the end of the congenital sensitive phase (4 days posthatch) 40% of these unimprinted APV-treated chicks could subsequently be imprinted on the 5th and 8th day posthatch (delayed imprinting). Furthermore, 2-deoxyglucose-autoradiography revealed that these chicks showed an increased MNH-activity when exposed to the imprinting tones. Successfully imprinted chicks with bilateral or unilateral injections of a single dose of 50μM APV into MNH no longer responded to the imprinting tones, but some of the unilaterally injected chicks still showed responses. After 24h, when the effects of APV had worn off, all chicks responded again. Our behavioral results together with the electrophysiological data indicate that NMDA-mediated LTP is one of the mechanisms which plays a role in auditory filial imprinting. *Supported by DFG (Br950/4-5) and BMFT.*

509.12

EXAMINATION OF METABOTROPIC RECEPTOR INVOLVEMENT IN SPATIAL LEARNING. T.C.Dumas*, R. Grimes and T.C. Foster Depart. of Psychology, U. of Virginia, Charlottesville, VA. 22901.

The glutamate metabotropic receptor is highly concentrated in the hippocampus. Receptor activation may influence synaptic plasticity during memory formation. Rats (n=12) were trained on the 8-arm radial maze. Prior to testing (30 min), the metabotropic receptor antagonist, 2-amino-3-phosphono-propionic acid (AP3) (1.0^{-6} - 0.1 M in ACSF, 3 L/cannula over 3 min) or ACSF was injected bilaterally ICV via chronic cannulae. Within dosages tested, AP3 had no effect on working memory errors or the latency to complete task. Acute injection did not influence acquisition of spatial information in a novel environment. No difference was observed between AP3 injection and control rats in latency to reentry on an inhibitory avoidance test. Rats chronically implanted with minipumps for ICV drug delivery (0.1 M AP3) exhibited visual cue and spatial discrimination learning on the Morris water task. Results indicate that AP3 does not effect spatial learning. Chronic effects on retention are currently being examined. FRS 441335.

509.13

EFFECTS OF PHENCYCLIDINE AND NALOXONE ON LEARNING OF A SPATIAL NAVIGATION TASK AND PERFORMANCE OF A SPATIAL DELAYED NON-MATCHING TO SAMPLE TASK.

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Rats were trained on a dry-land version of a spatial navigation task (cheese board) under the influence of 1 µl injection of 10 µg of Phencyclidine (PCP, an antagonist of the NMDA receptors), 13 µg of naloxone (an antagonist of the opiate receptors), or saline, directly into the dorsal hippocampus. Rats received 2 blocks of 4 trials with different starting points, relative to the goal (food) per day, for three consecutive days. Based on a distance traveled measure, results indicated that relative to saline controls, the PCP group learned normally within a day, but displayed forgetting between days. Conversely, the naloxone group displayed disruption of learning within a day, but displayed normal learning between days. These preliminary results indicate that the NMDA receptors mediate consolidation of spatial information, the opiate receptors mediate short-term memory representations, and that the processes can operate independent of each other.

This conclusion is further supported by results obtained from rats previously trained on an 8-arm spatial delayed non-matching to sample task with delays of 1 and 30 minutes. The rats were treated exactly the same as in the previous experiment. In this case, PCP had no effect, however naloxone disrupted performance on the 30, but not the 1 minute delay. Because it is assumed that the latter task measures the operation of short-term memory representations, but not consolidation of spatial information, the results lend additional support to the hypothesis that opiate receptors mediate short-term memory and NMDA receptors mediate consolidation of spatial information.

509.14

NITRIC OXIDE SYNTHASE INHIBITION BY N^{w}-NITRO-L-ARGININE IMPAIRS LEARNING OF RATS IN A 14-UNIT T-MAZE.

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N-methyl-D-aspartate (NMDA) receptor activation has been linked to enzymatic production of nitric oxide (NO) by nitric oxide synthase (NOS) (Garthwaite, *TINS*, 14:60, 1991). NMDA receptor antagonism retards formation of long-term potentiation (LTP) and impairs acquisition in maze tasks (Izquierdo, *TIPS*, 12:128, 1991). These observations have been linked to intracellular events involving NO production as a retrograde messenger increasing presynaptic glutamate release. Specifically, when NOS is inhibited, LTP is blocked *in vitro* (Schuman & Madison, *Science*, 254:1503, 1991). We examined whether NOS inhibition impairs learning of male F-344 rats (9 mo) in a 14-unit T-maze. Previous results in this maze indicated that NMDA receptor channel antagonism impairs acquisition but not retention (Spangler *et al.*, *Pharm. Biochem. Behav.* 40:949, 1991). In the present study, rats were pretrained in 1-way active avoidance to a criterion (13/15 avoidances) in a straight runway. The next day, rats received i.p. injections of 0.9% NaCl as controls or N^{w}-nitro-L-arginine (N-ARG) to block NOS (3.0, 4.5 or 6.0 mg/kg) 30 min before maze training. During 15 trials, rats were required to negotiate each of 5 segments within 10 s to avoid footshock (0.8 mA). Peformance variables included errors (deviations from correct pathway), runtime from start to goal, shock episodes and duration. N-ARG treatment impaired performance in all variables in a dose-dependent manner. Controls and rats treated with 3 mg/kg N-ARG were retested in the maze 7-10 days following training, with half being injected with N-ARG (6 mg/kg) 30 min in advance. Performance under these conditions was affected minimally indicating that NOS inhibition primarily impairs acquisition .

509.15

A NITRIC OXIDE SYNTHASE INHIBITOR RETARDS ACQUISITION OF THE CLASSICALLY CONDITIONED RABBIT EYELID RESPONSE.

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Nitric oxide (NO) may be involved in long term depression (LTD) processes in the cerebellum. In this present study, we tested the effects of L-nitro-arginine-methyl-ester (L-NAME), a NO synthase inhibitor on classical eyelid conditioning, a learning procedure thought to involve the cerebellum. Rabbits were given L-NAME at 3 doses (10, 25, 75 mg/kg) or the stereoisomer D-NAME (25 mg/kg) while being trained in the classically conditioned rabbit eyelid response paradigm (CS=tone, US=airpuff). The L-NAME groups had significantly lower percentages of conditioned responses than did the D-NAME controls over the first several days of training. No effect of L-NAME was obtained in rabbits that had been previously conditioned to criterion after injection of the control substance, D-NAME. Preliminary results from conditioning with L-NAME while recording multiple unit activity in the interpositus nucleus indicate that the training-related neuronal model characteristic of the interpositus nucleus is delayed similarly to the appearance of the conditioned response. These data suggest that blocking NO synthesis may disrupt cellular processes normally involved in the acquisition of classically conditioned responses.

BIOLOGICAL RHYTHMS AND SLEEP V

510.1

INHIBITORY RESPONSES TO PRESSURE EJECTED NEUROPEPTIDE Y IN RAT SUPRACHIASMATIC NUCLEUS NEURONES *IN VITRO*

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Neuropeptide Y (NPY) has been shown exclusively to increase the spontaneous firing rate of suprachiasmatic (SCN) neurones in hamster hypothalamic slices *in vitro* when administered by pressure ejection (1), but predominantly to inhibit the firing of both rat (2) and hamster (3) SCN neurones when perfused in the bathing fluid. Using hypothalamic slices prepared from rats, and standard extracellular recording techniques we found that pressure ejection of NPY (50-200µM, 10 psi, 0.1 to 3 seconds) inhibited the spontaneous firing rate of 75% (51/68) of SCN neurones tested. This refutes the suggestion that method of application explained the discrepancies between rat and hamster. No stimulatory responses were seen, the remainder of the cells being unaffected by NPY.

Neurones could be distinguished by the depth and duration of the response to NPY, and by a monophasic or biphasic return to previous firing rate. In a second series of experiments designed to investigate NPY responses in the SCN at various circadian times, we found no difference in the frequency of inhibitory responses (to 50µM NPY) during the light phase (83%) and the dark phase (81%). However, whereas the durations of responses during the light phase were equally distributed between short and long, during the dark phase they were predominantly long (20 to 45 min).

(1) Mason et al. (1987) Neuroscience Letters 80 173-179
(2) Albers et al. (1990) Am. J. Physiol. 258 R376-R382
(3) Liou and Albers (1991) Brain Res. Bull. 27 825-828

510.2

NEUROPEPTIDE Y RECEPTOR DISTRIBUTION IN THE SUPRACHIASMATIC NUCLEUS OF THE GOLDEN HAMSTER: RELATIONSHIP TO RETINOHYPOTHALMIC TRACT.

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The suprachiasmatic nucleus (SCN) receives direct visual afferent projections from the retina via the retinohypothalmic tract (RHT), as well as from the lateral geniculate nucleus via the geniculohypothalamic tract (GHT). Neuropeptide Y-containing fibers originating from the GHT appear to modulate circadian rhythmicity. Therefore we chose to investigate the relationship between neuropeptide Y (NPY) receptor density and RHT projections to the SCN in the golden hamster. The RHT was traced by implantation of carbocyanine dye DiI into the distal end of one optic nerve (n=7). NPY receptors were localized using receptor autoradiography (n=11). Sections were labeled with ^{125}I-peptide YY +/- unlabeled peptide YY. NPY receptor distribution was seen primarily in the ventral SCN in the rostral portion of the nucleus, but shifted laterally in more caudal sections. This pattern closely corresponded to RHT projections from the retina. These findings provide additional evidence that NPY is involved in circadian regulation within the SCN, and suggest that both the RHT and GHT project to the ventrolateral portion of the SCN. Supported by Hendricks Research Foundation,NS25512, AG09301.

510.3

SEROTONIN RECEPTOR GENE EXPRESSION IN THE RAT SUPRACHIASMATIC NUCLEUS. A.L. Roca*, D.R. Weaver and S.M. Reppert. Laboratory of Developmental Chronobiology, Children's Service, Mass. General Hospital, Boston, MA 02114.

The suprachiasmatic nuclei (SCN) receive a serotonergic (5HT) projection from the midbrain raphe nuclei. Quipazine phase shifts the SCN circadian clock in vitro in the presence of tetrodotoxin, suggesting that pacemaker cells in the SCN express 5HT receptors (*Brain Research* 573:336, 1992).

We used in situ hybridization to examine the expression of 5HT receptor subtypes in the rat SCN by film autoradiography. Rat serotonin receptor cDNAs were kindly provided by O. Civelli (5HT-1a) and D. Julius (5HT-1c, 5HT-2). Full-length ^{35}S-labeled cRNA probes were used for the 5HT-1a, -1c, and -2 receptors, while cRNA probes for the 5HT-1b and -1d receptors were generated from cDNA fragments cloned using PCR. 5HT-1c receptor mRNA showed intense hybridization in the SCN, as well as in caudate-putamen and choroid plexus. The mRNA for 5HT-1b receptor also displayed a consistent, though weak, signal in the SCN, and a stronger signal in the hippocampus, caudate-putamen, and thalamus. 5HT-1a and 5HT-2 receptor mRNAs were not readily detected in the SCN, even though an intense signal was seen in other brain regions. The 5HT-1d receptor mRNA was not detected in the SCN or anywhere else in the brain. There was no obvious circadian variation in signal intensity for any of the receptors examined. We conclude that 5HT-1c and -1b receptor mRNAs are the most highly expressed 5HT receptor subtypes in the rat SCN.

510.4

SEROTONERGIC REINNERVATION OF THE HAMSTER SUPRACHIASMATIC NUCLEUS AND INTERGENICULATE LEAFLET WITHOUT FUNCTIONAL CIRCADIAN RHYTHM RECOVERY. L.P. Morin and J. Blanchard*. Dept. Psychiatry, SUNY, Stony Brook, NY 11794.

Acute loss of serotonin changes several parameters of the circadian activity rhythm which persist in constant conditions (Brain Res. 515,9-19,1990; 566,173-185,1991). Serotonergic innervation of the suprachiasmatic nucleus (SCN) and intergeniculate leaflet (IGL), two nuclei involved in circadian rhythm regulation, may become re-established during long recovery periods. The present experiment tested a) whether serotonergic reinnervation occurs and b) if there is associated recovery of rhythm function.

Hamsters entrained to a LD 14:10 photoperiod received bilateral intraventricular injections of DHT after pretreatment with desmethylimipramine. The entrained circadian wheelrunning rhythm was studied for up to 20 wks post-lesion and related to the extent of serotonergic innervation.

Reinnervation of the SCN and IGL begins by 8 wks post-lesion and progresses to substantial, but not complete, levels by week 20. Four measures of the nocturnal activity phase of the circadian rhythm were rapidly modified by the lesions, consistent with previous studies, and persisted during the 20 wk recovery period. The circadian rhythm system of hamsters may be fundamentally different from other behavioral or neuroendocrine systems studied in rats with respect to its inability to recover from damage to its serotonergic innervation. Alternatively, the failure to demonstrate functional recovery may reflect a species difference or insufficient recovery time. Supported by NINDS 22168.

510.5

NMDA RECEPTORS IN RODENT SUPRACHIASMATIC NUCLEUS. M.D. Hartgraves, S.M. Grady and J.L. Fuchs*. Dept. Biological Sciences, University of North Texas, Denton, TX 76203.

NMDA receptors appear to be involved in mediating effects of light on circadian rhythms. Pharamacological studies indicate that glutamate may be a transmitter in the pathway from retina to suprachiasmatic nucleus (SCN), the primary circadian pacemaker. APV injected into the SCN diminishes light effects on the pineal (Ohi et al. '91), and systemic MK-801 injections can block effects of light on activity rhythms (Colwell et al. '90) and on fos-IIR in the SCN (Abe et al. '91).

The present study aimed to characterize NMDA receptors in the rat and hamster SCN. In addition, 34 rats were used to test for changes in NMDA binding which might contribute to the phase-dependency of light effects: comparisons were made between 4 time points in LD, and between L versus D near dawn or dusk. NMDA binding was also measured in 10 enucleated rats. Film autoradiographs were prepared from sections incubated with the NMDA antagonist [^{3}H]MK-801 (Monaghan '91). In both species, [^{3}H]MK-801 binding was moderately low in the SCN relative to other brain regions, was fairly uniform across the SCN region, and did not delineate the SCN. Scatchard analyses in SCN revealed similar binding properties in rat and hamster. ANOVAs showed no significant differences among treatment groups. The results demonstrate the presence of NMDA binding sites in the SCN; these binding sites could mediate NMDA antagonist-sensitive effects of light on rhythms. The absence of obvious changes attributable to enucleation, light condition or circadian time, suggests that in contrast to the phase-dependency of light effects on circadian rhythms, NMDA binding in the SCN is quite stable.
Supported by NIMH grant MH41865.

510.6

MELATONIN SENSITIVITY PERSISTS IN THE SCN IN VITRO. Russell R. Margraf and G. Robert Lynch*. Department of Biology, Wesleyan University, Middletown, CT 06459.

Past studies in the rat and Syrian hamster have shown that metabolic and electrical activity of the suprachiasmatic nucleus (SCN) is inhibited by melatonin (MEL) at about circadian time (ct) 8-11 h. This experiment examines the effect of MEL on the firing of SCN cells in brains slices prepared from 6 long day (LD 16:8) Djungarian hamsters (Phodopus sungorus). The primary aim of this study is to determine if MEL sensitivity persists in vitro with a period of about 24 h. Pressure ejection of MEL (0.2 mM in 165 mM NaCl) suppressed firing rate in 29% of SCN neurons, activated 9%, and had no effect on 62% (n=162). MEL elicited the highest percent response (71% of responding cells) during the 4 h time bin immediately preceding the projected "dark" phase on day 1 (ct 8-12 h). A similar pattern of MEL sensitivity was observed during this time bin on the second day of recording. Pressure ejection of vehicle alone had little effect (n=52) at all ct. These results demonstrate that MEL sensitivity persists with a period of about 24 h and does not require an endogenous MEL pulse the previous night to be expressed. Supported by NSF.

510.7

PARALLELISM BETWEEN NOCTURNAL CHANGES OF PLASMA MELATONIN AND TESTOSTERONE CONCENTRATIONS IN NORMAL MALE ADULTS. P.E. Schulz*, F. Chardon, M. Hugentobler and R.W. Rivest. Division of Clinical Psychopharmacology, Department of Psychiatry, Geneva University, IUPG, CH-1225 and Laboratory MNS, CH-1205, Geneva, Switzerland.

Differences or similarities in the temporal organization of hormones secretion in plasma reflect, among other phenomena, the activity of CNS pacemakers. We report on the simultaneous analysis of melatonin and testosterone concentrations. Five normal male subjects between 23 and 32 years old were studied during 2 non consecutive nights. Blood was withdrawn every 20 min during 12 hr, from 8:00 p.m. to 8:00 a.m. Testosterone concentrations during the night ranged from 3 to 10 ng/ml and melatonin from 20 to 200 pg/ml. The mean concentration of testosterone increased 1.5- to 2-fold during the second part of the night. For melatonin, this increase was 2.5- to 4-fold. The rhythms of melatonin and testosterone were similar. This was to the point of high synchrony in 5 of the 10 nights: the times of onset of secretion, as well as the slopes of increase in concentrations were the same for the 2 hormones. This positive relation between melatonin and testosterone nocturnal rhythms is in apparent contrast with the inhibitory action of melatonin on the reproductive system of several mammal species.
Dr. J. Arendt provided an antiserum for the assay of melatonin. The study was financed by grant No 3.811.0.87 from the Swiss national research foundation.

510.8

ROLE OF PROLACTIN AND TESTOSTERONE IN SEASONAL PHYSIOLOGICAL CHANGES IN COLLARED LEMMINGS. B. Gower*, T.R. Nagy, and M.H. Stetson. School of Life and Health Sciences, Univ. of Delaware, Newark, DE 19716.

Collared lemmings, *Dicrostonyx groenlandicus*, display several interesting physiological and morphological changes on a seasonal basis. During autumn and winter, or when exposed to an artificial short photoperiod, lemmings molt to a white pelage, increase in body mass, and develop a bifid "digging" claw. To determine the hormonal basis of these changes, we manipulated endogenous levels of prolactin (PRL) and testosterone (T) in animals exposed to short and long photoperiods. In animals transferred to 8L:16D, treatment with the dopamine antagonist sulpiride elevated PRL and prevented the molt to white pelage. Lemmings maintained under 16L:8D and displaying the summer pelage were treated with the dopamine agonist CB-154. When examined after both 3 and 6 weeks, treated animals had lower serum PRL than controls and had begun to develop the white winter pelage. Castration facilitated the pelage response to CB-154. CB-154 treatment had a negative effect on testes weights in intact animals, but had no effect on other parameters. Castration had a positive effect on body mass and claw size - an effect which was reversed by T administration. These results suggest that seasonal changes in PRL mediate changes in coat color, while body mass and claw size are regulated by steroid hormones.

510.9

ANDROGEN RECEPTOR MEDIATED SEXUAL DIMORPHISM OF SLEEP IN Tfm MICE. S.W. Yang*, J. Fang, and W. Fishbein. Dept of Psychology, The Graduate School of CUNY, N.Y., NY 10031

For the first time in 1987, we reported that sleep is sexually dimorphic in mice; prenatal stress sex reverses the sleep pattern of males, but not females. Prenatal stress reduces testicular enzyme activity with a reduction in plasma testosterone levels (Ward, 1984). In the present experiment we used androgen insensitive testicular feminized (Tfm) mice to investigate whether androgen receptors are involved in this phenomenon. EEG and EMG electrodes were implanted in four groups of adult animals: Normal males (Ta/y), normal females (Ta/Ta), heterozygous females (Tfm/Ta) and phenotypic female Tfm males (Tfm/y). Sleep recordings were taken continuously for 4 days.

Results: (1) Normal males spend less time in paradoxical sleep (PS) than normal females (3.95 vs 4.65 min/hr, p<.05). (2) PS in Tfm males is higher than all other groups (p<.05). (3) Daytime slow wave sleep is indistinguishable between all groups.

Conclusions: (1) The sexual dimorphism of sleep is replicated in another mouse strain. (2) Deficiency of androgen receptors causes genetic males to develop female sleep patterns.

510.10

NEUROCHEMICAL LESIONS OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS ACCELERATE PUBERTAL GONADAL GROWTH IN MALE FERRETS. Y.P. Tang* and C.L. Sisk. Neuroscience Program/Dept. Psychology, Michigan State University, East Lansing MI 48824.

Environmental photoperiod regulates the onset of puberty in male ferrets. Testicular growth is accelerated in ferrets that experience a transition from short to long days, compared to ferrets that are raised solely in short days. Daylength is transduced by the duration of the nighttime rise in pineal melatonin secretion. This circadian pattern of melatonin release is regulated by a neural circuit that includes the retina, suprachiasmatic nucleus (SCN), paraventricular nucleus (PVN), and superior cervical ganglion. In hamsters, the integrity of each of these structures is necessary for reproductive responses to photoperiod. In mustelids, the importance of the SCN for photoperiodic time measurement has been established, but other components of the circuit have not been functionally verified. In this experiment, axon-sparing neurochemical lesions of the PVN were made in short day-housed weanling (7 wk old) male ferrets by bilateral stereotaxic injections of N-methyl-D-aspartic acid (0.6 µl of 0.3M NMDA in artificial csf). The onset of pubertal testicular growth normally begins at about 18 wk of age in ferrets raised in short days. However, within 3 wk of the lesion, at 10 wk of age, mean testis width was significantly larger in ferrets with NMDA lesions compared to ferrets with sham lesions. At sacrifice (16 wk of age), mean testicular weight was greater in ferrets with lesions than in controls (0.92±0.08 vs 0.47±0.08 g; $p<0.05$), as was luteinizing hormone pulse frequency (5.0±1.6 vs 1.5±0.75 pulses/4 hr; $p<0.05$). Thus, pubertal activation of the reproductive system was accelerated by PVN lesions. These lesions result in reproductive responses that are similar to those induced in prepubertal ferrets by a short-to-long day transition. This is the first indication that the PVN is part of the photoperiodic time measurement system in a non-rodent species. Supported by HD26483 and HD00950.

510.11

THE EFFECTS OF ROTATING LIGHTING SCHEDULES ON ESTROUS CYCLICITY IN RATS. W.L. Woloshin and D.L. McEachron*.Biomedical Engineering and Science Institute, Drexel University, Philadelphia, PA 19104.

The activity patterns of females rats vary systematically with the days of the estrous cycle. Control of these sexual cycles is thought to involve a SCN-mediated circadian 'gate' interacting with an ovarian developmental clock. Given the strong ties between the SCN and photic environmental cycles,we examined the effects of different LD cycles on estrous rhythms.

Running wheel activity was monitored for 3 groups of female Sprague-Dawley rats. All were initially exposed to LD 8:16 for 4 weeks. All rats showed a circadian period of near 24 hours and an estrous cycle of 4 or 5 circadian cycles. Group 1 continued under this cycle for the remainder of the experiment, 13 weeks. Group 2 was exposed to rotating shift cycle which mimicked a typical human shiftworker's pattern, i.e., day, swing, and night shifts with weekends equivalent to day shift. Group 3 was exposed to a rapidly rotating schedule of 8 hour advances every 2 days.

Preliminary results indicate that Group 1 maintained a 24-hour circadian cycle and an estrous cycle equaling 4 or 5 circadian cycles. Group 2 animals consistently lengthened their circadian cycle to 25.2 hours and their estrous cycle to 5 circadian cycles. Group 3 animals displayed highly variable results: circadian cycles ranged from 24.2 to 25 hours and estrous cycles from 3 to 5 circadian cycles. We conclude that despite the speed of the 3-shift cycle, Group 2 animals entrained to the LD cycles. The consistent lengthening of circadian and estrous cycles in Group 2 and the discovery of estrous cycles equaling 3 circadian cycles (≈74 hrs) in Group 3 suggests that the relationship between circadian and reproductive cycles in female rats is more complex than has been suggested.

510.12

SEX DIFFERENCES IN SLEEP: EFFECTS OF ESTRUS CYCLE AND OVARIECTOMY. J. Fang*, S. J. Tien, S. W. Yang and W. Fishbein. Dept of Psychology, Graduate School of CUNY, New York, N.Y. 10031

Sleep is sexually dimorphic in rats: Males have more paradoxical sleep (PS) than females. However, it is not clear whether this sex difference is influenced by ovarian hormones and the 4-5 day estrus cycle. The present experiment examines this possibility.

Adult Male and female Sprague-Dawley rats were implanted with EEG/EMG electrodes. Sleep recordings were taken for 20-30 days in females and 4 days (randomly distributed in the same period) in males and ovariectomized females.

Results: (1) Males have more PS (3.86 min/hr) than females (2.98 min/hr; p<.02) in both daytime and nightime; (2) the sex difference in daytime PS is not influenced by estrus cycle or ovariectomy; (3) the sex difference in nighttime PS is due mainly to a decrease of PS during the proestrus phase (.88 min/hr vs 1.83 min/hr; p<.003); (4) ovariectomy eliminates the proestrus phase difference (2.06 min/hr); (5) SWS is indistinguishable between sexes.

Conclusion: (1) Nighttime PS depends upon the activational effect of ovarian hormones. (2) Ovarian hormones during daytime do not effect the sex difference in PS.

510.13

SUPRACHIASMATIC NUCLEUS (SCN) LESIONS INCREASE SUSCEPTIBILITY TO ACTIVITY-BASED ANOREXIA. E.Z. Stanley*, L.E. Doerries, T.S. Rieg and P.F. Aravich. College of William and Mary, Williamsburg, VA 23185; Christopher Newport University, Newport News, Va 23606; Eastern Virginia Medical School, Norfolk, VA 23501; & V.A. Med. Ctr., Hampton, VA 23667.

Suprachiasmatic hypothalamic nucleus (SCN) lesions eliminate a variety of circadian rhythms. Circadian rhythms are known to be perturbed in anorexia nervosa (AN). Because of interest in the relationship between exercise and AN we have been exploring activity-based anorexia (ABA) in the rat (1.5 h/day food; 22.5 h/day wheel access). We have previously found that constant bright illumination (LL) increases susceptibility to ABA. This experiment determined if SCN lesions have a similar effect. Circadian rhythms were disrupted in two ways, viz., LL and bilateral electrolytic lesions of the SCN, according to a 2 x 2 factorial design (SHAM vs SCN lesion x LL vs 12/12 hr light-dark illumination; LD). Following a postoperative recovery period, rats were subjected to ABA. Susceptibility to ABA was defined as the number of days to lose 25% of body weight. Analysis of ad lib food intake before and after surgery demonstrated that the circadian distribution of feeding was successfully altered by both the SCN lesion and LL treatments. When exposed to ABA, the SCN-lesioned groups required the least number of days to reach the weight-loss criterion, the SHAM LL group required an intermediate amount and the SHAM LD condition required the most days. There were no differences in terminal wheel revolutions or food intake across groups. These data indicate that a disruption of light-entrainable rhythms increases susceptibility to ABA. This raises the possibility that disrupted circadian rhythms are a novel risk factor for anorexia nervosa.

510.14

ABLATION AND PHARMACOLOGY STUDIES OF THE FOOD-ENTRAINABLE CIRCADIAN SYSTEM IN THE RAT. R.E. Mistlberger*[1], D.G. Mumby[2], T. Kippen[2] and I.Q. Whishaw[3]. Depts. Psychology, Simon Fraser U.[1], Burnaby, BC, U of British Columbia[2], Vancouver BC, and U. of Lethbridge[3], Lethbridge, Ab.

Rats anticipate a daily mealtime by entrainment of circadian oscillators outside of the suprachiasmatic nuclei (SCN). Neural mechanisms of this oscillator system are unknown. The present studies tested hypotheses concerning the possible role of sensory afferents and cortical, limbic and basal forebrain structures in the generation and entrainment of food-anticipatory rhythms. Food anticipation, measured by wheel-running and food-bin activity, was robust in rats sustaining (a) trigeminal nerve deafferentation, (b) complete ablations of the hippocampus and amygdala, their efferents and forebrain targets, (c) complete ablations of the nucleus accumbens, septum and adjacent medial forebrain and preoptic areas, or (d) complete removal of the neocortex. Intact rats receiving the dopamine antagonist haloperidol (IP, .3 or 2.0 mg/kg) 30 min prior to oral or intragastric feeding time showed some attenuation of anticipation and overall activity, but so did saline injected intragastric fed control rats. The results do not support previous suggestions that limbic or dopaminergic systems involved in memory and reinforcement processes have circadian functions necessary for food anticipation. Supported by NSERC, Canada.

510.15

EFFECTS OF THE PHOTOPERIOD ON WHITE ADIPOSE TISSUE CATECHOLAMINE CONTENT IN SIBERIAN HAMSTERS. T. G. Youngstrom* and T. J. Bartness. Depts. of Biology and of Psychology, Georgia State Univ., Atlanta, GA 30303.

We demonstrated previously that Siberian hamsters show impressive decreases in body weight, almost exclusively as body fat, during the first few weeks of short day (SD) exposure. This decrease in lipid stores is not accompanied by a significant decrease in food intake at this time. As an indication of sympathetic nervous system activity, and consequently lipid depletion, white adipose tissue (WAT) catecholamine (CA) content was measured in long day (LD)- and SD-housed hamsters. Male Siberian hamsters were killed 5 wk following transfer to SDs. Epididymal WAT (EWAT) and heart (control tissue) were harvested, and norepinephrine (NE), dopamine (DA) and epinephrine (EPI) content were measured by HPLC with EC detection. SD exposure increased NE content (ng/µg protein) in EWAT, but not in heart. DA content was not affected by the photoperiod for either tissue. For both photoperiods, the content of EPI was greater in EWAT than in heart. SD exposure increased EPI content in EWAT compared with that of the LD controls. Conversely, EPI content in heart was greater in LDs than in SDs. These results suggest that the rapid body weight (fat) decreases occurring during the first few wks of SD exposure may be due to an enhanced sympathetic nervous system drive on WAT. In addition, possible WAT *in situ* conversion of NE to EPI, or of EPI-containing neural input is suggested by the high EPI content in EWAT. Supported by NIMH RSDA MH 00841 and NIH DK 35254 to TJB.

510.16

PHOTOPERIODIC CONTROL OF BODY FAT REGULATION FOLLOWING LIPECTOMY IN SIBERIAN HAMSTERS. M. M. Mauer* and T. J. Bartness. Depts. of Biology and of Psychology, Georgia State Univ., Atlanta, GA 30303.

Siberian hamsters decrease body weight, primarily as body fat, when exposed to short, 'winter-like' days (SD). We were interested in testing whether the apparent ability to 'regulate' total body fat is photoperiod-dependent. In this experiment, male hamsters were housed in long days (light:dark 16:8) or transferred to SDs (light:dark 8:16) for 22 wks. SD-housed hamsters become photorefractory, showing gonadal recrudescence and body weight (fat) gain after ~20 wk of SD exposure. Therefore, we thought that surgical lipectomized (LIPX) hamsters undergoing naturally-occurring body weight (fat) increases would exhibit an enhanced ability to show seasonally-appropriate body fat levels. Paired epididymal white adipose tissue (EWAT) fat pads were removed (EWATx) or sham surgery was performed in LD hamsters and in SD hamsters exhibiting at least a 8% body weight loss across the 22wk SD period. Twelve weeks postsurgery some EWAT regrowth was seen in both LD and SD EWATx animals (47 & 23% of sham control values, respectively). Surprisingly, only LD EWATx animals returned to sham body weight and total carcass lipid levels. The LIPX-induced deficit in total body fat was compensated by retroperitoneal and dorsal WAT pad weight increases above sham values. These LD results suggest a seasonally-appropriate ability to regulate total body fat that is not seen in SD recrudescing hamsters. This inability of SD EWATx hamsters to regulate total body fat may reflect a permissive action of the gonadal steroids on fat accumulation, since their testes only were partially recrudesced (mean paired weight=~250mg). Testes of this size likely would be accompanied by relatively lower serum testosterone (T) concentrations. The possible role of T in the ability to regulate seasonally-appropriate body fat levels is currently being examined. Supported by NIMH RSDA MH 00841 and NIH DK 35254 to TJB.

BIOLOGICAL RHYTHMS AND SLEEP VI

511.1

HOST CLOCK RESETS PHASE OF METABOLIC ACTIVITY OF GRAFTED SCN. J. Servière[1], J. le Sauter[2] and R. Silver[2]. [1]Physiol. Sens. INRA, 78350 Jouy/Josas, France ; [2]Barnard College Columbia Univ., N.Y., N.Y. 10027.

In mammals, the suprachiasmatic nuclei (SCN) act as the dominant pacemaker controlling circadian rhythms (CR). In rodents, ^{14}C-deoxyglucose (2-DG) studies have demonstrated, both *in vivo* and *in vitro* that SCN energy consumption fluctuates with high levels of glucose utilisation during subjective day. In SCN lesionned animals, locomotor activity CR can be restored by transplantation of fetal whole tissue containing SCN. In intact animals, grafts can develop without altering period or phase of the host. To further explore interactions between host and donor SCNs, supernumerary SCN from neonates were implanted in intact adults male Syrian Hamsters ; the phase of the CR in host and donor SCNs were examined using 2-DG metabolic activity as an index.

Host and pregnant hamsters were housed in opposite light/dark cycles. On the day of birth, whole tissue containing SCN from neonates was implanted into the IIIrd ventricle of intact adults. Locomotor activity was recorded under constant darkness. 2-DG was injected during inactive (CT05) and active (CT14) periods. On the first day after grafting, the donor clock retained its phase indicating that isolation of the SCN from the fetal brain and implantation into the adult host animal did not disrupt circadian rhythmicity in the donor clock. From the 14th day after grafting, host and donor SCN were in synchrony, invariably with the phase of the host animal. The results indicate that the host SCN sends a signal which is effective in reseting the grafted SCN, and not vice versa. Further 2-DG injections at various delays after grafting will help to determine whether a diffusible signal is involved in synchronizing the two clocks. Supported by grants from INRA (J.S.) and AFOSR F49620 (R.S.).

511.2

SUPRACHIASMATIC NUCLEUS TRANSPLANTS NORMALIZE ENTRAINMENT IN AGED TAU MUTANT HAMSTERS. M. W. Hurd and M. R. Ralph*. Dept. of Psychology, Univ. of Toronto, Toronto, Ontario, Canada, M5S 1A1.

Fetal suprachiasmatic nucleus (SCN) transplants are able to restore circadian activity rhythms that have been eliminated by lesions. Using a period mutation in the golden hamster, tau, transplants between wild-type hamsters and mutant hamsters have demonstrated that period (24 vs. 20 or 22 hours) is an endogenous property of the tissue grafts. Expression of the donor rhythm requires at least a partial lesion of the host SCN and both donor and host rhythms may appear simultaneously if the host SCN is partially ablated. The partial SCN lesion causes reproducible changes in activity rhythms including shortened period, reduced amplitude and fragmentation. Similar changes accompany aging in animals which may reflect a progressive cellular and/or synaptic degeneration within the SCN. We have therefore investigated expression of fetal SCN grafts in intact, aged hosts and have found previously that grafts are capable of increasing total activity and altering the period of the host.

In these experiments, tau mutants that had received wild-type grafts were placed into a 14:10 light-dark (LD) cycle to assess entrainment patterns. Fetal tissue blocks from wild-type animals were harvested and transplanted into hosts of different tau genotypes using a standard protocol described elsewhere (Ralph et al., 1990). Preliminary results suggest that transplanted animals are able to entrain to a LD cycle. Normally, tau mutants are either unable to entrain to a LD cycle or they entrain with an abnormally advanced phase angle (Ralph & Menaker, 1988). However, mutant animals with wild-type implants exhibited entrainment patterns that are characteristic of the wild-type. These results suggest that the SCN graft is able to integrate functionally with the circadian system of the aged host.

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511.3

CIRCADIAN RHYTHMICITY RESTORED BY RAT-TO-HAMSTER ANTERIOR HYPOTHALAMIC (AH) HETEROGRAFTS IS NOT ABOLISHED BY INDUCTION OF ANTIGENIC RESPONSE TO RAT. P.J. Sollars* and G.E. Pickard. Department of Psychiatry, Univ. of Penn., Philadelphia, PA 19104

We have reported that rat-to-hamster heterografts of fetal AH tissue containing the suprachiasmatic nucleus (SCN) are able to restore circadian rhythmicity to arhythmic, SCN-lesioned hosts. To determine the extent to which the rat implant is responsible for generating the restored rhythm, seven successful restorations of rhythmicity were followed after several weeks by application of rat skin grafts onto the dorsal surface of the hamster host to trigger an immunological rejection of the AH heterografts. In animals with the most robust restored circadian patterns of locomotor activity, this procedure failed to abolish or even to disrupt the rhythm. This suggests either that the skin grafts did not induce a rejection of the implanted neural tissue, or that the observed rhythmicity arose from an oscillatory capacity remnant in the brain of the SCN-lesioned hamster hosts. Histological evaluation of the damage to the host SCN and of the viability of the neural implant will be conducted to distinguish between these possibilities. Supported by MH47501 to GEP.

511.4

ALTERATIONS IN NEURONAL EXCITABILITY IN VENTROBASAL COMPLEX OF THE RAT ACCOMPANYING CHANGES IN STATE OF AROUSAL. G.A.Marks' and H.P.Roffwarg. Dept. of Psychiatry, Southwestern Med. Sch., Dallas, TX 75235.

Neurons of the thalamus are influenced by state of arousal. Transfer of information through the thalamus is subject to state-specific alterations related both to inhibition and excitation. The absence of inhibitory interneurons in many dorsal thalamic nuclei, including ventrobasal complex (VB), of rat provides a specialization for studying the consequences of altered inhibitory mechanisms. Spontaneous activity of VB neurons in the rat and in neurons in thalamic nuclei that contain interneurons is similar in that discharge rate is high in active waking and REM sleep and low in SW sleep. However, bursting discharge occurs in rat VB neurons during REM sleep rather than the single spike discharge that characterizes the REM sleep in other thalamic nuclei. Inasmuch as bursting activity is associated with inhibition in thalamic neurons, we attempted to uncover whether the pattern of excitability across states of arousal is different for VB neurons in rat.

Excitability of individual VB neurons was measured by the proportion of responses to near threshold level shocks (50% in SW) applied to the medial lemniscus during different states of arousal. In the majority of cases, we observed high levels of excitability in waking and REM sleep and relatively low levels in SW sleep. These are the same excitability changes observed in other thalamic nuclei. We conclude that the altered discharge pattern of VB neurons in rat does not make for a major functional change in state-dependent-information transfer through this thalamic nucleus.

511.5

A CONTROLLED WAVELET TRANSFORM FOR BIOLOGIC TIME-SERIES. S. J. Schiff*, Dept. of Neurosurgery, Children's Nat. Medical Center, Washington, D.C. 20010.

Fourier transforms have had wide application to detect periodic components in biologic time-series, and are most accurate when applied to long time-series with "stationary" mean values. When faced with non-stationary, erratic biological signals, wavelet transformations permit one to continuously explore along a signal for meaningful patterns at progressively smaller time scales. Recent use has been made of pseudo-random number controls to identify non-random clustering in 2 dimensional maps of galaxy and asteroid distribution using wavelet transformations. This same control technique is here applied to 1 dimensional time-series. The method is developed and verified with transient periodic functions, using sine functions contained within Gaussian envelopes contaminated with added noise. Noise can be effectively filtered out using pseudo-random number time series as controls, and non-random clustering of points and periodicity detected. The ability to detect fractal and chaotic behavior is explored. The method is then applied to a set of experimental time-series of spinal cord reflexes that has been previously characterized as a linearly correlated stochastic process. This method is widely applicable to experimental data from many biologic systems.

511.6

SPONTANEOUS PHASIC ACTIVITY IN TWO THALAMIC NUCLEI DIFFERS ACROSS BEHAVIORAL STATE. L.D. Sanford, A.R. Morrison*, W.A. Ball, R.J. Ross and G.L. Mann. Depts. of Anim. Biol., and Psychiatry, Univ. of Penn. and VAMC., Phila., PA 19104.

Ponto-geniculo-occipital waves (PGO) recorded from the lateral geniculate body mark the onset and course of rapid eye movement sleep (REM). PGO-like waves occur in many brain areas including the thalamic central lateral nucleus (CL). Both PGO and CL waves occur spontaneously and may be elicited by "alerting" stimuli in all behavioral states. Both nuclei receive cholinergic input from the mesopontine region thought to be responsible for PGO generation. To determine whether waves in CL and LGB could be produced by common generator mechanisms we compared rate/min of each wave in non-REM (NREM), transition from NREM into REM (T) and REM. The subjects were 5 cats with standard sleep recording electrodes (EEG, EOG, and EMG) and with depth electrodes in LGB and CL. Rate/min of both waves was consistent across episodes and within cats during each test state. PGO rate/min was greater in REM (58 $\pm$ 2.0) and T (36 $\pm$ 3.7) than in NREM (9 $\pm$ 0.71). CL wave rate/min was somewhat greater during NREM (10.7 $\pm$ 3.4) than T (2.8 $\pm$ 0.8) and REM (0.9 $\pm$ 0.5) although the difference did not reach significance ($p < .08$). PGO rate/min was higher than CL wave rate/min during T ($p < .01$) and during REM ($p < .001$). There was no significant difference between PGO and CL waves during NREM. Although similar rates/min of PGO and CL waves were found in NREM, waves in each nucleus occurred concurrently or independently. CL waves were infrequent in T and REM, when PGO were most frequent. The results indicate that macropotentials in CL and LGB are not the same waves propagated anteriorly from a common brainstem generator region, and that their mechanisms are differentially active across behavioral state. We suggest that phasic activity during REM, which may be recorded in many areas of the brain, is not a unitary phenomenon. Supported by MH-42903, MH-18825 and the D.V.A. Med. Res. Serv.

511.7

ONTOGENY OF THE CIRCADIAN LOCOMOTOR ACTIVITY RHYTHM IN CRAYFISH. M. L. Fanjul-Moles*, M. Miranda-Anaya and J. A. Prieto-Saqredo. Lab. de Neurofisiología Comparada, Fac. Ciencias, UNAM., México, D. F., 04510, MEXICO.

The aim of this work was to investigate the temporal organization of locomotor activity during ontogeny in crayfish. *Procambarus clarkii* juvenile instars, aged between 10 and 120 days after hatching, were individually housed in especially designed activity recording cages under constant temperature conditions. The animals were divided in two experimental groups (n=20). In a first group each crayfish was left in free running condition under darkness (DD) during 30 days. In a second group each crayfish was in DD free running for at least 10 days and afterwards changed to a light dark cycle (LD 12:12) during the next 20 days. In general the activity rhythms of juvenile instars were not nearly as robust as those expressed by adults[1], and activity onsets were not well defined. 58% of animals examined between 10 and 90 days exhibited a circadian activity rhythm in DD (τ=23.3 hrs). Only the 50% of this age animals showed the ability to synchronize to LD cycle (τ=24.1 hrs). The juvenile crayfish 120 days old displayed a free running clearer and shorter circadian activity rhythm (τ=21.7 hrs) and fully synchronized to the LD cycle (τ=23.7 hrs). This result , although preliminary seems to indicate that the expression of the overt activity rhythm in crayfish is related to the postembryonic development.

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[1]Page, T. and Larimer, J. (1972). J. Comp. Physiol. 78:107-120.

511.8

CIRCADIAN REGULATION OF EFFERENT OPTIC NERVE ACTIVITY IN *LIMULUS*

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A circadian clock in the brain modulates sensitivity of the *Limulus* lateral eye via efferent fibers traveling in the optic nerve. The rhythmic efferent signals regulate numerous structural and functional properties of the retina that combine to enhance nighttime sensitivity as much as 10^6 times (Barlow *et al., J Gen Physiol* 89: 353-378, 1987). We analyzed the temporal characteristics of the clock's output by recording for several days from efferent fibers of the lateral optic nerve in restrained animals partially submerged in a seawater aquarium placed in a light-proof Faraday cage.

No efferent activity is detected during the animal's subjective day, but a structured firing pattern is observed during the animal's subjective night. Throughout most of the night, efferent spikes are fired in highly synchronous bursts of about 10-12 spikes each. Clusters of 6-12 bursts are grouped into packets separated by long intervals of inactivity ("dead time"). The intervals between spikes, bursts, clusters, and packets are shortest in the early evening and lengthen throughout the night. The burst rate is highest following the onset of activity, decreases as the night progresses, and stops by dawn. Prior to dawn, the interspike interval can lengthen to the point that discrete bursts are no longer distinguishable suggesting a gradual uncoupling of efferent cell bodies in the brain. This complex temporal organization of activity may indicate the presence of nested oscillators that control the various periods of efferent activity: interspike (ms), interburst (1-7 sec), intercluster (>10 sec), interpacket (mins), and circadian (~24hr).

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511.9

EXPRESSION OF PERIOD (*PER*) REPEAT SEQUENCE WHICH EXISTS IN *DROSOPHILA* CLOCK GENE IN THE RAT BRAIN. N.Shibata*[1], S.Noji[2], K.Ono[3], T.Nohno[5], N.Ishida[4], Y.Mitsui[4], A.Tokunaga[3], S.Taniguchi[2] and T.Ohmoto[1] Dept. of Neurological Surgery[1], Dept. of Third Anatomy[3], Okayama Univ. Med. Sch., Dept of Biochemistry[2], Okayama Univ. Dental Sch., Okayama, 700, Cell Science and Technology Division[4], Fermentation Research Institute, Agency of Industrial Science and Technology, Tsukuba Science City, 305, Dept. of Pharmacology[5], Kawasaki Medical College, Kurashiki, 701-01, Japan

This study aims to elucidate circadian rhythm in mammals. *Per* repeat sequence, which is a hexamer repeat sequence (ACNGGN)n, exists in the clock gene of *Drosophila*. A fragment of the genomic DNA containing *per* repeat was cloned from mouse liver DNA library, and was identified to be cp2.2. Eleven cDNA recombinants were cloned with cp2.2 from rat brain cDNA library. One of them was designated pRB15, which contained *per* repeat. The temporal and spatial expression of genes hybridized with pRB15 was examined in the adult rat brain by *in situ* hybridization. Hybridization signals were obserbed in almost all neurons. Fluctuation of the signals under the light-dark cycle was apparently observed in the suprachiasmatic nucleus; the hybridization signals was intense in the middle of the day, but became weak in the middle of the night. However, the signal stayed relatively constant in other brain regions. The signal was detected in some glial and ependymal cells in the day but few in the night. The present findings suggest that genes hybridized with the *per* repeat sequence may be involved in the circadian rhythm in the rat nervous system.

511.10

THE GENICULOHYPOTHALAMIC COMPONENT OF THE CIRCADIAN SYSTEM IN A MUTANT ANOPHTHALMIC MOUSE: CYTOARCHITECTURE AND IMMUNOCYTOCHEMISTRY FOR NEUROPEPTIDE Y (NPY). L. K. Laemle* and J.E. Ottenweller. Dept. of Anatomy, Cell Biology and Injury Sciences and Dept. of Neuroscience, New Jersey Medical School, Newark, NJ 07103.

Circadian rhythms generated by the suprachiasmatic nuclei (SCN) are entrained to environmental light. Light information normally reaches the SCN through two visual pathways: a direct pathway (RHT) from the retina and an indirect pathway (GHT) from the intergeniculate leaflet (IGL). The mutant anophthalmic mouse is characterized by the absence of the RHT.The development of the GHT in these animals has not been explored. In the normal mammalian system GHT terminals in the SCN are known to contain NPY. We have used light microscopic methods to examine the GHT in anophthalmic mice. Serial sections were stained with cresyl violet for study of cytoarchitecture or with immunocytochemical methods for localization of NPY. Our data demonstrate that the IGL is well developed in anophthalmic mice. Perikarya and fibers in the IGL are immunoreactive for NPY, and based on a robust plexus of NPY fibers and terminals in the SCN, we conclude that a GHT develops in the absence of visual input. (Supported by a grant from the Whitehall Foundation).

511.11

BIOCHEMICAL MODELING OF THE BULLA GOULDIANA OCULAR CIRCADIAN CLOCK, Michael H. Roberts*, Dept. of Biology, Clarkson University, Potsdam, NY, 13699

The eyes of several marine snails contain circadian pacemakers. Although these clocks have been extensively studied, no detailed model of the cellular mechanism regulating the circadian rhythm has been explicitly formulated. Based upon some recent inhibitor studies, we have proposed that the cellular mechanisms generating the circadian and cell division cycles may be similar (Roberts et al., 1992). In support of this proposal, a 29kDa homolog of the cell-cycle kinase, p34cdc2, is found in the Bulla eye (Leader and Roberts, in prep.).

In an attempt to further support our proposal, a mathematical simulation derived from a model of the cell cycle (Tyson, 1991) is used to model the molluscan circadian rhythm. Manipulation of rate constants in the model corresponding to protein synthesis and tyrosine kinase activity produces results which match the effects of protein synthesis and tyrosine kinase inhibitors on the phase and the period of the circadian rhythm. In addition, period stability in spite of rate constant manipulation can be observed, suggesting a mechanism for the temperature compensation of circadian rhythms. These simulations provide support for the hypothesis that circadian rhythms in the molluscan eye are generated by biochemical processes similar to those generating the cell division cycle. Supported by NS26272.

511.12

ANISOMYCIN BLOCKS PHASE SHIFTS OF THE *BULLA* CIRCADIAN PACEMAKER TO LOW EXTRACELLULAR CA^{2+} PULSES. S.B.S. Khalsa* & G.D. Block. Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

Circadian studies have revealed 2 types of phase response curves (PRC's) which are shifted 12 hrs in time from each other. In *Bulla* and *Aplysia* pulses of light, depolarizing seawater, cGMP (*Aplysia*) and depolarizing current injection (*Bulla*) yield PRC's with phase shifts in the subjective night (depolarizing-type PRC). Pulses of cAMP, hyperpolarizing seawater, serotonin (*Aplysia*), hyperpolarizing current injection (*Bulla*), low extracellular Ca^{2+} (*Bulla*) and FMRFamide (*Bulla*) yield PRC's with phase shifts largely in the subjective day (hyperpolarizing-type PRC). It has been hypothesized that the membrane potential rhythm drives a transmembrane Ca^{2+} flux rhythm, and these agents all phase shift by perturbing Ca^{2+} flux. Depolarizing-type PRC agents phase shift by generating a Ca^{2+} influx during the subjective night when it is normally low; hyperpolarizing-type PRC agents phase shift by inhibiting Ca^{2+} influx during the subjective day when it is normally high.

Studies in *Aplysia* have shown that serotonin and cAMP-induced phase shifts are blocked with anisomycin. This study addresses whether a similar blockade can be observed in *Bulla* for hyperpolarizing-type PRC agents. Pulses of low Ca^{2+} seawater (with EGTA) were applied in the late subjective day from CT 8-14 to yield phase advances relative to controls. When this experiment was repeated with additional 0.75 uM anisomycin applied to both experimental and control eyes from CT 7-14, no phase shift was apparent. Anisomycin applied alone at this phase has little effect. Preliminary data suggest that FMRFamide-induced phase advances may also be blocked with anisomycin.

These data provide support for the hypothesis that agents generating a hyperpolarizing-type PRC act via a common mechanism: inhibition of transmembrane Ca^{2+} flux, which in turn generates a phase shift via a protein synthesis-dependent mechanism. *Supported by NS15264 to G.D.B.*

511.13

THE TRANSCRIPTION INHIBITOR, 5,6-DICHLORO-1-ß-D-RIBOFURANOSYLBENZIMIDAZOLE (DRB) BLOCKS THE PHASE-SHIFTING EFFECT OF cGMP ON THE CIRCADIAN RHYTHM OF NEURONAL ACTIVITY IN RAT SCN *IN VITRO*. C. Liu* and M.U. Gillette. Neuroscience Program and Dept. of Cell and Structural Biology, Univ. of Illinois, Urbana, IL 61801.

A phase advance of the circadian rhythm of neuronal activity in the SCN is induced with subjective night-time treatment with cGMP analogs. The phase-shifting effect of cGMP could be mediated by regulating transcription or changing post-transcriptional processes. To study the two possibilities, we have examined the effect of the reversible trancription inhibitor DRB on the SCN circadian rhythm and tested whether it could block the phase-shifting effect of cGMP.

Coronal hypothalamic slices containing the SCN were prepared from 8 wk old female Long Evans rats housed in a 12L:12D cycle. The medium in the brain slice chamber was replaced with fresh medium containing DRB, 8-Br-cGMP or both for designated periods. The neuronal activity rhythm in SCN was measured by monitoring firing rates the following day.

A two-hour pulse of DRB (100 μM) given at CT 13-15 had no detectable effect, however, when given at CT 1-3, CT 5-7, CT 9-11, CT 17-19 and CT 21-23, it produced either arrythmicity or multiple activity peaks. DRB (10 μM) had no effect at CT 13-15 and CT 17-19 but disordered the circadian rhythm when given at CT 5-7 and CT 9-11. Treatment with 1-hr 8-Br-cGMP(0.5 mM) at CT 17-18 caused 6-7 hr phase advance. This phase-advance effect was totally blocked in the presence of 10 μM DRB (CT 17-19). When a DRB pulse was given either before the 8-Br-cGMP pulse at CT 15-17 or 1 hr late at CT 18-19, the effect of the cGMP anolog was not blocked. These results demonstrate: 1) the SCN circadian rhythm is insensitive to DRB treatment during early to middle subjective night but interrupted by the treatment at other circadian times; 2) DRB blocks the resetting effect of cGMP. They suggest that transcriptional events are involved in maintaining the circadian oscillator and that immediate early gene transcription may mediate the phase-shifting effect of cGMP. (Supported by PHS NS 22155).

511.14

CIRCADIAN VARIATION IN S100 PROTEIN IN HAMSTER CNS. U. Vaidya*, L. Morin and M. R. Wells. Nerve Regeneration Research Laboratory, Dept. of Veterans Affairs Med. Center, Northport, N.Y. 11768 and Depts. of Neurology and Psychiatry, SUNY, Stony Brook, N.Y. 11794.

Astrocytes participate in a variety of localized functions including the inactivation of neurotransmitters, extracellular ionic concentrations, and possibly the supply of trophic factors to neurons. A calcium binding, astrocyte-specific protein, S100b, has recently been reported to have neurotrophic activity in both the peripheral and central nervous systems. We now report preliminary evidence that S100b production varies during the circadian cycle in both the suprachiasmatic nucleus and in other areas of CNS. Adult, male hamsters (n=7 per group) were maintained on a (10:14) light /dark cycle. Tissue samples, consisting of spinal cord, visual cortex, somatomotor cortex, optic nerves, suprachiasmatic nucleus including a portion of the underlying chiasm, anterior hypothalamus, and hippocampus were taken at the midpoint of the light and dark cycles. Samples were homogenized, and the soluble fraction examined for content of S100b protein using an enzyme-linked immunoassay. Content of S100b protein was expressed as a function of total protein content in the soluble fraction. Significant light-dark (L>D)differences were observed in visual cortex and suprachiasmatic nucleus ($p<0.05$). Optic nerve also showed a similar trend which was not statistically significant. Spinal cord and anterior hypothalamus showed a significant D>L difference ($p<0.05$). These data provide evidence for a greater content of S100b protein in astrocytes at times of increased neuronal activity at different points in the circadian cycle. Supported by the Dept. of Veterans Affairs.

511.15

H8 BLOCKS QUIPAZINE-INDUCED PHASE ADVANCES OF THE MAMMALIAN CIRCADIAN CLOCK IN VITRO. R.A. Prosser*, H.C. Heller, and J.D. Miller. Dept. of Biological Sciences, Stanford University, Stanford CA 94305.

The mammalian circadian clock located in the suprachiasmatic nuclei (SCN) produces a 24 hr rhythm in spontaneous activity when isolated *in vitro*. This clock can be phase-shifted *in vitro* by a variety of agents, including cAMP analogs and the serotonin agonist quipazine. cAMP analogs induce phase advances in the subjective daytime, while quipazine induces daytime phase advances and nighttime phase delays. The daytime effects of quipazine are mimicked by the 5-HT_{1A} agonist 8-OH-DPAT and blocked by the 5-HT_{1A} antagonist NAN-190. Since 5-HT_{1A} receptors stimulate cAMP production in some systems, we investigated whether the effects of quipazine could be blocked by the protein kinase A inhibitor H8.

Coronal brain slices containing the SCN, prepared from male Wistar rats housed in a 12:12 LD cycle, were maintained in constant perifusion conditions. At a specified time during day 1 *in vitro* the tissue was treated for 1 hr with either quipazine (1 uM), H8 (5 uM), or quipazine + H8. The activity of single cells was recorded extracellularly on day 2 and their firing rates were averaged into 2 hr intervals to determine the pattern of SCN neuronal activity. This rhythm was then compared to that seen in untreated slices.

We find that, while H8 has no effect by itself at CT 6, it blocks the phase advance induced by quipazine at this time (2.88±0.13 hr advance with quip., N=2, vs 0.83±0.13 hr advance with quip+H8, N=3). In contrast, preliminary results suggest that H8 does not block quipazine-induced phase delays at CT 15. Other preliminary results suggest that H8 alone induces significant phase-delays when applied at CT 10. These results suggest that the chain of events through which quipazine phase-advances the SCN clock includes the following: bind to 5-HT_{1A} receptor -- incr. cAMP -- stimulate protein kinase A. (This work was supported by NRSA fellowship 08905 to RAP and the Upjohn Company.)

511.16

ALTERATIONS IN CIRCADIAN CONTROL IN SPONTANEOUSLY HYPERTENSIVE RATS. R.V. Peters[1]*, E.G. Stopa[2], G. Anderson[3] and H.E. Albers[1]. [1]Lab. of Neuroendocrinol. & Beh., Depts. Biol. & Psych., GA State University, Atlanta GA 30303; [2]Dept. Path. & [3]Dept. Med., SUNY Health Science Center, Syracuse, NY 13210.

The suprachiasmatic nuclei (SCN) of spontaneously hypertensive rats (SHR) contain higher levels of vasoactive intestinal peptide (VIP) mRNA than do the SCN of normotensive Wistar-Kyoto (WKY) control rats. Since the SCN is the site of a putative circadian pacemaker, and VIP is hypothesized to play an important role in the control of circadian rhythms, we have investigated whether circadian control is altered in SHR rats. Previously, we have reported that the onset of activity occurs nearly 1.5 hrs earlier relative to lights off in SHR rats than in WKY controls. The present study examined whether the difference in the timing of activity onset might result from differences in the free-running period of the underlying circadian pacemaker. The free-running period of the circadian activity rhythm in constant darkness was significantly shorter in SHR rats than in WKY controls. The free-running period was lengthened by constant light in both groups but remained comparatively shorter in SHR rats. Following the experiments, the SHR rats were confirmed to have higher blood pressure than WKY controls. These data suggest that the circadian period may be related to the levels of VIP in the SCN. (Supported by AG09301).

511.17

DIURNAL RHYTHM OF GALANIN-LIKE IMMUNOREACTIVITY IN HYPOTHALAMIC NUCLEI OF THE RAT. A. Akabayashi[1], J.I. Koenig[2], J.T. Alexander[1], S.E. Kyrkouli*[1], and S.F. Leibowitz[1]. [1]The Rockefeller Univ., NY, NY 10021 and [2]Georgetown Univ. Sch. Med., Wash DC 20007

Galanin (GAL) is found to be abundant in the hypothalamus and has a range of functions in the physiological control of various endocrine and behavioral process. The purpose of this study is to examine the diurnal rhythm in hypothalamic GAL levels to explore possible new roles for this brain peptide. Male Sprague-Dawley rats, maintained on a 12:12 hr light/dark cycle, were sacrificed by decapitation at light (N=10) and dark (N=10) onset. Ten hypothalamic nuclei, and the anterior (AP) and posterior (PP) pituitary were microdissected. GAL was measured by RIA. Circulating serum corticosterone (CORT), aldosterone (ALDO), insulin, and glucose levels were also determined.

GAL levels were significantly higher at dark onset relative to light onset in the suprachiasmatic nucleus (24.5 ± 4.4 vs. 10.9 ± 1.8 ng/mg protein, in the light, $p<0.02$) and the supraoptic nucleus (30.7 ± 4.3 vs. 15.3 ± 2.0 ng/mg protein, $p<0.01$). While a reverse trend, higher GAL levels at light onset, was apparent in the magnocellular paraventricular nucleus (PVN; 25.0 ± 5.1 vs. 19.1 ± 4.6 ng/mg protein, $p>0.1$), other areas (parvocellular PVN, arcuate nucleus, AP, and PP) exhibited no diurnal rhythm of GAL levels. Measurement of circulating levels of hormones revealed strong diurnal light/dark rhythms for CORT and ALDO, with peak levels at dark onset. No significant relationship between these hormones and GAL levels was detected. These results indicate possible roles of GAL in biological circadian rhythm.

511.18

EARLY DESIPRAMINE EXPOSURE ALTERS CIRCADIAN RHYTHMS IN RATS. A. M. Rosenwasser* and M. J. Hayes, Dep't of Psychology., Univ. of Maine, Orono, ME 04469.

Rats exposed to desipramine during early postnatal development show neuro-behavioral alterations in adulthood that may model human depression. In the present study, we applied this model to the study of free-running circadian drinking rhythms. Neonatal rats were cross-fostered and culled into litters. Male pups were given daily desipramine (5.0 mg/kg, s.c.; N=6) or saline (N=6) injections on postnatal days 8 through 22. After weaning, animals were housed individually and drinking was continuously monitored during extended exposure to constant darkness (DD), constant light (LL), and re-exposure to DD. Desipramine-treated rats showed unusual instability of free-running period and generally longer periods than saline-treated rats under DD, but periods did not differ in LL. Desipramine-treated rats also showed increased intake of a 10% alcohol solution, consistent with a previous report. These results indicate that developmental perturbation of monoaminergic systems can alter circadian rhythmicity and behavior in adulthood.

BIOLOGICAL RHYTHMS AND SLEEP VII

512.1

A PARADOXICAL SLEEP WINDOW FOR PLACE LEARNING IN THE MORRIS WATER MAZE. G.M. Rose* and C. Smith, Medical Research VAMC, Denver, CO, U.S.A. and Dept. of Psychology, Trent University, Peterborough, Ontario, Canada

Paradoxical (PS) sleep, or REM sleep, deprivation is known to disrupt learning in both human beings and laboratory animals. Recently it has been shown that loss of PS during distinct periods, termed PS or REM windows, is as effective in inducing learning impairments as is total PS deprivation. In rats, PS is accompanied by rhythmical slow activity ("theta" rhythm) in the hippocampus. Electrical stimulation patterned to mimic this theta rhythm is very effective in inducing long-term potentiation (LTP), a lasting increase in synaptic strength which is thought to serve as a memory encoding device. Taken together, these observations suggest the possibility that endogenous plasticity mechanisms are engaged during the PS window. However, a necessary step in verifying this hypothesis involves demonstrating the existence of a PS window for a hippocampus-dependent learning task.

Long-Evans rats were given 4 trials/day in the place learning version of Morris water maze. The rats were then selectively deprived of PS for a specific 4-hour interval after training. It was found that PS deprivation during the window 5-8 hours after training, but not at other intervals, delayed the learning of the location of the hidden platform. Thus, PS during a particular period after training is necessary for normal acquisition of this hippocampus-dependent place learning task. Studies are currently in progress to evaluate whether NMDA-receptor antagonists, administered during the PS window, will also retard place learning in the water maze.

512.2

CORONARY FLOW SURGES ENHANCED DURING THE PHASIC MUSCLE TWITCHES OF REM SLEEP. **LW Dickerson, MM Thurnher, RL Verrier.* Georgetown University School of Medicine, Department of Pharmacology, Washington DC 20007**

Previous studies in dogs showed dramatic increases in coronary blood flow (CBF) coupled with episodes of sinus tachycardia during REM sleep. The present report found that 90% of these surges were concentrated in periods of phasic REM sleep and only 10% in tonic REM sleep. The incidence of these surges was related to the degree of phasic eye movement activity, in that the surges were three times more frequent during intensely phasic REM sleep than during moderately phasic REM sleep. However, the magnitudes of heart rate and CBF surges were unaffected by the particular substage of REM sleep in which the surge occurred.

An additional enhancement of the magnitude of CBF surges was associated with the presence of phasic muscle twitches and not with frequency of eye movements or PGO waves. The increase in CBF (29.3% ± 1.8%) was significantly greater ($p<0.006$, unpaired t-tests) in phasic REM sleep with muscle twitches (n=100 events) than in phasic REM sleep without twitches (21.6% ± 1.8%) (n=62 events). There were no changes in blood pressure in either group and no significant differences in the magnitudes of the increase in heart rate between these two groups. CONCLUSIONS: (1) A mechanism other than enhanced cardiac metabolic demand (HRxSBP) is implicated in the additional 8% increase in CBF concomitant with muscle twitches during REM sleep. (2) The tachycardia-associated surges in CBF represent part of the repertoire of autonomic responses intrinsic to the phasic periods of REM sleep in dogs. (3) The phasic events of REM sleep (eye movements vs. muscle twitches) affect the incidence and magnitude of CBF surges differentially.

512.3

SLEEP APNEA PATTERNS IN LEAN AND OBESE ZUCKER RATS. **S. De Mesquita*, K.A. Burgess and E. A. Schoene. Dept. of Physiology, Marshall University School of Medicine, Huntington, WV 25755-9340.**

Sleep apnea is strongly associated with obesity in humans. This study investigated the frequency and type of sleep apneas in obese and lean Zucker rats. Nine lean (475±6g) and six obese (743±20g) Zucker rats were implanted with EEG and EMG electrodes and allowed to recover. All rats were monitored for sleep-wake pattern for 4 hr on five consecutive days. Prior to each sleep recording each rat was fitted with a chest pneumograph.

Two types of apneas were identified: Quiet Apneas (QA) and Augmented Apneas (AA).

	Quiet Apnea		Augmented Apnea	
(sec)	NR	R	NR	R
lean	2.4±.3	1.7±.1	2.8±.2	2.3±.2
obese	1.5±.1	1.4±.1	1.9±.2	1.7±.1

Both types of apneas tended to be longer in the lean rat during NR (Non-Rapid Eye Movement) sleep. The frequency of apnea occurrence was not significantly different between the lean and obese rats, however there were significantly more apneas during REM than Non-REM sleep.

512.4

ABSENCE OF REBOUND AND INCREASE OF VIP WHEN CSF IS EXTRACTED AFTER SLEEP DEPRIVATION. A. Jiménez-Anguiano, A. Báez, R. Aguilar-Roblero* and R. Drucker-Colín. 1. Depto. de Neurociencias, Inst. de Fisiología Celular and 2.Inst. de Invest. Biomédicas, UNAM, México, D.F.,México.

Cerebrospinal fluid (CSF) from sleep-deprived (SD) animals and VIP has been shown to increase REM sleep periods in normal and insomniac animals. The aim of this study was to determine the effects of CSF extraction (EXT) from SD cats on REM sleep rebound and to quantify by RIA the concentration of the VIP-LS in the CSF from SD cats. 43 adult cats implanted with a cannula in 4th V were used. EXP I: 23 cats were additionally implanted with electrodes for standard sleep-wake cycle recordings and were studied under the following conditions: control (CC), under SD by the water tank method for 24, 48 and 72 h and under SD for 24, 48 and 72 h with EXT of 100 ul of CSF. They were recorded during 12 hours. EXP II: 20 cats were studied in the following conditions: CC, under SD for 24, 48 and 72 h and CC of SD with a large platform (LP), for 24, 48 and 72 h. After the SD or CC, CSF (200-400 ul) was withdrawn. Then, all samples were analyzed with VIP 125 I RIA. Results showed that SD produces an increase of REM sleep which was abolished by the EXT of CSF. Moreover, this rebound is related to an increase in the concentration of VIP in the CSF from SD cats and this effect was independent of stress. These results suggest that during SD, the VIP is accumulated in the CSF and this may be causally related to REM sleep rebound.

512.5

EFFECTS OF CHRONIC SLEEP DEPRIVATION ON CENTRAL ADRENOCEPTORS IN RAT BRAIN. *L.-L. Tsai, B.M. Bergmann, B.D. Perry* and A. Rechtschaffen. Depts. of Psychiatry and Psychology, The University of Chicago, Chicago IL 60637.*

We deprived rats of sleep (SD) for 10 days by the disk-over-water method (Bergmann et al. Sleep 12:5-12, 1989) and compared their regional adrenoceptor binding sites with those of yoked control (YC) and home cage control (CC) rats (n=7, each group). Relative to baseline, the SD rats lost 73±7% (Mean±SD) of total sleep and 90±7% of paradoxical sleep. As in previous studies (Everson et al., Bergmann et al. Sleep 12:13-21, 31-41, 1989), food intake and energy expenditure increased and waking body temperature eventually decreased more in the SD rats than in the YC rats. For 11 brain regions, six concentration saturation assays using ^{3}H-prazosin, ^{3}H-rauwolscine, and ^{125}I-iodocyanopindolol were used to determine B_{max} and K_D values for α_1, α_2, and ß receptor binding sites, respectively. Adrenoceptor binding site B_{max} and K_D values were significantly different among groups only for the cerebellum and the hypothalamus. In the cerebellum, higher α_2-binding site density (SD: 24.7±4.1, YC: 26.6±4.5, CC: 19.1±2.7 fmol/mg protein, $p < 0.05$) with lower affinity (SD: 0.47±0.12, YC: 0.59±0.2, CC: 0.25±0.08 nM, $p < 0.05$) and lower ß-binding density (SD: 70.0±19.7, YC: 75.0±8.5, CC: 97.8±25.1 fmol/mg protein, $p < 0.05$) were seen in the SD and YC rats than in the CC rats. This pattern is attributable to apparatus effects common to the SD and YC rats. In the hypothalamus, α_2-binding site density (SD: 133.7±68.9, YC: 70.2±19.9, CC: 75.0±20.3 fmol/mg protein, $p < 0.05$) was higher in the SD rats than in the YC and CC rats without differences in affinity. The hypothalamic binding site differences are attributable to sleep loss in the SD rats. Thus, the present results did not support a functional role of sleep in the upregulation of adrenoceptors as proposed by Siegel and Rogawski (Brain Res. Rev. 13:213-233, 1988). However, the upregulation of hypothalamic α_2 receptor binding sites after prolonged sleep loss suggests a possible role of hypothalamic adrenoceptors in sleep-related mechanisms and functions.

512.6

HUMAN AND RABBIT INTERFERONS INDUCE SLEEP IN RABBITS. M. Kimura-Takeuchi*, J. A. Majde, L. A. Toth, M. R. Opp and J. M. Krueger, Univ. of TN, Memphis, TN 38163, and Office of Naval Res., Arlington, VA 22217.

Interferon (IFN) is usually defined by its antiviral activity; it also has many other biological activities. Human recombinant (hu-r) IFNα2 enhances non-rapid-eye-movement sleep (NREMS) in rabbits and rats and reduces latency to REMS in monkeys. Patients undergoing IFN therapy complain of sleepiness. The somnogenic effects of IFNß have not heretofore been documented nor has a species-specific IFN been assayed for sleep promoting activity in the species of origin. Thus, hu-rIFNß and rabbit (rb) IFN were tested in male rabbits implanted with EEG electrodes, a brain thermistor and intracerebroventricular (ICV) cannula. Each received pyrogen-free saline solution on day 1, and one of 3 IFN preparations on day 2: Hu-rIFNß (Berlex Lab., Inc.; 300,000 or 1,125,000 U), rb-IFNα/ß (Lee Biomolecular Res. Inc.; 640 or 6,400 U) or rb-IFN (NIH standard; 25 or 250 IU). Sleep-waking activity was monitored for 6 h after each injection. The high dose of hu-rIFNß, but not the low dose, significantly increased NREMS and brain temperature (T_{br}); neither dose altered REMS. All doses of rb-IFN enhanced NREMS and elicited fever; none affected REMS. Further, in an antiviral assay using rabbit RK-13 cells, hu-rIFNß was much less potent than the rabbit IFNs. In conclusion, data suggest that the potencies of IFNs are relatively species-specific.
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512.7

REM SLEEP DEPRIVATION INCREASES CATECHOLAMINE TURNOVER AND TYROSINE HYDROXYLASE TRANSCRIPTION IN THE RAT. S.E.Smith, T.Porkka-Heiskanen†, T.Taira, J.Toppila and D.Stenberg*. Dept. of Physiology, Univ. of Helsinki, 00170 Helsinki, Finland, and †Dept. Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60208-3520.

Adult male rats were deprived of REM sleep (REMs) for 8, 24, or 72 hours, or were allowed to sleep for 8 or 24 hours after 72 hours of deprivation. The deprivation group (DEP) was kept on small platforms (diameter 6.5 cm) in a water bath, while the control animals were kept on large platforms (diameter 11 cm, CON1) or in a dry cage (CON2). In some rats the hypothalamus was rapidly dissected and frozen for catecholamine measurement while in other rats the whole brain was removed for measurement of tyrosine hydroxylase (TH) mRNA. Catecholamines were measured from homogenized tissue using HPLC with EC-detection. The DOPAC/DA ratio was significantly higher in the posterior hypothalamus (PH) of DEP animals after 24 hours of deprivation ($p<0.05$) and significantly lower after 24 hours of recovery sleep ($p<0.05$). In addition, norepinephrine was significantly elevated in the PH after 72 hours of deprivation ($p<0.05$). TH mRNA was significantly elevated in the locus coeruleus in the DEP animals after 72 hours of deprivation ($p<0.05$). These findings suggest that catecholamine function is enhanced during REMs deprivation.

512.8

RELATIONS BETWEEN REM SLEEP AND THE PONTINE AUDITORY P1 EVOKED POTENTIAL INDUCED BY CHOLINERGIC AGONISTS INJECTED INTO THE PONS. **Z. Elazar* and Y. Navat, Dept. of Physiology and Pharmacology, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv 69978, Israel.**

P1 potential, the 20-30 msec midlatency component of the vertex auditory evoked potential, was found to decrease in slow wave sleep and increase in wakefulness and REM sleep (Chen & Buchwald, 1986). We recorded this potential from the pons of cats chronically prepared with electrodes and cannulas for sleep studies. The pontine P1 potential was similar in shape but of shorter latency than that recorded from the vertex. Its amplitude varied with the behavioral state and was always higher in REM sleep than in slow wave sleep. Injections of carbachol or neostigmine (2-10 µg) in the vicinity of the electrode in pons induced REM sleep and an increase in the amplitude of the P1 potential up to twice that recorded during natural REM sleep. The amplitude of P1 decreased gradually to reach the level of the natural potential after 60 to 120 minutes, depending on the dose. The amplitude of potential P1 was dose dependent. The degree and time course of the amplitude change was correlated with the degree and time course of REM sleep enhancement. These results suggest that the amount of REM sleep is related with the intensity of cholinergic synchronous activation of pontine neuronal populations expressed by potential P1.

512.9

IL-1ß ANTIBODIES REDUCE SLEEP AND ATTENUATE IL-1ß-INDUCED ENHANCEMENT OF SLEEP IN THE RAT. Mark R. Opp* & James M. Krueger. Dept. Physiol. & Biophys., Univ. of TN, Memphis, TN 38163

Interleukin-1 (IL-1) is a cytokine with pleiotropic biologic actions (1). It is hypothesized that IL-1 is involved in the physiological regulation of sleep (2); if this is the case, then reduction of IL-1 levels at appropriate receptors should result in less sleep. To test this hypothesis, male Sprague Dawley rats were intracerebroventricularly (ICV) injected with 3 doses of anti-rat IL-1ß antibody (Cytokine Sciences; 5-, 10-, 20 µg) at light onset and sleep-wake activity determined for the next 12-hr. The amount of time spent in non-rapid-eye movement sleep (NREMS) or in REMS was not greatly affected by the two lowest doses of anti-IL-1ß. However, after the 20 µg dose of the antibody, the amount of time spent in NREMS was reduced by 61-min across the 12-h recording period, relative to control values; REMS was not greatly affected. To determine if anti-IL-1ß could antagonize the effects of exogenously administered hu-r-IL-1ß, another group of rats was pretreated with 20 µg of the antibody 1-h prior to "lights off" and injected just prior to "lights off" with 10 ng hu-r-IL-1ß. The characteristic enhancement by hu-r-IL-1ß of NREMS was completely abolished by this pretreatment. These data support the hypothesis of a role for IL-1 in sleep regulation. *Supported in part by: NS 25378 and MH 47103.* *1) Dinarello, CA. Blood 77:1627-1652, 1991. 2) Krueger, JM, et al., Yale J Biol Med 63:157-172, 1991.*

512.10

REM-ENHANCING EFFECTS OF ADRENERGIC ANTAGONIST IDAZOXAN INFUSED INTO THE MEDIAL PONTINE RETICULAR FORMATION IN THE FREELY MOVING CAT. M. Bier*and R.W. McCarley. Harvard Medical School/VAMC, Brockton, MA 02401

Adrenergic input to the medial pontine reticular formation (mPRF) has been postulated to play a role in the control of REM sleep and the sleep-wake cycle. We have previously reported (Soc. Neurosci. Abstr., 1991) the effects on sleep-wake states of adrenergic agonists norepinephrine (NE, mixed α_1,α_2 agonist), phenylephrine (PE, an α_1 agonist) and clonidine (CL, an α_2 agonist) microinjected into the mPRF. These data showed that CL is REM suppressive, presumably by action at α_2 receptors. The REM suppressive effect was localized to those regions of the mPRF which give short latency, long duration increases in REM following infusion of carbachol. Here we describe the effect of the α_2 antagonist idazoxan (IZ) on REM sleep. A series of microinjections into the mPRF of IZ (55 nmol and 550nmol in 0.5 µL/ 1 minute) were performed. In the first hour REM sleep was decreased following IZ (55nmol, -38.6% : 550nmol, -86.9%). In the second hour IZ at a dose of 55nmol decreased REM -56.9% while the higher dose of 550nmol increased REM by 44.1%. In the third hour REM sleep was increased at both doses of IZ (55nmol, 24.4% : 550nmol, 40.0%). In the fourth hour IZ increased REM at both doses (55nmol, 250% : 550nmol, 625%). Across the entire 4 hours of recording REM sleep was significantly increased by IZ at 55nmol (44.7%; n=12; $p<0.05$) while the higher dose also produced an increase (155%), but this effect was not significant with n=8. These data indicate a REM-enhancing role of IZ. This action is presumably mediated through antagonism of α_2 receptor activation but heterogeneity of this receptor and binding of IZ to imidazoline sites pharmacologically distinct from the α-adrenoceptors complicates interpretation of action in-vivo. SUPPORTED BY NIMH Grant MH18825

512.11

EFFECTS OF A LOW DOSE OF 8-OH-DPAT ON SLEEP IN THE RAT. RH Pastel* and P Covington. Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307.

8-OH-DPAT (8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide) is a selective and potent 5-HT_{1A} agonist. One previous study demonstrated that 2.5 mg/kg of 8-OH-DPAT suppressed both rapid-eye movement (REM) and non-REM (NREM) sleep. However, that dose also induces the serotonin behavioral syndrome, hypothermia and increased feeding. We investigated the effects of 10 ug/kg of 8-OH-DPAT, a dose which does not produce these effects.

Three male rats were given saline (sc) and 24 hr later 8-OH-DPAT (10 ug/kg, sc). Treatments were administered at light onset and EEG was measured for 6 hr following injection. Administration of 8-OH-DPAT increased waking and decreased both NREM and REM sleep. The suppression of REM and NREM sleep, reported previously using a 2.5 mg/kg dose, could have been due to the induction of the serotonin behavioral syndrome, feeding behavior and/or hypothermia. The present results, using a smaller dose of 8-OH-DPAT, suggest that the effects of 8-OH-DPAT on sleep may be due to an effect on 5-HT_{1A} receptors.

512.12

DISRUPTION OF LIGHT-INDUCED C-FOS IMMUNOREACTIVITY IN THE SUPRACHIASMATIC NUCLEI (SCN) OF MIDDLE-AGED FEMALE RATS. J.M. Lloyd*, A. Cai and P.M. Wise. Department of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201

Mammalian circadian rhythmicity of various biological functions is maintained by a light-entrained pacemaker in the hypothalamic suprachiasmatic nuclei (SCN). Recent evidence suggests that this light-entrainment may involve the induction of specific immediate early genes such as c-Fos. To determine if age-related deficits in SCN function involve altered levels of neuronal activation we compared c-Fos expression in the SCN of young and middle-aged animals. Regularly cycling or ovariectomized estradiol-treated young (3-4 month) and middle-aged (10-12 month) animals were administered an overdose of pentobarbital on proestrus or after 2 days of estradiol and perfused 75-90 min before and after lights on. Brains were processed for immunocytochemical localization of c-Fos (Cambridge Research, OA-11-823). In agreement with previous studies, in young animals c-Fos expression was evident only after lights on. However, in middle-aged animals, (a) in some instances, expression of c-Fos can be observed prior to lights on, (b) the intensity of light-induced c-Fos expression appears to decrease, and (c) the number of c-Fos containing neurons decreases. These data demonstrate that there is a disruption in light-induced c-Fos expression the SCN of middle-aged animals. Supported by NIH AGO2224 and NRSA AGO5525.

512.13

INDUCTION OF FOS IN THE SUPRACHIASMATIC NUCLEI IN VITRO. M.R. Dwyer*, E.L. Meyer, M.E. Harrington and E.L. Bittman. Dept. of Psychology, Smith College, Northampton, MA 01063 and Zoology Dept., University of Massachusetts, Amherst, MA 01003.

Light pulses which phase shift circadian rhythms also induce several immediate early genes, including c-fos, in the suprachiasmatic nuclei (SCN, Kornhauser et al, Science, 255, 1992). Both carbachol (an acetylcholine agonist) and forskolin (an adenylate cyclase activator) have been reported to phase-shift circadian rhythms. We used an in vitro slice preparation to determine if these pharmacological agents would also be associated with increased Fos-like immunoreactivity (Fos-lir) in the SCN.

Brain slices (600 μm) obtained from male golden hamsters housed under 14L:10D at either Zeitgeber time (ZT) 0-1 or ZT 8-9 (ZT 12 = lights off). The slices were maintained as described previously (Harrington et al, Soc. Res. Biol. Rhythms abs. 1992). Carbachol (10 mM) and forskolin (24.4 μM) were applied 6 h post-dissection. Control slices received no drug application. Thirty minutes later all slices were placed in cold 4% paraformaldehyde for 4 h. After storage in 30% sucrose, brain slices were sectioned (30-45 μm) into cryoprotectant (-20°C) and stored until staining with a rabbit polyclonal antibody (dch-1) raised against the N terminal (2-17) of rat Fos (D. Hancock and G. Evan, ICRF, London).

Control slices showed little Fos-lir in the SCN. Carbachol was able to induce Fos-lir at ZT 14. Forskolin was able to induce Fos-lir at both ZT 6 and ZT 14. Photic induction of Fos in vivo is only observed in the subjective night. Our results indicate that this may be due to a circadian fluctuation antecedent to or not involving adenylate cyclase activation. Supported by NIH NS26496 (MEH) and NIMH 44132 (ELB).

512.14

c-fos PROTO-ONCOGENE CHANGES AFTER REM SLEEP INDUCED BY VASOACTIVE INTESTINAL POLYPEPTIDE (VIP). H. Merchant-Nancy, A Jiménez-Anguiano and R. Drucker-Colín* Instituto de Fisiología Celular. Universidad Nacional Autónoma de México. México D.F. México.

Since it has been showed that intraventricular administration (IVA) of VIP induce a selective increase in REM sleep, we used Fos like inmunostaining (FLI) for constructing maps of the patttern of second messenger activity in the brain stem, after REM sleep induction by this manipulation. 12 male Wistar rats were implanted for conventional sleep recorgins. Aditionaly, a stainless steel cannula was implanted into the 4th ventricule. Two groups were used: a control, in wich an IVA of 5ul saline was applied, and a VIP group where 100 ng of VIP in 5 ul was inyected IVly. After 4 hr of sleep recording, the animals were anesthetised and perfused with 4% parafolmaldehyde. The brains were processed for inmunohistochemistry with the ABC technique of Hsu et al., 1982.We used a Fos antibody provided by Tom Curran. The results showed an increase in REM sleep frequency in VIP group. Moreover, this group showed a significant increase in FLI in several brain stem structures. These results suggest that VIP sleep inducing properties may depend on an increment in the number of active neurons in a withspread network in brain stem.

512.15

DEVELOPMENT OF FOS-LIKE IMMUNOREACTIVITY IN DORSOLATERAL PONTINE REGIONS ASSOCIATED WITH REM SLEEP. **Priyattam J. Shiromani*, Munazza Malik, Stuart Winston & Robert W. McCarley**, *VA Med Ctr & Harvard Med School, 940 Belmont St, Brockton, MA 02401*

We sought to determine the time course of activation of the immediate-early gene, *c-fos*, in conjunction with rapid-eye movement (REM) sleep. Carbachol, a mixed cholinergic agonist, was used to produce a sustained REM sleep episode. Fos-like immunoreactivity (Fos-LI) was assessed at various times after the end of REM sleep.

Cats were implanted (pentobarbital) with sleep recording electrodes and a guide cannula in the medial pontine reticular formation (medial PRF). Two weeks later, microinjections of vehicle (0.25ul;n=3) or carbachol (0.2-2.0 ug/0.25ul;n=9) were made. The animals were euthanized by overdose of pentobarbital at various intervals after end of REM sleep.

In vehicle controls, few Fos-LI cells were seen in the dorsolateral pons. Carbachol elicited 15 min to 70 min long REM sleep bouts. In carbachol animals examined immediately upon end of carbachol-REM sleep, counts of Fos-LI cells in the raphe, LC, LDT-PPT and the medial PRF were higher compared to vehicle controls. One carbachol animal with a 45 min long REM sleep bout showed few Fos-LI cells 24 hrs after end of REM sleep. A lower dose (0.2 ug/0.25 ul) of carbachol produced no REM sleep and no Fos-LI cells compared to vehicle controls. One carbachol animal had no REM sleep and no Fos-LI cells.

Fos-LI occurs in association with cholinergically-induced REM sleep in pontine regions implicated in REM sleep. A minimum duration of REM sleep appears to be necessary for Fos-induction. However, a plateau in number of Fos-LI cells is reached after about 45 minutes of continuous REM sleep. Some of the Fos-LI cells are cholinergic or noradrenergic.
Supported by DVA Research Service, NS30140, and NS25212

512.16

BASAL AND PHOTIC-INDUCED EXPRESSION OF IMMEDIATE EARLY GENES IN YOUNG VS. AGED RAT BRAIN. E.L. Sutin*, B.F. O'Hara, F.L. Watson, and T.S. Kilduff, Sleep Res. Ctr, Stanford Univ., Stanford, CA 94305.

Many of the sleep disturbances of the elderly such as the fragmentation of the major sleep period and early morning insomnia may be due to a deterioration in the circadian system that underlies the timing of sleep and wakefulness. In an effort to address this issue at the molecular level, we have focused on circadian aspects of "immediate-early" gene (IEG) expression which may be involved in both input and output mechanisms of the circadian pacemaker. Two approaches have been utilized: 1) The photic-induction of IEGs in the suprachiasmatic nucleus (SCN) was examined in young vs. old rats. In a pilot study, young (4 months) and aged (22 months) male Fischer-344 rats were given either a 30 min light-pulse late in the subjective night at circadian time (CT) 22 or left in darkness. Message levels were identified by *in situ* hybridization using a [^{35}S] cRNA probe to c-*fos*. Aged rats that received photic stimulation at CT 22 demonstrated a blunted response of c-*fos* mRNA expression in the ventrolateral SCN relative to young rats. Neither young nor old rats remaining in darkness showed any detectable IEG expression. 2) IEG expression in extra-SCN regions was investigated across the circadian cycle using Northern blot analysis. Nocturnal rats have the highest levels of c-*fos* message during the night while maximal levels were observed in diurnal ground squirrels during the day, suggesting a correlation with activity. In contrast, c-*jun* mRNA was invariant in the rat but increased during the active phase in squirrels. Surprisingly, in older rats, which have weaker activity rhythms, a somewhat higher level of c-*fos* message was observed. In addition, two larger messages cross-hybridized with c-*fos* that were not apparent in the younger rats. Taken together, these results suggest alterations in circadian aspects of IEG regulation which may help elucidate mechanisms underlying the age-dependent decay of circadian organization.
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512.17

NEURONAL GENE EXPRESSION DURING SLEEP DEPRIVATION. B.F. O'Hara*, K.A. Young, F.L. Watson, L. A. Roldan, W.C. Dement, and T.S. Kilduff. Sleep Research Center, Stanford University, Stanford, CA 94305.

The two-process model of sleep regulation posits that a homeostatic drive to sleep, referred to as Process S, increases with time spent awake. The purpose of this study was to evaluate whether immediate early gene expression is increased in brain in proportion to time spent awake, when Process S would be expected to increase. Rats were deprived of sleep by gentle handling beginning at light onset for 45 min, 3 hr or 6 hr. The duration of sleep deprivation in each of the three groups was chosen to correspond to the previously described response of EEG delta power to sleep deprivation. At the end of the deprivation period, animals were sacrificed by decapitation, the brain dissected into subregions and frozen. An equal number of control rats were sacrificed at each time point. To date, Northern blots have been prepared from cortex, thalamus, cerebellum and hypothalamus. c-*fos* expression was detectable in all brain regions from all animals, however, the sleep deprived animals showed higher expression than the control animals at all time points. Curiously, the highest levels of expression were observed in the 45 min as well as 6 hr deprived rats. The high c-*fos* mRNA expression in the 45 min animals may be attributable to the transient stress of movement to the deprivation room. The high c-*fos* mRNA in the 6 hr animals may reflect either (1) increased c-*fos* expression in parallel with build-up of Process S or (2) increased expression due to stress induced by increased handling of the deprived animals. Preliminary results indicate that c-*jun* does not undergo the same changes exhibited by c-*fos* whereas *jun*-B is more similar to c-*fos*. Further efforts will be directed towards (1) other modes of sleep deprivation in an effort to minimize stress, and (2) examination of other IEGs (Supported in part by the Upjohn Company).

512.18

NEURONAL GENE EXPRESSION ACROSS THE HIBERNATION CYCLE. T.S. Kilduff*, B.F. O'Hara, F.L. Watson, L. Bitting, E.L. Sutin, S.K. Welch, and H.C. Heller. Sleep Research Center, Stanford University, Stanford, CA 94305.

Changes in arousal state such as sleep and hibernation are likely to be accompanied by changes in gene expression, some of which may facilitate state transitions. The goal of this project is to evaluate whether gene expression is altered in specific brain regions across the hibernation cycle. Our initial focus is on immediate early gene (IEG) expression because IEGs are known to play a critical role in the regulation of long-term changes in gene expression. Golden-mantled ground squirrels (*Citellus lateralis*) were implanted with abdominal telemeters to monitor body temperature (T_b) continuously. Animals were placed in a constant temperature environment at 5°C under LD 12:12 photoperiod. Four animals were sacrificed between 1200-1600 P.S.T. during each of five phases of the hibernation cycle: euthermia (T_b=37°C); entrance to hibernation (T_b=20°C); day 1 of deep hibernation (T_b<8°C); days 4 or 5 of deep hibernation (T_b<8°C); and arousal (T_b=20°C). A sixth group of summer euthermic animals was also sampled. The brain was dissected into subregions, frozen on dry ice and RNA extracted. Northern blots were then prepared for subsequent hybridization with [^{32}P] c-*fos* and c-*jun* probes. Expression of c-*fos* and c-*jun* show clear changes throughout the hibernation cycle. For example, both genes exhibit a rapid increase of message in the hypothalamus during arousal from hibernation. This observation is of particular interest because previous work, including our 2-deoxyglucose studies, has shown this brain region to play a critical role in the arousal process. Preliminary results from *in situ* hybridization studies suggest that the suprachiasmatic nucleus is among the hypothalamic regions exhibiting this increase (Supported in part by the Upjohn Company).

512.19

LIGHT ACTIVATION OF IMMEDIATE EARLY GENE EXPRESSION OCCURS PREDOMINANTLY IN INTRINSIC NEURONS OF THE SUPRACHIASMATIC NUCLEUS. E. L. Bittman, M. H. Hastings, and F. J. P. Ebling*, Dept. of Anatomy, University of Cambridge, U.K.

Light exposure during the subjective night activates the expression of several immediate-early genes (IEGs) in the suprachiasmatic nucleus (SCN) of the Syrian hamster and causes phase shifts in circadian activity rhythms. The role of these light-responsive cells of the circadian pacemaker in the generation of behavioral and endocrine rhythms is not known. We have combined retrograde labelling of SCN neurons with photic induction and immunocytochemical detection of Fos and Egr-1 in an attempt to determine whether induction of IEGs occurs in cells which project to identified targets of this nucleus or to the contralateral SCN.

Hamsters (n=10) maintained on a 16L:8D cycle received unilateral stereotaxic injections (0.1-0.3µl) of fluorescent latex microspheres (Lumaflour, Inc.) aimed at the septum, preoptic area, hypothalamic paraventricular or ventromedial nuclei, subparaventricular area, intergeniculate leaflet (IGL), or SCN. After a survival period of 1.5-3 weeks, animals were exposed to light for 1h commencing 3 h after lights off and were then anesthetized and perfused with 4% paraformaldehyde. Frozen sections (40µm) were processed for Fos and Egr-1 immunoreactivity (-ir) and examined for fluorescent label.

Light exposure reliably induced Fos-ir and Egr-1-ir in the SCN and the IGL. As reported previously , the greatest concentration of IEG-ir neurons was seen in the ventrolateral SCN. Immunoreactive cells also occurred in the surrounding anterior hypothalamus. Retrograde labelling of SCN and subparaventricular neurons revealed projections to each of the areas targetted. Less than 1% of the Fos-ir or Egr-1-ir cells contained fluorescent microspheres. It is concluded that photic induction of these IEGs occurs principally in intrinsic cells of the SCN rather than its projection neurons. Supported by SERC (GR/H08716) and The Royal Society.

INGESTIVE BEHAVIOR: WATER AND SALT INTAKE

513.1

OSMOSENSITIVE VAGALLY-MEDIATED CONTROL OF DRINKING IN ADVANCE OF SYSTEMIC DEHYDRATION IN RATS. F. S. Kraly*, Y.-M. Kim and L. M. Dunham. Dept. of Psychology, Colgate Univ., Hamilton, NY 13346.

Intragastric infusions of hypertonic solutions activate splanchnic afferents to increase plasma vasopressin without changing plasma osmolality in rats (Choi-Kwon & Baertschi, Am. J. Physiol. 261: E18, 1991). We examined whether such infusions elicit drinking without systemic dehydration. Adult Sprague-Dawley male rats (n=32) were surgically prepared with a chronic gastric catheter. A test in rats not deprived of food or water was initiated by a 2 ml (in 1 min) infusion of 300, 600, 1200 or 1800 mOsm NaCl or sucrose, mannitol, LiCl or Na isethionate. The latency to initiate drinking was decreased ($p<.05$) and 60-min water intake was increased ($p<.05$) by 600, 1200 and 1800 mOsm NaCl compared to 300 mOsm NaCl. Plasma osmolality at initiation of drinking was not changed from baseline (300 mOsm NaCl) by 600 or 1200 mOsm ($p>.10$) but was increased by 1800 mOsm NaCl ($p<.05$). Equiosmotic solutions of sucrose, mannitol, Na isethionate and LiCl also elicited drinking. Drinking elicited by 600, 1200 or 1800 mOsm NaCl was abolished by total abdominal vagotomy ($p<.05$). Our results are consistent with the hypothesis of a vagally-mediated osmosensitive gastrointestinal and/or hepatic-portal mechanism for eliciting drinking in advance of systemic dehydration in the rat.

513.2

ROLES OF AT-1 AND AT-2 ANGIOTENSIN (ANG) II RECEPTOR SUBTYPES IN EXPERIMENTALLY-INDUCED WATER INTAKE IN RATS. M.J. Fregly, A. Rozelle, L. Han, K. Greenwood & N.E. Rowland*. Depts Psychol & Physiol., Univ of Florida, Gainesville, FL 32611.

Water intake induced by either central or peripheral injection of ANG II is blocked by the AT-1 receptor antagonist losartan potassium (DuP 753). We report the effects of acute cerebroventricular (ICV) injection of losartan (50 ug: 500x the dose that blocks water intake to ICV ANG II) on various types of thirst in rats. ICV losartan had no effect on water intake following either hypertonic NaCl, isoproterenol, carbachol (ICV), or on food intake in a dessert test. ICV losartan blocked drinking caused by either SC injection or IV infusion of ANG II. The antidipsogenic effect of central AT-1 blockade thus is selective for ANG II.

In contrast, ICV administration of the AT-2 receptor antagonist, PD 123319 (100 ug) blocked water intake following injection of hypertonic NaCl, isoproterenol, ICV carbachol and ANG II. ICV PD 123319 did not reduce food intake in a dessert test. Thus, the AT-2 antagonist may have a common action on the various mechanisms of water intake, but it does not nonselectively reduce food ingestion. We speculate that an AT-2 receptor may be on a "final common pathway" for the integrated thirst signal. **Supported by the American Heart Assoc., Florida affiliate.**

513.3

ANGIOTENSIN RECEPTORS IN SFO BUT NOT OVLT MEDIATE ISOPROTERENOL-INDUCED DRINKING. D.A. Fitts*. Dept. of Psychology, Univ. of Wash., Seattle, WA 98195.

Rats having lesions of the subfornical organ (SFO) drank an average of 0-2 ml water after isoproterenol, 10-50 mg/kg SC, compared with mean intakes of 5-7 ml in sham controls. The doses of isoproterenol were much lower than previously reported in SFO-lesioned rats (160 μg/kg, Simpson et al, JCPP, 1978), so this lesion-induced reduction in intake is not specific to high doses of isoproterenol.

Another experiment compared the effects of 90-min infusions of the angiotensin (ANG) receptor blocker [Sar^1,Thr^8]-ANG II (sarthran), 20 pmol/hr at 0.8 μl/hr, into SFO, organum vasculosum laminae terminalis (OVLT), or lateral ventricles after 20 mg/kg SC isoproterenol. A sarthran infusion into SFO significantly reduced drinking from 4.2 ± 0.4 ml in the saline vehicle control condition to 2.3 ± 0.6 ml (mean ± SE, $p < .01$); the same infusion into OVLT (4.6 ± 0.7 vs 5.0 ± 0.6 ml, respectively) or into the dorsal third ventricle caudal to the SFO (3.5 ± 0.9 vs 3.6 ± 0.4 ml) did not. Sarthran was ineffective at 20 (4.8 ± 0.5 vs 4.3 ± 1.0 ml) or 500 (3.3 ± 0.6 vs 4.4 ± 0.5 ml) pmol/hr in the lateral ventricles.

ANG accounts for at least 47% of the drinking observed after isoproterenol treatments in rats, and the receptors critical for this drinking are in SFO rather than OVLT. The study also confirms that blood-side ANG receptors in SFO are inaccessible to moderate doses of ventricularly applied blockers (Fitts & Masson, Behav. Neurosci., 1990). This explains some negative results in previous studies attempting to block peripheral ANG from the ventricles.

Supported by NS-22274.

513.4

THE HYPERDIPSIA INDUCED BY INJECTION OF MUSCIMOL INTO THE MEDIAN RAPHE NUCLEUS (MR) IS ATTENUATED BY LESIONS OF THE SUBFORNICAL ORGAN (SFO) OR LATERAL HYPOTHALAMUS (LH). T.R. Stratford* and D. Wirtshafter, Department of Psychology, University of Illinois at Chicago, Chicago, IL 60680

We have previously reported intense hyperphagia and hyperdipsia in rats following injections of the GABA-A agonist muscimol into the MR. In the current study, we lesioned various forebrain structures which have been reported to play a role in the mediation of water intake and observed the effects on muscimol-elicited drinking. Electrolytic lesions of the SFO or ibotenic acid lesions of the rostral pole of the LH significantly attenuated the hyperdipsia. While control animals drank an average of 11.8 mls of water during the 60 min test period, the mean intake of SFO-lesioned rats was 2.1 mls and that of LH-lesioned rats was 3.0 mls. In contrast, electrolytic lesions of the precommissural median preoptic nucleus (MnPO) or excitotoxic lesions of the lateral preoptic area (LPO) significantly increased water intake during the test. Mean water intake was 20.8 mls for the MnPO-lesioned group and 21.4 mls for the LPO-lesioned group. Lesion effects on a series of regulatory challenges suggest that muscimol-induced drinking may be related to the renin-angiotensin system.

513.5

CHANGES OF DRINKING BEHAVIOR IN THE RAT AFTER SEPTAL LESIONS. R.-M. Liao*& C.-C. Yeh; Dept. of Psychology, National Cheng-Chi University, Taipei, Taiwan, R.O.C.

The septum has been suggested to play an inhibitory role in the drinking behavior. It is also known that the septum is heterogeneous in terms of neuroanatomical perspective. The present study examined the water intake and the locomotor activity of rats lesioned with kainic acid (0.5 ug/0.5 ul/ site) on three septal subregions: anterio-medial (MSa), posterio-medial (MSp), and lateral (LS) sites. There were significant differences between groups on both measurements in the postlesion tests. Drinking volume was enhanced mostly by the MSp lesion, so was the locomotor activity. Another experiment further determined the dipsogenic effects of polyethylene glycol (PEG; 20%, SC) and hypertonic saline (NaCl; 1M, IP) in the MSp lesioned rats. These cellular and extracellular thirst stimuli were given on the 18th postlesion day. Water intake was significantly increased by the hypertonic treatment, but not by the injection of PEG. In addition to showing the septal hyperdipsia, these data suggest that the septum can be functionally heterogeneous in drinking behavior.

(supported by NSC 80-0412-B004-01)

513.6

SUPRACHIASMATIC LESIONS EFFECT UPON THE LATERALIZED PREFERENCE IN DRINKING BEHAVIOR OF RATS. R. Díaz-Pérez, P. Vergara-Aragón and B. Barrera-Mera*. Depto. de Fisiología. Fac. de Medicina. U.N.A.M. A. Postal 70250 México 04510 DF

Electrolytical lessions selectively applied on either left (L) or right (R) suprachiasmatic nuclei (SCN) have revealed direct SCN control on rodents locomotor circadian activity. Initial and final half-portions of the total amount of loco motor activity in a 24-h period of time, apparently receive a direct neural program from each L or R SCN. These evidences claim an evaluation of hypothalamic bilateral control upon volitive tasks drived by specific procencephalic nuclei, and upon SCN mutual bilateral interaction, judged by the lateralized preference. A preferential lateralization task was measured in 42 Wistar rats of either sex. These animals (60-190 g bw) were individually housed under a L-D 12-12 photoperiod. They obtained food ad libitum, and were furnished with a couple of water bottles for drinking. Each 24 h, we measured the amount of water drinked by the animals. The preference was 27% for R, 40% for L, and 33% for both bottles. Under ether anaesthesia all the R and L animals were lesioned at their contralateral SCN. Differing from the lesion effects upon other diencephalic nuclei, SCN lesions did not induce any modification on lateral preference in drinking for either side. These results suggest that rodent SCN activity does not control the lateral drinking preference.

513.7

ANGIOTENSIN II AT1 RECEPTOR BLOCKADE WITH DuP 753 ABOLISHES SODIUM INTAKE IN THE ADRENALECTOMIZED RAT. O. G. Galaverna*, C. Polidori, S. Y. Chow, D. H. Yi , A. N. Epstein, R. R. Sakai and S. J. Fluharty. Leidy Labs, Biology Dept., Univ. of Penn., Phila. PA 19104-6018.

In the intact sodium depleted rat, sodium intake is driven by the synergistic actions of angiotensin II (Ang II) and aldosterone on the brain. Removal of the adrenal glands results in a chronic loss of sodium and an increased sodium consumption. Our previous study has shown that central administration of Sarcosine^1-Isoleucine^8-angiotensin II (SARILE), a non-selective Ang II receptor antagonist, suppressed sodium intake in the adrenalectomized male rat. The suppression was dose dependent and the highest dose of SARILE resulted in a minimal intake, which was still present after aldosterone replacement, suggesting that the residual intake was need-free intake. In the present experiment we show that blockade of AT1 receptors inhibits the sodium intake of adrenalectomized rats. Adrenalectomized female (n=6) and male rats (n=6) were given a 3% NaCl solution 2 hours per day, and received daily injections of dexamethasone (20μg/day) to compensate for the lack of corticosterone, and a prophylactic treatment of gentamicin (4mg/day). On the day of the experiment, 15 minutes before the access to 3% NaCl, the animals were given a pulse intracerebroventricular injection of DuP 753, a selective AT1 receptor antagonist at doses of 30nM/2μl or 100nM/2μl (females), 50nM/2μl or 100nM/2μl (males), counterbalanced by an injection of 2μl of saline vehicle. The intake of salt was recorded thereafter at 15 min., 30 min., 1 hour and 2 hours. At all doses of DuP, sodium intake was significantly suppressed in both males and females. At the dose of 100nM, the intake was completely abolished in males, but a residual intake persisted in females which may be an expression of need-free sodium intake. It is concluded that in both female and male adrenalectomized rats, brain Ang activation is responsible for the increased sodium intake, and that AT1 receptors are involved in the natriorexigenic effect of the brain Ang activation.

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513.8

EFFECT OF CENTRALLY ADMINISTERED MINERALOCORTICOID OR GLUCOCORTICOID RECEPTOR ANTAGONIST ON ALDOSTERONE-INDUCED SODIUM INTAKE IN RATS. L.Y. Ma, C. Polidori, J. Schulkin, A.N. Epstein, E. Stellar* B.S. McEwen and R.R. Sakai. Departments of Biology and Anatomy, University of Pennsylvania, Philadelphia PA 19104 and Lab. of Neuroendocrinology, The Rockefeller Univ., New York, NY 10021.

We have previously reported that intravenous (IV) infusion of aldosterone (ALDO) induces sodium intake in rats. Here we studied the role of central mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) in mediating this ALDO-induced sodium intake. Male rats were fitted with an intracerebroventricular (ICV) cannula and an IV catheter. Water, 3% NaCl and Purina Chow were available ad libitum. Rats received a continuous ICV (cICV) infusion of the Type I, MR antagonist, RU-28318 (10μg/hr) combined with concurrent cIV infusion of ALDO (3.3μg/hr). Central administration of RU-28318 completely blocked ALDO-induced 3% NaCl intake (cIV ALDO = 9.5±3.1 mls, vs cIV ALDO + cICV RU-28318 = 0.4±0.2 mls 3% NaCl). The suppression of cIV ALDO-induced 3% NaCl intake was reversed once the cICV RU-28318 infusion was stopped. Continuous ICV infusion of the Type II, GR antagonist, RU-38486 (10μg/hr) was ineffective in suppressing ALDO-induced 3% NaCl intake. Together, the data provide evidence for a role of brain Type I MR involvement in ALDO-induced sodium intake in the rat. Supported by MH 43787 and NSO 3469.

513.9

BRAIN SODIUM AND SODIUM APPETITE IN THE RAT. S.Y. Chow,*A.N. Epstein, B.S. McEwen, S.J. Fluharty and R.R. Sakai. Univ. of Pennsylvania, Phila, PA. 19104 and The Rockefeller Univ., New York, NY. 10021.

We examined the effects of modifying brain cerebrospinal fluid (CSF) sodium concentration during sodium depletion on the expression of sodium appetite in the rat. Male rats fitted with intracerebroventricular cannula were sodium depleted by combining treatment with furosemide and removal of all ambient sodium. They received either 2M NaCl, 0.7M mannitol or isotonic saline (5µl/hr) by continuous infusion 2 hours prior to the beginning and throughout the depletion treatment. Sodium appetite, as measured by their ingestion of 3% NaCl, was recorded the following day. Infusion of 2M NaCl decreased 3% NaCl intake (5.7 ± 1.9 mls) and infusion of 0.7 M mannitol increased 3% NaCl intake (12.4 ±1.2 mls) as compared to infusion of isotonic saline (9.4 ± 1.7 mls). Infusions of either 2M NaCl or 0.7M mannitol had no effect on the rats' daily need-free sodium intake. In addition to the changes in sodium appetite, parallel changes in the expression of hypothalamic angiotensinogen mRNA were evident. Regulation of angiotensin II receptor binding in this brain area was also examined.

Together, the data show 1) that direct manipulation of CSF sodium concentration during sodium depletion affects the expression of sodium intake in the rat and 2) that the mechanism by which modification of CSF sodium affects sodium intake may be through its effects on the brain angiotensin system which can then act as a synergist with aldosterone to elicit sodium appetite in the rat.

(Supported by MH43787)

513.10

AMYGDALA PATHWAYS INVOLVED IN SODIUM APPETITE IN THE RAT, ANATOMICAL CONSIDERATIONS. G.F. Alheid*,[1], J. Schulkin[2], and A.N. Epstein[3,†]. [1]Univ. Va. Health Sci. Ctr., Charlottesv. Va 22908, and Depts. [3]Biol. and [2]Anat. Univ. Penn, Phil. Pa. 19104. ([†]deceased).

It is known that destruction of the stria terminalis does not interfere with sodium appetite while large knife cuts in the ventral amygdalar association pathways do. In this study, we are reexamining the role of the ventral pathways with respect to DOCA and AII induced sodium appetite by placing small knife cuts in the trajectory of amygdalo-fugal and -petal pathways. In addition, behavioral testing is followed by the intracranial injection of fluorescent neuronal tracers for selected animals, in order to provide detailed information about the particular pathways spared by our knife cuts. We observed that small cuts medial to the central amygdaloid nucleus result in a decrease in sodium appetite after fluid volume depletion or repeated $Captopril_R$ injection, but normal responding to DOCA injections. Surprisingly, in all animals so far examined (n=8), these lesions did not block the projection of the central nucleus to the brainstem, since relatively complete retrograde labeling of the nucleus was possible in every case. Subsequently, we have also injected anterograde tracers into the central nucleus of knife-cut animals with the preliminary results (n=2) suggesting that these cuts might interrupt associative connections with the forebrain, and possibly central amygdalar afferents to the hypothalamus. Supp. NINCDS #NS17743 (GFA) and NIMH #MH-43787 (ANE and JS).

513.11

SALT-TASTE RESPONSES OF FOREBRAIN NEURONS, WHICH ALSO RESPOND TO IONTOPHORETIC APPLICATION OF ANGIOTENSIN II, IN AWAKE RATS. S. Nicolaïdis*, M.-C. Mousseau and S.N. Thornton. CNRS URA 637, Neurobiologie des Régulations, Collège de France, 11 pl. Marcelin Berthelot, 75231 Paris CEDEX 05, France.

Pretreatment with deoxycorticosterone acetate (DOCA) for 3 days sensitises the rat anterior forebrain to a subsequent intracerebro-venticular injection of a below threshold dose of angiotensin (AII) such that a strong appetite for sodium is generated. We have investigated electrophysiologically the effects on neurons of the septum and preoptic area of iontophoretic application (Io) of AII and of giving salt solutions to awake rats before and after pretreating them with DOCA.

Male wistar rats were anaesthetised with ketamine (0.3 ml/100 g body weight) and a specially adapted cranioplastic prosthesis fixed to the skull after exposure of the dura. This prosthesis subsequently permits restraint of the rat in a stereotaxic apparatus while allowing the animal to move its body and limbs and to drink water or solutions of salt or glucose. A 7 barrelled microiontophoretic electrode sealed to an extracellular recording electrode was then advanced through the cortex into the septal then medial preoptic regions where unit activity was recorded at the same time as different solutions were offered to drink.

In the medial septal and medial preoptic areas, but not in other areas, neurons were recorded that differentially responded to salt and to water. Some of these salt vs water taste receiving neurons also responded to Io AII which elicited a "salt" type response. In these conscious rats DOCA pretreatment produced an enhancement of neuronal reponsiveness to AII as we have shown in anaesthetised preparations.

In these areas implicated in sodium regulation and appetite, specific gustatory projections onto AII responsive neurons that can be enhanced by DOCA pretreatment suggest a mechanism by which these hormones enhance preference for salty solutions.

(Supported by MH 43787)

513.12

THE EFFECT OF MINERALOCORTICOID (MC) TREATMENT ON SUBSEQUENT INDUCED SODIUM APPETITE. T.M. Nicholson * and N.E. Rowland. Dept. of Psych., U. of FL., Gainesville

Previous studies have shown that rats acutely depleted of sodium drink more NaCl on the second depletion than the first. It has been proposed that the high levels of hormones of sodium depletion (i.e. MC and angiotensin II {Ang II}) act synergistically to sensitize brain mechanisms for sodium appetite. In order to assess the generality of this behavioral sensitization as well as the relation of the two hormonal mechanisms, two groups of female Sprague-Dawley rats were treated for 1 week with either an MC (DOCA) or the Ang CEI, enalapril (which is thought to increase brain Ang II activity). Both groups showed the expected salt appetite as measured by 24 hour intakes of 0.2M NaCl and distilled water. After a 1 week rest, a second 1 week treatment was performed, with half of the rats receiving the same treatment, and the other half receiving the other (viz: DOCA-DOCA, DOCA-enalapril, enalapril-enalapril, enalapril-DOCA). After a further 2 week rest, the treatments in phase 2 were repeated. The rats that received DOCA on all 3 treatments showed a steady increase across the phases. The rats that received enalapril on all 3 treatments showed no change across the phases. The rats that were switched from DOCA to enalapril showed a paradoxical decrease in salt appetite compared with rats receiving enalapril without prior DOCA. Rats with prior DOCA treatment did not show an abnormal plasma renin (PRA) response to the acute enalapril treatment. The mechanism and time course of the paradoxical decrease in salt appetite will require further scrutiny because MC treatment and/or NaCl intake up-regulates Ang II receptors in many brain regions. Supported by NSF grant BNS 89-09439.

513.13

FOURTH VENTRICLE MICROINJECTIONS OF DIAZEPAM ENHANCE HEDONIC REACTIONS TO TASTE. S. Peciña* and K.C. Berridge, Dept. of Psychology, University of Michigan, Ann Arbor, MI 48109.

Benzodiazepine administration (e.g. 5mg/kg diazepam, ip) enhances hedonic reactions to tastes (e.g. tongue protusions; Treit & Berridge, 1990). Benzodiazepines also enhance hedonic taste reactivity in decerebrate rats, which suggests that systems within the brainstem mediate this effect (Berridge, 1989). To test this hypothesis in normal animals, we compared the effects on hedonic taste reactivity of intracranial diazepam injections into the 3rd or 4th ventricles. Twenty rats were implanted with 3rd and/or 4th ventricle cannulae and with oral cannulae. Diazepam (0, 5, 15, 25, 40, 50, 75, and 100ug) was injected into either the 3rd ventricle or the 4th ventricle seven minutes before the rats were tested for taste reactivity to 0.3M sucrose (1ml/min).

Diazepam was more effective at enhancing hedonic reactions when injected into the 4th ventricle than when injected at the same dose into the 3rd ventricle: low diazepam doses (40ug) enhanced hedonic taste responses only when injected into the 4th ventricle and produced no hedonic enhancement when injected into the 3rd ventricle. Higher doses (50, 75ug) were effective in both ventricles. These results support the conclusion that benzodiazepine receptors in the brainstem mediate this enhancement of hedonic reactions to taste.

513.14

NEONATAL CHORDA TYMPANI TRANSECTION ALTERS ADULT PREFERENCE FOR NH_4Cl IN THE RAT. S.I. Sollars & I.L. Bernstein*. Dept. of Psychology, University of Washington, Seattle, WA 98195.

The chorda tympani nerve (CT) is considered the main gustatory pathway for NaCl taste stimulation. In the adult rat, bilateral transection of the CT does not affect preference for salts. The current study examined the effect of transection of CT in 10-day-old rats on adult preference for salts. This approach was based on anatomical evidence that primary gustatory nerves play an inductive role in taste bud development with the sensitive period for this effect spanning the first 10 days postnatal in the rat. Ten-day-old Wistar rats were given bilateral transection of the CT (CTX; N=13) or sham operations (SHAM; N=12). When 60-days-of-age the animals had two-bottle access to salt solutions (NaCl, NH_4Cl, KCl, $CaCl_2$) and water. CTX animals displayed a significantly higher preference for NH_4Cl at all concentrations tested (.05M, .1M, .15M, .2M) while their preference for other salts was not consistently altered. Following the series of two-bottle tests, animals were tested for generalization of conditioned taste aversions (CTA) to salts. LiCl was used to condition a significant CTA to .1M NaCl. Tests for generalization were performed by giving one-bottle access to NH_4Cl or KCl and a two-bottle test with NH_4Cl and NaCl. CTX and SHAM animals showed similar patterns of CTA generalization and the two-bottle test revealed that CTX animals were clearly able to discriminate between NaCl and NH_4Cl. The results suggest that neonatal transection of the CT significantly enhances adult preference for NH_4Cl and that this effect is not due to the animals' inability to distinguish between NH_4Cl and NaCl.

513.15

PSYCHOPHYSICAL EVIDENCE THAT SENSATIONS OF ASTRINGENCY ARE DUE TO DECREASES IN ORAL LUBRICATION. P.A.S. Breslin*, M.M. Gilmore, G.K. Beauchamp, and B.G. Green. Monell Chemical Senses Center, Phila., PA 19104

Aluminum potassium sulphate ($AlK(SO_4)_2$ - 'alum') was tested, in three experiments, for its ability to elicit sensations of oral astringency ('dryness') independently of its ability to elicit taste sensations. First, the perceived astringency of alum (10 g/l) was compared to water on a nongustatory surface by rubbing a cotton roll saturated with stimulus between the gum and upper lip. Astringent sensations were rated on a visual analog scale that ranged from 'no sensation' to 'extremely intense'. Alum elicited strong sensations of astringency when the lip was moved laterally against the gum to increase friction. Second, subjects were asked to identify which of two solutions elicited a sensation of astringency in a forced-choice, two-alternative paradigm by simply dipping the anterior of the tongue into two cups: one containing alum, the other a mixture of sucrose, citric acid, and caffeine that approximated the taste elicited by alum. Subjects were as likely to identify the mixture as astringent as they were the alum, when only the anterior of the tongue was stimulated. These two studies support the notion that friction is important in the identification of astringency, whereas taste cues are not. Third, after sensations of astringency had been elicited, several rinses were compared for their ability to decrease astringency. Water, cellulose, corn oil, and the subjects' own saliva were compared when rubbed between the gum and upper lip in a cotton roll. Water did not decrease oral astringency as effectively as the other lubricants. Together these three studies point to tactile sensations caused by increased friction between oral membranes as the primary basis of astringent sensations.

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INGESTIVE BEHAVIOR: NUTRIENTS, SEROTONIN AND INSULIN

514.1

EFFECT OF DILUTIONS OF CORN OIL ON INTAKE AND PREFERENCE IN LEAN AND OBESE MALE ZUCKER RATS. D. Greenberg*, D.R. Lewis, J.M. Philopena, and G.P. Smith. Bourne Laboratory, Dept. of Psychiatry, New York Hospital-Cornell Medical Center, White Plains, NY 10605.

In dietary selection studies obese Zucker rats choose a greater proportion of their diets as fats than do lean rats. To investigate the contribution of the orosensory effects of fats for the control of fat ingestion in these rats, we measured the sham fed intake of several dilutions of corn oil in one-bottle intake tests and two-bottle preference tests.

Male Zucker rats (n=11 obese, n=7 lean) were fitted with gastric cannulas for sham feeding. In 1-bottle sham feeding intake tests, eight concentrations of corn oil, decreasing in concentration by half and ranging from 100% to 0.78% were offered to rats in descending order. At the completion of this series of tests, 2-bottle preference tests were performed on two consecutive days comparing preference for 100% and 75% corn oil. There were no clear differences in 2-bottle preference. In 1-bottle tests, although intake was an inverted-U function of concentration for both genotypes, obese rats required a larger D_{50} to increase intake 50% of maximum (12.5% vs. 6.25%), and obese rats achieved maximal intakes at one-half the concentration required by lean rats (25% vs. 50%). The steeper intake response function for obese rats may be relevant to their increased fat intake.

Corn Oil Concentration		
	Obese	Lean
D_{50}	12.5%	6.25%
smallest dose for maximal intake	25%	50%
peak preference (2 bottle test)	n=5, 75%; n=6, 100%	n=7,75%

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514.2

DOSE-DEPENDENT EFFECTS OF DUODENAL AND ILEAL INFUSION OF GLUCOSE AND OLEIC ACID ON MEAL PATTERNS IN RATS. T.A. Woltman and R.D. Reidelberger*. Veterans Administration Medical Center and Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE 68105.

The mechanisms mediating the inhibition of feeding by luminal nutrients in the proximal and distal small intestine are not clearly understood. In the present study we determined the dose-dependent effects of duodenal and distal ileal infusion of glucose and oleic acid on meal patterns in ad libitum feeding rats. Animals (n=8-12) with chronic cannulas in both the duodenum and ileum (14 cm proximal to the cecum), received a 2-h infusion (0.13 ml/min) of glucose (0, 0.01, 0.02, 0.04, 0.08, and 0.16 kcal/min) or oleic acid (0, 0.003, 0.017, and 0.085 kcal/min) at the start of the dark period, and meal patterns were monitored for 19 h. ***Results***: Three-hour cumulative intake was inhibited dose-dependently by ileal as well as duodenal infusion of both glucose and oleic acid. Ileal glucose was more inhibitory than duodenal glucose, while duodenal fat was more inhibitory than ileal fat. Duodenal glucose and fat inhibited feeding by decreasing meal frequency; ileal fat decreased only meal size, while ileal glucose reduced both meal size and frequency. ***Conclusion***: It appears that distinctly different mechanisms mediate the inhibitory effects of duodenal and distal ileal glucose and fat on food intake.

514.3

INTESTINAL CAPSAICIN ATTENUATES OLEATE-INDUCED SUPPRESSION OF FOOD INTAKE WITHOUT CAUSING ANATOMICALLY DETECTABLE NEURONAL DAMAGE OR IMPAIRED OLEATE ABSORPTION. C.S. Tamura and R.C. Ritter.* Dept. of V.C.A.P.P. and Pharmacology/Toxicology Graduate Program, Washington State University, Pullman, WA 99164.

We have previously reported that intraintestinal infusion of capsaicin (5mg/rat) attenuates suppression of sham feeding by intraintestinal oleate infused 24h post-capsaicin (Soc. Neurosci. Abstr. 17:542, 1991). The attenuation is transient and oleate-induced suppression is reestablished by 48h post-capsaicin. To determine whether capsaicin's desensitization of oleate-induced suppression was due to destruction of vagal sensory neurons, we examined the dorsal hindbrain for evidence of neuronal degeneration following intestinal capsaicin infusion. Intestinal capsaicin (5mg) produced no histochemical evidence of sensory neuron degeneration in the dorsal hindbrain. In contrast intraperitoneal injection of capsaicin, 5mg/rat or 50mg/kg, produced degeneration of terminals and fibers in the nucleus of the solitary tract and the spinal trigeminal nucleus. To assess the possibility that intestinal capsaicin damages enteric neural fibers, we examined substance P-like immunoreactivity (SPLI) in whole mounts of intestinal myenteric and submucous plexes. Intestinal capsaicin caused no apparent loss of SPLI in either plexus one hour after intestinal capsaicin. Finally, we measured intestinal absorption of ^{14}C oleate in vehicle- and capsaicin-infused rats. Preliminary results indicate that intraintestinal capsaicin does not alter oleate absorption. We conclude that capsaicin attenuates oleate-induced suppression of sham feeding by a mechanism other than vagal sensory neuron destruction, substance P depletion or impairment of intestinal absorption. Supported by NIH NS20561 to R.C.R.

514.4

SUBSTRATE SPECIFIC REVERSAL OF GLUCOPRIVIC BUT NOT LIPOPRIVIC FEEDING. L.K. Singer* and S. Ritter. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

Food intake is stimulated by drugs which block glucose or fatty acid utilization (glucoprivic and lipoprivic feeding, respectively). The purpose of the present experiment was to identify the metabolic fuels capable of modulating glucoprivic and lipoprivic feeding and eventually, to determine the metabolic specificity of receptors which monitor glucose and fatty acid utilization for control of food intake. Glucose and fatty acid utilization were blocked with 2-deoxy-D-glucose (2DG, 200 mg/kg) and 2-mercaptoacetate (MA, 600 μmol/kg), respectively. Rats with intraatrial catheters were infused (0.1 ml/min) with 0.3M saline, glucose or lipids (Intralipid), beginning 15 min before injection of 2DG or MA and for the 2 h post drug period during which food intake was measured. We found that glucose infusion blocked the feeding response to 2DG, but that equicaloric lipid infusion did not (saline = 2.55g, glucose = 0.65g, lipid = 2.1g). Preliminary data indicate that glucose infusion also blocked the response to MA. These data may indicate that receptors controlling glucoprivic feeding have a high degree of metabolic specificity, while those controlling lipoprivic feeding may respond to a broader spectrum of metabolic fuels.

514.5

POSTMEAL FATTY ACID OXIDATION DEPENDS ON MEAL COMPOSITION. D.M. Surina*, W. Langhans, R.P. Reeves, C. Wenk. Institute of Animal Sciences, Swiss Federal Institute of Technology, 8092 Zurich, Switzerland.

The influence of macronutrient content of a meal on fatty acid oxidation was investigated in 13 Caucasian males after consumption of a high fat (HF) breakfast (33% CHO, 52% Fat, 15 % Pro), containing also short- and medium-chain fatty acids, and after a high carbohydrate (HC) breakfast (78% CHO, 6% Fat, 15% Pro). Respiratory quotient (RQ) and plasma beta-hydroxybutyrate (BHB) were measured during the three hours following the meal as indicators of whole body substrate oxidation and hepatic fatty acid oxidation, respectively. Plasma insulin, glucose, lactate, free fatty acids (FFA), and triglycerides were also determined. RQ was significantly lower and plasma BHB was higher after the HF meal than after the HC meal, implying that more fat is burned in general and in the liver after a HF meal. As expected, plasma FFA and triglycerides were higher following the HF meal, and insulin and lactate were higher after the HC meal. In sum, considerable fat oxidation occurred in response to a single high fat meal, without prior adaptation to a high fat diet. The short- and medium-chain fatty acid content of the HF meal may be the primary contributor to the observed increase in plasma BHB, which reflects elevated hepatic fatty acid oxidation. This is interesting in relation to previous studies which link changes in hepatic fatty acid oxidation to the control of food intake.

514.6

CELIAC GANGLIONECTOMY DECREASES THE HUNGER-STIMULATING EFFECT OF FAST HEPATIC PORTAL MONOSACCHARIDE INFUSIONS IN RABBITS. L. O'Farrell* and D. Novin. Neuroscience Program and Psychology Dept., UCLA, Los Angeles, CA 90024.

In previous experiments, fast (3 ml/min, 3.3 ml/kg, 0.3 *M*) hepatic portal vein glucose and fructose infusions increased subsequent chow intake in *ad libitum* fed rabbits. One mechanism responsible for this effect may be altered hepatic metabolism. Previous experiments showed that these hunger-stimulating infusions caused increased hepatic conversion of the monosaccharides into lipid, and decreased mitochondrial uptake of the sugars. Therefore, hunger may be associated with increased hepatic storage and decreased hepatic oxidation of nutrients. The present experiment tested whether these changes in hepatic metabolism might be causually related to the increased food intake. Because the hepatic branch of the vagus nerve could not be reliably located in rabbits, the celiac ganglion was removed. Like the vagus, this ganglion contains afferents arising in the liver. Celiac ganglionectomy decreased the hunger-stimulating effect of fast hepatic portal glucose and fructose infusions in rabbits relative to sham ganglionectomy. The results suggest that hepatic sensory innervation may be necessary for the increased chow intake following fast glucose and fructose infusions in rabbits.

514.7

ANORECTIC EFFECTS OF A NOVEL DOPAMINE-FATTY ACID BIOCONJUGATE. G.W. Hesse* and V.E. Shashoua. Ralph Lowell Laboratories, McLean Hospital, Harvard Medical School, Belmont, MA 02178.

A novel pharmacological agent consisting of a fatty acid amide linked to dopamine has been synthesized. This novel compound has been found to have anorectic effects. It inhibits food consumption by fasted mice in a dose dependent manner with an ED50 of about 15 umol/kg. The anorectic effects of this compound are antagonized by sulpiride, but not by domperidone, indicating that central nervous system dopaminergic receptors are involved. Chronic administration of this compound produces little or no tolerance. During daily administration of the compound for 21 days no loss of efficacy in appetite suppression in fasted mice was observed. At the end of the 21 day period, food consumption returned to the baseline level within 24 hours. Finally, the compound does not induce hyperactivity, stereotypy or catalepsy even at doses up to 100-fold above the ED50. This novel compound is an effective appetite suppressant which appears to lack the excitatory effects on behavior often associated with dopaminergic anorectics.

514.8

INFLUENCE OF INTRAVENOUS NUTRIENTS ON FOOD INTAKE AND ENERGY EXPENDITURE. E.K. Walls* & H.S. Koopmans. Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Metabolic theorists propose that satiety is produced by relative increases in the rate of oxidation of fuels. To determine if intravenous (iv) nutrient-induced satiety is dependent upon increased fuel oxidation, daily food intake (17 hr/day) and 24 hr energy expenditure (EE) via indirect calorimetry were measured during iv nutrient infusions in rats. Infusion of 52 kcal of glucose and amino acids (GAA) for 4 days immediately reduced food intake by 38.4 kcal or 74% of the nutrient calories infused. EE on day 1 of GAA (57.8 $\pm$ 1.2 kcal) was not greater than saline control (58.1 $\pm$ 1.3 kcal) but increased to 61.0 $\pm$ 1.3 kcal on day 4 ($p < .05$) whereas RQ values increased from .938 during saline to 1.07 and 1.08 on days 1 and 4 of GAA infusion ($p < .001$). Infusion of 40 kcal of iv lipid for 6 days reduced food intake by 21.9 kcal or 55% of the lipid calories infused. EEs on day 1 and 6 of the lipid infusion (62.7 $\pm$ 2.2 and 62.6 $\pm$ 2.4 kcal) were not greater than during saline control (62.6 $\pm$ 2.1 kcal), whereas RQ values were reduced from .904 to .848 and .811 on day 1 and 6 ($p < .001$). While the shifts in RQ indicate enhanced fat utilization in the lipid condition and increased carbohydrate utilization in the GAA condition, the fact that no changes in EE accompany large changes in food intake suggests that signals for iv nutrient-induced satiety are not generated by large increases in fuel oxidation.

514.9

PERIPHERAL CHRONIC CLONIDINE DOES NOT AFFECT SUSCEPTIBILITY TO ACTIVITY-BASED ANOREXIA. T.S. Rieg*, J. Choi, and P.F. Aravich. Department of Anatomy & Neurobiology, Eastern Virginia Medical School, Norfolk, VA 23501; Medical Research Service, VA Medical Center, Hampton, VA 23667.

Activity-based anorexia (ABA) is an animal model of anorexia nervosa (AN) with two characteristics of the disorder, decreased food intake and increased activity. We have previously shown that chronic stimulation of the paraventricular hypothalamus with the noradrenergic agonist clonidine exacerbates ABA rather than ameliorates it as predicted. This study determined if peripheral chronic administration of clonidine would affect ABA. Rats were implanted subcutaneously with osmotic minipumps infusing saline, 30, or 300 ug/kg/day. Following postsurgical recovery, all animals were exposed to ABA (1.5 hrs/day/ad lib food; 22.5 hrs/day ad lib wheel access). Susceptibility was defined as days to a 25% body weight-loss criterion. Results showed that clonidine did not affect susceptibility or food intake, but substantially increased wheel activity in a dose related fashion. These findings are perplexing in that susceptibility to ABA was not increased although the high dose animals were running more. This may be due to an inhibition of sympathetically mediated energy expenditure by clonidine in ABA.

514.10

CONCURRENT TAMOXIFEN AND FLUPHENAZINE: EFFECTS ON FOOD INTAKE, BODY WEIGHT AND ^{3}[H]-TAMOXIFEN BINDING IN BRAIN. J.M. Gray* and M. Bishop. Dept. Psych./Prog. Biopsych., Vassar College, Poughkeepsie, NY 12601

Ovariectomized (OVX) rats given daily injections of 50 ug fluphenazine (FLU) showed only slight decreases in food intake, although their body weights were significantly lowered by the end of the first week of treatment and the differences between drug-treated and control animals increased over the treatment period. OVX rats given daily injections of either 2 ug estradiol benzoate (EB) or 1 mg tamoxifen (TAM) showed transient, but highly significant, decreases in food intake and rate of body weight gain. The effects of TAM or EB on both food intake and body weight were additive with the effects of FLU in rats receiving concurrent administration of the drugs.

Prolonged treatment with FLU, TAM or a combination of the drugs had no effect on ^{3}[H]-TAM binding to 'anti-estrogen binding sites' (AEBS) in selected areas of the brain (hypothalamus-preoptic area and area postrema-nucleus of solitary tract region) and pituitary, although both unlabelled TAM and FLU compete for ^{3}[H]-TAM binding in the in vitro binding assay. (Supported by BNS-9011263).

514.11

OBESITY INDUCED BY MANIPULATIONS OF THE PARENTAL ENVIRONMENT RESULT IN ELEVATED MEDIAL HYPOTHALAMIC NOREPINEPHRINE, Alan Jones*, Emmanuel Pothos, Pedro Rada, Bartley G. Hoebel, and Deborah Olster. Pitzer College, Claremont, CA, Princeton Univ. Princeton, NJ, and University of California at Santa Barbara.

In previous work it has been shown that adult male offspring of rats that have either been injected with 6IU/Kg/day of Protamine Zinc insulin during days 15-20 of gestation (Jones & Dayries, 1990) or undernourished to 50% of baseline intake during the first two weeks of gestation only (Jones & Friedman, 1982) develop significant obesity commencing at about 50 days of age. The present experiment examined the question of whether rats with these two forms of obesity display abnormalities in neurochemical release in areas of the brain known to influence feeding and body weight. Twenty-one gauge stainless steel guide shafts, were chronically implanted using standard stereotaxic procedures. One week later 3mm 26 ga microdialysis probes were lowered into the medial hypothalamus. Dialysate collection began 12 hours later. Dialysates collected from both male and female animals in the two experimental conditions contained significantly higher Norepinephrine levels ($p<.05$) than did controls. It would appear that in addition to sharing a similar time course of onset and a sex dependent expression of obesity (only males become obese), both of these models of obesity are also characterized by elevated medial hypothalamic norepinephrine. Since expression of this obesity is sex dependent, we investigated whether sex steroids might be mediating the effect. Gonadal weights were measured and plasma estrogen and testosterone levels were measured. These data will be presented.

514.12

EFFECTS OF SYSTEMIC TRYPTOPHAN ON EXTRACELLULAR LEVELS OF NEUTRAL AMINO ACIDS IN PVN AND CAUDATE PUTAMEN. C.E. Greenwood[1]*, L.T. NG[1], P.J. Currie[2], D.V. Coscina[2], G.H. Anderson[1]. Dept Nutr Sci[1], Univ of Toronto & Clarke Inst of Psychiatry[2], Toronto, Canada.

Intraperitoneal (IP) injections of a variety of amino acids (AA), including tryptophan (TRP), increases brain AA levels and results in food intake suppression. While brain levels of AA remain elevated for up to 2 hrs after IP injections, the distribution of AA between the intra- and extra- cellular pools and possible regional differences in these responses are unknown. This study examined the effects of IP TRP (100 mg/kg) on extracellular levels of TRP and the large neutral amino acids (LNAA), which compete for the same brain uptake carrier, in separate brain regions known to regulate food intake. Microdialysis probes were simultaneously placed in the hypothalamic paraventricular nucleus (PVN) and caudate/putamen (CP) of anaesthetized rats and dialysates collected for 1 hr prior to and 2 hrs after IP TRP. Plasma TRP levels and TRP/LNAA ratio rose immediately ($p<0.05$) following TRP administration and remained elevated for 100 min. A similar time course was observed with extracelluar PVN and CP TRP levels increasing significantly within 40 min of injection and remaining elevated for 100 and 40 min ($p<0.05$), respectively. Consistent with the concept of competition for brain uptake carriers, extracelluar levels of the LNAAs were decreased following TRP administration. In all cases, no significant interaction was observed when comparing responses between the PVN and CP suggesting that these two regions responded similarly to systemic TRP administration. However, due to a larger variation in the PVN compared to the CP, a significant decline in individual LNAA was not observed. These results demonstrate that alterations in brain levels of AA following systemic AA administration is reflected in the extracelluar fluid and suggest that there is little or no difference in response between the PVN and CP. (supported by NSERC).

514.13

SEROTONIN IN THE PARAVENTRICULAR NUCLEUS (PVN) AND THE SEROTONIN ANTAGONIST METERGOLINE INJECTED PERIPHERALLY (I.P.) PRODUCE OPPOSITE EFFECTS ON MEAL PATTERNS IN RATS. J.T. Alexander, *G.B. Brennan, W.K. Cheung and S.F. Leibowitz, The Rockefeller University, New York, N.Y. 10021

Studies have shown that carbohydrate is the preferred macronutrient in the first meal of the nocturnal feeding cycle. It has been proposed that serotonin (5-HT) is involved in switching off this initial carbohydrate meal, acting through hypothalamic 5-HT_1 receptor mechanisms that control satiety. To provide a further test of this hypothesis, we have examined, in male albino rats, the impact of PVN injections of 5-HT (2.5 nmoles) and i.p. injections of the 5-HT antagonist, metergoline (MTG, 1.0 mg/kg), administered at dark onset, on the microstructure of feeding over the 12 hr nocturnal cycle. Serotonin in the PVN produced a selective decrease in carbohydrate intake. This effect, while still evident 8 hrs after injection, was exhibited primarily during the first meal of the feeding cycle. During this meal, there occurred a selective decrease in size, percent composition, feeding time and feeding rate for carbohydrate, as well as an increase in the satiety ratio (post-meal interval/meal size) for this nutrient. In contrast, i.p. MTG injection produced opposite effects, namely, a selective stimulation of carbohydrate ingestion, an increase in the rate of carbohydrate feeding, and a decrease in the satiety ratio for this nutrient. This effect was followed 3 hrs later by a potentiation of protein, but not fat, intake. These results indicate that 5-HT may be involved in switching off preference for the carbohydrate diet at the onset of the natural feeding cycle.

514.14

NEURAL SITES OF ACTION OF DEXFENFLURAMINE: c-Fos IMMUNOHISTOCHEMICAL STUDY IN RATS. B.-H.Li*, L. Han & N.E.Rowland. Dept Psych, Univ Florida, Gainesville FL 32611

The critical sites of anorectic action of the serotonin-ergic agent dexfenfluramine (DFEN) are unclear. We have examined rat brain for the presence of c-Fos following acute and chronic regimens of DFEN. C-Fos was localized in vibratome sections using antibodies (F.R. Sharp and Oncogene Ab2) to the N-terminus of Fos protein.

Saline-treated control rats showed minimal c-Fos product. Low doses of DFEN (2-3 mg/kg) increased c-Fos in several brain regions, with maximal responses after 2-6 hr; the signal was undetectable after 26 hr. Brain regions most strongly activated were: nucleus (N) of the solitary tract; lateral parabrachial N; paraventricular thalamic N; central N amygdala; bed N of stria terminalis; rostral striatum, and orbitofrontal cortex. Many of these regions receive visceral or gustatory input, suggesting a functional relationship to the anorectic action of DFEN. Anorectic tolerance occurs with repeated injection of DFEN and a parallel decrease in DFEN stimulation of c-Fos occurs after chronic low dose DFEN. Preliminary studies with C-Jun antibody (Oncogene Ab2) shows presence in the central N amygdala and rostral striatum after acute DFEN. Food deprivation induces c-Fos in several brain regions; that in the supramammilary area is reversed by acute DFEN. Supported by IRIS (Servier).

514.15

HYPOTHALAMIC 5-HT_{1A} RECEPTOR SUB-TYPES ARE INVOLVED IN CONTROL OF GLUCOSE RELATED FEEDING. B. Moorjani, P. Lacy *, and M. Jhanwar-Uniyal. The Rockefeller Univ. New York, NY 10021 and Osteopathic Med/Hlth Sci, Des Monies, IA.

The sulphonylurea tolbutamide (TOL), a hypoglycemic agent, and the 5-HT_{1A} agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), cause hyperphagia. This study explored the mechanism common to these hyperphagic actions. The objective was to examine the effect of chronic and acute TOL, and glucose (GLU) replacement therapy, on the 5-HT_{1A} receptor density, in the medial hypothalamus (MH) and lateral hypothalamus (LH) of male Sprague-Dawley rats. Rats were given one of the following treatments: a) 5 chronic injections (i.p.) of either propylene glycol (vehicle), TOL (25mg/kg), or TOL + GLU (20%); b) acute injections (i.p.) of vehicle or TOL (50 mg/kg). Rats were sacrificed 30 mins post-injection, their brains were removed, and the MH and LH were dissected. The radioligand binding technique was used to estimate the binding of [3H] 8-OH-DPAT (2 nM) to 5-HT_{1A} receptor sites (in the presence or absence of 10μM 5-HT). Trunk blood was collected for estimation of plasma GLU. The results show: 1) TOL induced a significant decrease in GLU levels in both chronic (-57.8%) and acute (-52%) states; 2) TOL produced an increase in 5-HT_{1A} receptor density in the MH, under both chronic (234%;$p<0.01$) and acute (132%;$p<0.01$) conditions; 3) GLU replacement caused a partial restoration of receptor density (-45% from the TOL treated rats). These findings suggest that the medial hypothalamic 5-HT_{1A} receptor sub-types may interact with blood glucose in the control of feeding behavior, and TOL may induce hyperphagia via activation of 5-HT_{1A} receptors.

514.16

REDUCTION IN DARK ONSET FEEDING AND CARBOHYDRATE INTAKE IN GENETICALLY OBESE (ob/ob) AND LEAN (+/?) MICE INJECTED WITH 5-HYDROXYTRYPTAMINE. P.J.Currie[1]* and L.M. Wilson[2]. [1]Clarke Institute of Psychiatry, University of Toronto, Toronto ON M5T 1R8 and [2]Department of Psychology, University of Manitoba, Winnipeg MB R3T 2N2 Canada.

The genetically obese (ob/ob) mouse exhibits multiple disturbances in neural systems putatively involved in the control of feeding, including altered levels of brain 5-hydroxytryptamine (5-HT) and reduced 5-HT metabolism. 5-HT has also been implicated in the control of carbohydrate ingestion in nonobese rodents, specifically at the onset of the dark cycle. This study examined the effects of intraventricular injection of 5-hydroxytryptamine creatinine sulphate on food intake (lab chow diet) and macronutrient (carbohydrate, protein, and fat) selection in free-feeding obese mice ($n=7$) and lean controls ($n=7$). Mice were injected with 5-HT (35-140 nmol) or sterile physiological saline, in counterbalanced order, immediately prior to dark onset. 5-HT decreased feeding and carbohydrate ingestion dose-dependently ($p<.05$), and as a result, tended to enhance the proportional intake of protein and fat. Obese mice, however, showed a reduced sensitivity to the anorectic effect of exogenously administered 5-HT. Although these results are consistent with a role for serotonin in the control of feeding and carbohydrate ingestion in mice, the altered sensitivity of the ob/ob to 5-HT treatment may result, in part, from an impaired satiety control mechanism in this genetic strain. (Supported by NSERC of Canada to LMW and a MHRC predoctoral scholarship to PJC).

514.17

Insulin Receptor Binding is Altered in Artificially Reared Rats Overfed as Neonates. E. Taylor*, J. Diaz, G. Watkins, and D. Figlewicz Lattemann, Dept. of Psychology, Univ. of Washington, Seattle, WA 98195 and VA Med. Center, Seattle, WA 98108.

Freinkel (1980) proposed that the fuel rich environment experienced by a fetus of a gestational diabetic could result in an abnormal insulin system, causing the offspring to be obese throughout life and more likely to develop gestational diabetes. Critical events in neural and hormonal development during late gestation in humans occur in early postnatal life in rats. We have shown that overfeeding rats during this critical time produces adult rats that are frankly obese and either unable to carry pregnancies to term or producing significantly smaller pups.

The purpose of this study was to examine the immediate effects of overfeeding on insulin receptor binding in brain and liver. At postnatal day four, rats were assigned to one of 3 groups: (1) mother-reared (MR); (2) Gastrostomy-fed to weight match MR's (WM); (3) Gastrostomy-fed with excess formula (OF). On day 14 the animals were sacrificed and cerebral cortices and livers were assayed for insulin receptor binding.

At sacrifice OF animals were significantly larger than MR's or WM's. Insulin binding was greatest for MR's and least for OF's. A similar but not significant trend was seen in cortical binding.

Hyperinsulinemia is seen in babies of gestational diabetics. Our data suggest that: (1) overfeeding rats during the second postnatal week may induce hyperinsulinemia leading to a decrease in insulin binding in liver; (2) formula feeding in itself may cause a lesser degree of hyperinsulinemia.

514.18

DISCRIMINATION OF INSULIN-PRODUCED HYPOGLYCEMIA P.M.DUNCAN* and W.LICHTY. Psychology Dept Old Dominion University. Norfolk, Va. 23508.

Eight rats were trained to discriminate the normal state of euglycemia from the hypoglycemia produced by injection of 6 units/kg insulin. A "drug discrimination" procedure was used with two-lever Skinner boxes and a food-motivated operant. A 60-sec no-reinforcement was followed by 6 min during which either left, or right lever presses were reinforced on a VI 10-sec schedule. Insulin or water injected 25 min prior to the operant sessions determined whether left, or right lever presses were reinforced. During 40 training sessions, reliable discrimination of the insulin-produced cue developed, with a mean of 73% responses on the "insulin lever" after insulin injection, and only 18% after water injection. After insulin, or water injection mean blood glucose levels dropped to 75%, or rose to 127% pre-injection values. When injected with 800 mg/kg ethanol, most rats chose the non-insulin ("euglycemic") lever. These results show that insulin produces a specific interoceptive cue, presumably involving hypoglycemia.

514.19

PARAVENTRICULAR HYPOTHALAMIC INJECTION OF 7-CHLOROKYNURENIC ACID (7CK) STIMULATES FEEDING IN SATIATED RATS. *T.L. Sorrels and E. Bostock, Psychology Dept., Queens College, CUNY, Flushing, NY 11367.

7CK (3ug & 6ug) and 2-amino-5-phosphonovaleric acid (AP5; 5ug & 10ug) were injected into the following hypothalamic sites: anterior h.(AH), lateral h.(LH) ventromedial n.(VMN) dorsomedial n.(DMN), and the paraventricular nucleus (PVN) of satiated rats and 1 hour consumption of pelleted food was measured. Rats with cannulae in the third ventricle anterior or posterior to the PVN were also given 7CK. AP5 failed to increase feeding at any site at the doses tested. 7CK data

Area(n=)	LH(7)	AH(6)	VMN(8)	DMN(6)	PVN(9)
7CK 3ug	0.64±.29	0.38± 25	0.71± 32	0.05±.05	¶2.66±.62
7CK 6ug	0.68±.19	1.51±.54	0.77± 41	0.93±.44	0.77±.40

Area(n)	3V(11)Anterior	3V(5)Posterior	3V(6)
7CK 3ug	0.86±.23	0.68±.19	*1.01±38
7CK 6ug	**1.78±.25	**2.33±.38	*1.31±.19
Veh	0.44±.14	0.46±.23	0.41±.18

¶ sig. dif. from each other, 3ug gp. at p<.01
* sig. dif. veh p<.05 ** sig. dif. veh. p<.01
Feeding in hypothalmic gps. following vehicle was 0.39±.09g.
PVN animals also received a test dose of norepinephrine 40 ug which produced a feeding response of 4.11±.54 gms.

DRUGS OF ABUSE: ETHANOL AND GABA

515.1

THE EFFECTS OF ALCOHOL ON INHIBITORY MECHANISMS IN RAT HIPPOCAMPAL CA1 NEURONS IN VIVO. J.R. Criado* and R.Thies. Depts. Psychiatry & Behav. Sci. and Physiology & Biophysics, Univ. of Okla. HSC, Okla. City, OK 73190.

Alcohol typically inhibits neurons, which may be due to potentiation of inhibitory GABAergic systems. Alcohol also may excite neurons, which could be due to either direct facilitation or disinhibition of tonically-active inhibitory GABAergic interneurons. Potentiation of the effects of GABA by alcohol has been shown in neurochemical and behavioral but not in electrophysiological studies. This study examined the acute effects of various doses of i.v. alcohol on GABAergic inhibitory mechanisms in hippocampal CA1 neurons.

The paired pulse paradigm was used to test the effects of alcohol on the activity of local inhibitory circuits in the CA1 region. Local groups of neurons (recording population spikes) were activated by paired pulses from the same source (orthodromic-orthodromic) or from two different sources (antidromic-orthodromic). Local inhibitory circuits were characterized by manipulating the period of inhibition with GABAergic agonists and antagonists released from a multibarrel pipette.

Recurrent inhibition was unaffected by alcohol, but alcohol prolonged the inhibition in response to feedforward and recurrent inhibition together. Consequently, alcohol probably potentiates feedforward inhibition, possibly by affecting post- or pre-synaptic $GABA_B$ receptors (Supported by the Department of Psychiatry and Sigma Xi).

515.2

GABAERGIC AND NONGABAERGIC SYNAPSES IN THE DENTATE MOLECULAR LAYER ARE DIFFERENTIALLY AFFECTED BY CHRONIC ETHANOL ADMINISTRATION. E. Fifková*, H. Eason, J. Lanman and K. Bueltmann. Dept. of Psychology, University of Colorado, Boulder, CO 80309.

The effect of chronic alcohol exposure and withdrawal on the GABAergic and nonGABAergic synapses of the dentate molecular layer (DML) has been studied in the alcohol-sensitive LS_{IBG} and alcohol-insensitive SS_{IBG} mice, respectively (28 animals total). Mice were fed for 4 mo either with a control isocaloric liquid diet or with an isocaloric liquid diet containing ethanol (23.5% ethanol derived calories). Half of the ethanol-treated mice were withdrawn from the diet for 1 mo. All animals were treated for GABA immunoelectron microscopy in the postembedding procedure.

Out of the total synaptic population in the middle and distal thirds of the DML, 91% and 87% of synapses are on dendritic spines and 9% and 13% on dendritic shafts, respectively. Within the population of axodendritic and axospinous synapses, the GABAergic contacts represent 60% and 3%, respectively. There are more synapses on dendrites in the distal than in the middle third, while on spines, the contacts are equally distributed across both thirds. During ethanol exposure, there was a significant loss (24%) of axospinous synapses in the distal third of the DML. This change was transient and returned to control values during withdrawal. However, in the GABAergic synapses there was a progressive loss of axodendritic contacts which peaked during the withdrawal period (41% and 37% in the middle and distal thirds of the DML, respectively). Given that in the DML the major synaptic input is on dendritic spines, the ethanol-induced loss of the excitatory axospinous synapses could affect the activity of dentate granule cells. The loss of the GABAergic inhibition could be secondary to the reduced excitation. The sequence in which these changes occur supports such a conclusion. Functional validation of these synaptic changes remains to be established in the dentate fascia. However, in chronic ethanol preparations, the CA1 pyramids display a reduced capacity to induce long-term potentiation which could result from a decreased excitatory synaptic input (Tremwel and Hunter, Alcoholism, 15:336A, 1991). Supported by #AA06196.

515.3

ETHANOL DIFFERENTIALLY MODULATES SYNAPTICALLY EVOKED $GABA_A$ RECEPTOR-MEDIATED RESPONSES FROM HIPPOCAMPAL, CORTICAL AND SEPTAL NEURONS IN RAT BRAIN SLICES. B.L. Soldo*, W.R. Proctor and T.V. Dunwiddie, and University of Colorado Health Sciences Center, and Veterans Admin. Medical Research Services, Denver, CO.

Previous electrophysiological studies have reported variable results concerning the effects of ethanol on $GABA_A$ receptor-mediated responses in the CNS. The present study was designed to determine whether ethanol modulation of GABA responses is brain region dependent, and to identify factors that might regulate ethanol sensitivity. We examined the effects of ethanol on synaptically evoked $GABA_A$ IPSCs, which were studied with whole-cell voltage clamp recordings from neurons in three brain regions (hippocampus, cerebral cortex, and septum) that have been reported to differ in their degree of ethanol modulation. Bicuculline-sensitive IPSCs elicited by local stimulation were isolated by pretreatment with the glutamate specific antagonists, DNQX and APV. Ethanol (20-160 mM), did not significantly modulate evoked $GABA_A$ IPSCs in CA1 pyramidal neurons. In contrast, ethanol potentiated these responses in cortical neurons (layer V), and in medial and lateral septal neurons. However, even in these areas, there were neurons whose IPSCs were unaffected by ethanol. These results suggest that ethanol modulates responses to endogenous GABA released during synaptic activation, that there are overall differences in $GABA_A$ receptors from various brain regions, and that there may be additional factors (such as heterogeneous subpopulations and local endogenous neuromodulators) that affect ethanol sensitivity within certain brain regions.

Supported by grant AA03527 and the V.A. Medical Research Services.

515.4

A SLICE PATCH ANALYSIS OF ETHANOL EFFECTS ON AMINO ACID-INDUCED SYNAPTIC CURRENTS. R. A. Morrisett,* Dept. Pharmacol., Univ. Nebraska Med. Ctr., Omaha, NE 68198-6260.

It is generally agreed that the physiologic expression of NMDA-receptor mediated synaptic currents is dependent upon a decrease in GABAergic inhibition (disinhibition). Priming stimulation paradigms (5 Hz) produce this loss of inhibition presumably through the activation of presynaptic $GABA_b$ receptors which subsequently inhibit the release of GABA. We have previously demonstrated that such NMDA receptor-mediated synaptic responses and the resultant synaptic plasticity (LTP) is blocked by ethanol in a dose-dependent manner. This inhibitory effect of ethanol could be due to a direct action of ethanol upon the NMDA receptor channel complex and/or ethanol could directly potentiate $GABA_a$ mediated synaptic currents. The present work utilized patch clamp recordings to determine the site of action of ethanol at both perforant path-dentate granule and Schaffer collateral-CA1 pyramidal synapses.

Synaptic currents were pharmacologically isolated to verify the identity of the glutaminergic and GABAergic components. Pairing of stimuli over the range of intervals of 150-1000 msec resulted in a profound depression of the outward (GABAergic) currents. The decrease in GABAergic currents was completely reversed by $GABA_b$ antagonists. Ethanol (25-100 mM) had no effect on the outward GABAergic currents or upon the fading the these currents when stimuli were paired at 200 msec. Conversely, in the presence of DNQX, pairing of stimuli revealed a slow inward current that was highly D-APV or ethanol sensitive. These data strongly suggest that the contribution of $GABA_a$ conductances to the acute effects of ethanol in the hippocampal formation is limited, and that the NMDA channel is the major ligand-gated ion channel affected by ethanol. (Supported by the State of Nebraska and the Alcoholic Beverage Medical Research Foundation)

515.5

ETHANOL ENHANCEMENT OF GABA-MEDIATED CHLORIDE CURRENTS IN ISOLATED CEREBELLAR PURKINJE CELLS IS EXERTED BY AN INCREASE IN BURST DURATION AND STEPS TO MULTIPLE LEVELS OF THE PREDOMINANT SINGLE CHANNEL CONDUCTANCE. M.F. Pacheco* and D.J. Woodward. CUIB-Univ. de Colima, COL., 28045 Mexico and Dept.of Physiol. and Pharmacol., Bowman Gray School of Medicine, Wake Forest Univ., Winston-Salem, NC 27106.

This work was undertaken in order to characterize, under patch clamp conditions, the actions of ethanol on GABA-mediated chloride currents (I_{GABA}) in dissociated Purkinje cells from 1-8 days old rats. In the whole-cell configuration, short pulses (1 psi, 17 sec) of ethanol (10 to 50 mM) enhanced up to 127 % the currents elicited by GABA (10 μM, 2 psi, 100 msec). Repetitive (every 30 sec) ethanol pulses or continuous bath perfusion induced a gradual potentiation (dose-dependent) of I_{GABA}, up to 300 % above control after 20 min of alcohol (10-50 mM) application. In cell-attached (V_H=E_m) and inside-out (V_H=-40 mV) configurations, ethanol (10-50 mM) induced initially an increase in the burst duration of GABA-gated channels (GABA concentration in pipette: 0.5 μM), followed by steps to multiple levels of the predominant single channel conductance. We conclude that ethanol enhancement of I_{GABA} takes place by direct interaction of ethanol with the GABA receptor, which increases the frequency of occurrence of channel opening and/or a change in the gating properties, as well as by inducing recruitment of additional channels. Partially supported by DGICSA-SEP, CONACYT (MFP); NIAAA 03901-11 (DJW).

515.6

ETHANOL POTENTIATION OF GABA RESPONSES IN CEREBELLUM: INVOLVEMENT OF cAMP. **Ronald K. Freund*, Anya M. Y. Lin, Michael R. Palmer, Dept. of Pharmacology, Univ. of Colorado Health Sciences Center, Denver, CO 80262 and Institute of Biomedical Sciences Center Academia Sinica, Taiwan**

A number of studies have demonstrated the involvement of the $GABA_A$ receptor complex in the actions of ethanol (EtOH). While we have been able to antagonize EtOH-induced inhibition of Purkinje neuron firing in vivo with the $GABA_A$ receptor antagonist bicuculline, it has been difficult to consistently demonstrate an EtOH-induced potentiation of GABA inhibitions, unless those inhibitions are modulated by a β-adrenergic agonist. In the present study we investigated the potentiative effect of EtOH on modulated GABA responses to determine whether cyclic adenosine monophosphate (cAMP) might be involved. GABA, 8-bromo-cAMP (8-BrcAMP) and EtOH were applied locally from multibarrelled pipettes by iontophoresis or electroosmosis. Similar to the effects of norepinephrine or isoproterenol, continuous application of the membrane-permeable cAMP analog, 8-BrcAMP was able to sensitize the GABA mechanism of cerebellar Purkinje neurons to the potentiating effects of EtOH. Preliminary data indicated that 8-BrcAMP is not acting through an adenosine mechanism, since the modulation by 8-BrcAMP, as well as the EtOH-induced potentiation, were still observed after systemic injection of the adenosine antagonist theophylline. The present data suggest that the catecholaminergic sensitization of GABA receptors to EtOH involves a second messenger action of cAMP. (Supported by USPHS grants AA05915 and AA00102.)

515.7

PRE- AND POSTSYNAPTIC EFFECTS OF ETHANOL ON THE INTERACTION OF NOREPINEPHRINE WITH GABA RESPONSES IN THE CEREBELLUM OF YOUNG AND OLD F344 RATS: ELECTROPHYSIOLOGICAL AND IN VIVO ELECTROCHEMICAL STUDIES. **A.M.Y. Lin*, M.N. Friedemann, P.C. Bickford, G.A. Gerhardt, R.K. Freund and M.R. Palmer. Veterans Admin. Med. Ctr., and Depts. of Pharmacology and Psychiatry, Univ. of Colorado Health Sci. Ctr., Denver, CO 80262, and Institute of Biomedical Sciences Center, Academia Sinica, Taiwan.**

In previous electrophysiological studies, we have shown that ethanol potentiates GABA-induced depressions of cerebellar Purkinje neurons when these responses were simultaneously augmented (modulated) by a β-adrenergic agonist, such as norepinephrine (NE) or isoproterenol (ISO). Aged F344 rats have been shown to have deficits in the postsynaptic function of β-adrenergic mechanisms in the cerebellum and, thus, might be expected to be less sensitive to the ethanol-induced potentiation of GABA effects in this brain area. In the present study, we found electrophysiological evidence which suggests that not only does the efficacy of locally-applied ISO for potentiating cerebellar GABA responses decrease with aging in F344 rats, but also that ISO frequently causes antagonism of GABA effects in the aged animals. In contrast, ethanol enhanced both the potentiating and inhibitory interactions of ISO with GABA responses in the young (6 months) and old (24 months) F344 rats, respectively. Similarly, our in vivo electrochemical data indicate that the clearance of locally applied NE was inhibited by ethanol applications in both young and aged F344 rats. These data suggest that ethanol inhibits NE uptake in the cerebellum, since the time-course of NE clearance was significantly lengthened by ethanol. Furthermore, this effect of ethanol appears to be preserved in the aged animals. (Supported by the VAMRS and by USPHS grants AG04418, AG06434, AG00441, AA05915 and AA00102)

515.8

EFFECTS OF MICROINJECTION OF EXCITANT AMINO ACID ANTAGONISTS OR GABA AGONISTS INTO THE BRAINSTEM RETICULAR FORMATION ON AUDIOGENIC SEIZURES DURING ETHANOL WITHDRAWAL A. Riaz*, D. N. Chakravarty and C.L. Faingold. Dept. Pharmacol. Southern Illinois Univ. Sch. Med. Springfield, IL 62794.

Ethanol is reported to alter the action of excitant amino acids (EAAs) and GABA in several brain sites. The brainstem reticular formation (RF) plays an important role in propagation of generalized seizures, including audiogenic seizures (AGS). Previous studies indicate that microinjection of an EAA antagonist, AP7, into the pontine RF blocks AGS in the GEPR. Effects of microinjection of GABA agonists into the RF of the GEPR have not been reported. In the present study, Sprague Dawley rats were implanted with guide cannulae bilaterally over the pontine RF (nucleus gigantocellularis) stereotaxically. Ethanol was administered intragastrically (9-15 g/kg/day). At the end of day 4, ethanol was withdrawn. Animals exhibiting AGS at 10h after ethanol withdrawal (ETX) received infusions of vehicle (phosphate buffer or dimethylsulfoxide) into the RF without effect. Vehicle infusion was followed by infusion of an NMDA antagonist (CPP), a non-NMDA antagonist (CNQX) or a GABA-A agonist (THIP). Both CPP (10nmol/side) and CNQX (30nmol/side) suppressed AGS during ETX. THIP (10nmol/side) also blocked AGS during ETX. These results support a vital role of enhanced EAA and reduced GABA neurotransmission in the RF in AGS susceptibility during ETX. These findings further support the importance of the RF in several forms of AGS and audiogenic-like seizures. (Support NIAAA AA08591).

515.9

EFFECT OF ALCOHOL DEPENDENCE AND WITHDRAWAL ON mRNA FOR $GABA_A$ RECEPTOR SUBUNITS AND CCK IN MICE.
H. Day, E. Pettersson, M. Field, J.A. Poat and J. Hughes*. Parke-Davis Neuroscience Research Centre, Addenbrookes Hospital Site, Hills Rd., Cambridge CB2 2QB.

A period of chronic ethanol treatment followed by withdrawal from the drug, results in profound behavioural symptoms and changes in $GABA_A$ receptor activity. Recently we have shown that the novel anxiolytic, CI-988, a selective CCK_B receptor antagonist, inhibits withdrawal induced behaviours. The present study extends these observations to examine the effect of ethanol at various time intervals, on levels of mRNA for CCK and $GABA_A$ receptor $\alpha 1$, $\alpha 6$ and $\gamma 2$ subunits in the brain. Mice were exposed to 8% ethanol, given as a liquid diet, for 1-7 days. After 7 days, animals were withdrawn for 16 hours and then slowly infused i.v. with N-methyl-DL-aspartate (NMDLA) until a clonic seizure was induced. CI-988 (0.1-1 mg/kg s.c.) was given at the start of the withdrawal period and 40 minutes prior to testing for spontaneous and NMDLA-induced seizure activity. mRNA for $GABA_A$ receptor subunits and CCK was measured by Northern blot analysis and *in situ* hybridisation using oligonucleotide probes labelled at the 3' end with [^{32}P] or [^{35}S]dATP respectively. Ethanol treatment and withdrawal produced a number of changes in the mRNA for $GABA_A$ receptor subunits although mRNA for CCK was unaffected at any phase of the experiment. During withdrawal, the mean convulsive dose (MCD) of NMDLA decreased from a control value of 143 ± 8.9 to 72 ± 8 mg/kg. CI-988 dose-dependently antagonised the proconvulsant actions of NMDLA, with a minimum effective dose of 0.3mg/kg s.c. (MCD of NMDLA = 112 ± 6.4 mg/kg), although the drug is without anticonvulsant activity *per se*. The results suggest that CCK_B receptor activation may play a role in alcohol withdrawal, although this does not appear to be via an increase in CCK mRNA. It is possible that CCK_B receptor activation is the more important function.

515.10

EFFECT OF ACUTE ETHANOL EXPOSURE ON GABA-ACTIVATED CHANNEL ACTIVITY IN GANGLION CELLS OF RAT RETINA.
H. H. Yeh*, Dept. Neurobiology & Anatomy, University of Rochester Medical Center, Rochester, NY 14642.

This laboratory has previously demonstrated that acute exposure to ethanol potentiates GABA-activated whole-cell current responses in ganglion cells isolated from the rat retina. Despite this and similar reports by others on select central nervous system neurons, issues related to the cellular and subcellular mechanisms underlying this effect of ethanol are outstanding. Here, results are presented on ongoing work aimed at testing the effect of ethanol on GABA-activated channel currents in membrane patches isolated from retinal ganglion cells.

Ganglion cells were isolated by treating postnatal or adult rat retina with either papain or pronase E and thermolysin. GABA-activated channel activity, elicited in cell-attached and inside-out patches by including 2-5 uM GABA in the recording pipet, was monitored continuously before, during and after bath application of 50 mM ethanol.

A reversible effect of ethanol could be detected in approximately 60% of the patches examined (14 of 24). Cell-attached and inside-out patches gave the same results. Generally, ethanol increased to varying degrees the probability of channel opening or burst duration. This occurred without alterations in the mean amplitude of GABA-activated channel current. The degree of change during exposure to ethanol was subtle except in two cases, where dramatic increases in channel activity were observed in the inside-out patch configuration. It is proposed that these changes may account for the modulation of GABA currents observed under whole-cell voltage clamp. Importantly, the finding that ethanol can exert effects on GABA-activated channel activity in inside-out patches leads to the postulate that ethanol could interact directly with the $GABA_A$ receptor complex independent of intracellular intermediaries which may contribute indirectly by priming the state of neuronal receptivity to GABA.

515.11

INTERACTION OF TEMPERATURE AND ETHANOL ON 5α-PREGNAN-3α-OL-20-ONE (3α,5α-P)-INDUCED ALTERATIONS OF GABA-STIMULATED CHLORIDE UPTAKE IN LS AND SS MICE: PRELIMINARY STUDIES.
M. Bejanian*, D.A. Finn, K.W. Gee and R.L. Alkana. Dept. Molecular Pharmacology and Toxicology, Univ. So. California, Los Angeles, CA 90033.

Offsetting hypothermia during intoxication increases sensitivity to ethanol-induced loss of righting reflex in SS mice and decreases sensitivity in LS mice. Recent studies suggest genotypic differences in the interaction of temperature and ethanol on the $GABA_A$ receptor complex (GBRC). The present study investigated the interactive effects of temperature and ethanol on 3α,5α-P-induced alterations of GABA-stimulated chloride uptake in LS and SS mice synaptoneurosomes. 3α,5α-P (10 μM) produced a significant increase in GABA-stimulated chloride uptake in LS mice and a non-significant increase in SS mice at 30, 34 and 38°C. Ethanol (100 mM) did not significantly alter the effects of 3α,5α-P on the GBRC at 30 and 34°C. At 38°C, ethanol significantly decreased the effects of 3α,5α-P on GABA-stimulated chloride uptake in LS mice and produced a non-significant increase in SS mice. In the absence of 3α,5α-P, ethanol (25-100 mM) produced a significant increase in GABA-stimulated chloride uptake at 30°C, but not 34 and 38°C in LS mice, and had no effect at any of the tested temperatures in SS mice. Although speculative, these preliminary results suggest that the effects of temperature on behavioral sensitivity to ethanol may involve temperature-induced alterations in the 3α,5α-P and GABA site in LS mice, and the 3α,5α-P site in SS mice (Supported by USPHS grants AA03972 AA05234, NS25986 and NS24645).

515.12

FAILURE TO "PRECIPITATE" SIGNS OF ETHANOL WITHDRAWAL WITH A GABA ANTAGONIST AND A BENZODIAZEPINE INVERSE AGONIST.
S. Rassnick*, J. Krechman and G. F. Koob. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Previous work has shown that spontaneous withdrawal from ethanol (E) and precipitated opiate withdrawal produce a decrease in responding for food reinforcement, suggesting that this effect reflects a disrupted motivational state during withdrawal from chronic drug administration. The present study was designed to test whether administration of bicuculline methiodide, a competitive GABA receptor antagonist, or RO 15-4513, a benzodiazepine inverse agonist, would "precipitate" signs of withdrawal during chronic E intoxication. It was hypothesized that an increased sensitivity to the response-disruptive effects of these drugs during E intoxication would provide evidence for a "precipitated" E withdrawal syndrome. Rats were trained to lever-press for food on a fixed ratio-15 schedule of reinforcement, then maintained E dependent for 2 weeks on a liquid diet containing 35% E-derived calories, or maintained on a control liquid diet. E-dependent rats displayed a decreased sensitivity, rather than an increased sensitivity to the response-disruptive effects of bicuculline methiodide (100 ng ICV) and RO 15-4513 (3 and 6 mg/kg). The inability of these drugs to "precipitate" E withdrawal is consistent with recent biochemical studies which show that chronic E intoxication produces a down-regulation or uncoupling of activity at the GABA/benzodiazepine receptor complex (Supported in part by NIAAA grants: AA 05297, AA 06420, and The Alcoholic Beverage Medical Research Foundation).

DRUGS OF ABUSE: COCAINE'S INTERACTION WITH NON-DOPAMINE SYSTEMS

516.1

BLOCKADE OF SENSITIZING EFFECTS OF AMPHETAMINE PREEXPOSURE ON COCAINE SELF-ADMINISTRATION BY THE NMDA ANTAGONIST MK-801.
S. Schenk*, A. Valadez, C. McNamara and B.A. Horger. Dept. Psychology, Texas A&M Univ., College Station, TX 77843.

Rats preexposed with amphetamine acquired cocaine self-administration (0.125, 0.25 or 0.5 mg/kg/infusion) at a faster rate than saline preexposed rats. This suggests that repeated exposure to this drug prior to self-administration testing sensitized the rats to the reinforcing effects of cocaine. Co-administration of MK-801 (0.25 mg/kg, IP), a non-competitive NMDA antagonist, blocked the ability for chronic exposure to amphetamine to sensitize rats to cocaine; these rats' rate of acquisition of cocaine self-administration did not differ from saline preexposed controls. Thus glutamatergic systems and the NMDA receptor in particular may play a critical role in the sensitization produced by intermittent exposures to amphetamine. The NMDA antagonist also effected the motor activating effect of an acute injection of amphetamine. A suppression of the activating effects during the first 30 min post-amphetamine and an enhancement of the activating effects during the last 30 min post-amphetamine was produced by MK-801. These data suggest that the behavioral expression of an acute exposure as well as chronic administration of this drug (ie., sensitization) may rely on an intact NMDA receptor system. In experienced self-administering rats, MK-801 also shifted the dose response curve for cocaine self-administration to the right, suggesting an antagonism of the reinforcing effects of cocaine even in rats that had already undergone a form of sensitization. These data are discussed in terms of similarities between pharmacological sensitization and long-term potentiation resulting from electrical stimulation of limbic sites.

516.2

BUPRENORPHINE MIMICS MORPHINE ACTIVATION OF DOPAMINE NEURONS, IN VIVO.
S.J. Grant*, G. Sonti, D.A. Highfield Dept. Psychology and Prog. in Neuroscience, Univ. Delaware, Newark, DE. 19716.

Buprenorphine (BUP) is a synthetic opioid proposed as a potential treatment for cocaine craving. BUP is commonly described as a partial opioid agonist, but little is known of its electrophysiological effects. We previously reported that BUP, like morphine, completely suppresses the spontaneous activity of noradrenergic neurons of the locus coeruleus. We now report that BUP also mimics the morphine-induced activation of dopamine cells.

Extracellular single unitactivity was recorded from dopaminergic (DA) neurons in the substantia nigra (SN) and Ventral Tegmental Area (VTA) of chloral hydrate anesthetized rats. Standard physiological and anatomical criteria were used to identify DA neurons. Like morphine, BUP (10-400 μg/kg, i.v.) activated DA neurons in the VTA, but not the SN. As previously seen in the LC, BUP had a long duration of action and could not be reversed by the opioid antagonists naloxone or naltrexone (up to 10 mg/kg).

These studies demonstrate that buprenorphine has morphine like effects in both the VTA and the LC. Since BUP does not exhibit limited efficacy, partial agonist, or mixed agonist-antagonist properties on neural activity, these results are consistent with the hypothesis that *in vivo* BUP acts acutely as an agonist. The effects of acute and chronic BUP pre-treatment on morphine actions in the VTA and LC are in progress.

Supported by NIMH, the State of Delaware, and ICI Pharma.

516.3

SELECTIVE REGULATION OF MU AND KAPPA OPIOID RECEPTORS FOLLOWING REPEATED EXPOSURE OF GUINEA PIGS TO COCAINE. Y. Itzhak* and I. Stein, Dept. of Biochemistry & Molecular Biology, REPSCEND Labs, University of Miami School of Medicine, Miami, FL. 33101

The precise neurochemical mechanisms involving cocaine-induced psychological and neurophysiological addictions are not entirely clear. Since a few studies implied the involvement of the opioid system in the reinforcing effects of cocaine, we sought to investigate the regulation of opioid receptors following repeated exposure to the drug. Guinea pigs were treated with either saline or cocaine (40 mg/kg/day; i.p.) for 7 days, sacrificed 24 h after the treatment, and various brain regions were dissected and prepared for opioid receptor binding. The three subtypes of opioid receptors, mu, delta and kappa, were labeled with [^{3}H]DAGO, [^{3}H]DPDPE and [^{3}H]U69 593, respectively. A significant down-regulation of mu-opioid receptors (40-60% of control Bmax) was observed in the frontal cortex, amygdala, hippocampus and thalamus. No change in mu-opioid receptor binding was detected in caudate putamen, substantia nigra, neocortex and hypothalamus. Delta-opioid receptor binding in the various brain regions of cocaine-treated animals did not differ from control. Kappa-opioid receptor binding was significantly increased (135±5% of control) only in the cerebellum, a region that contains primarily kappa-opioid receptors in guinea pig brain. Taken together, these findings indicate that repeated cocaine administration induces distinct alterations in opioid receptors which may be associated with the neurochemistry of cocaine-addiction. Supported by NIDA DA07589.

516.4

SYSTEMIC ADMINISTRATION OF DYNORPHIN A(1-13) MARKEDLY INHIBITS DIFFERENT BEHAVIORS INDUCED BY COCAINE IN THE MOUSE. M. Ukai *, T. Kamiya, T. Toyoshi, M. Mizutani and T. Kameyama. Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Meijo University, Nagoya 468, Japan.

The effects of systemic administration (i.p.) of dynorphin A(1-13) on the cocaine-induced behavioral alterations in the mouse were determined by using multi-dimensional behavioral analyses based upon a capacitance system. A 1.0 mg/kg dose of cocaine did not influence various behaviors, while increasing doses to 3-30 mg/kg produced a significant increment in the frequency of behaviors such as linear locomotion, circling, rearing and grooming. Although a 1.0 mg/kg dose of dynorphin A(1-13) alone produced a significant decrease in grooming behaviors, higher doses (3.0 and 10.0 mg/kg) of the peptide failed to affect different behaviors. The cocaine (3.0 mg/kg)-induced increases in linear locomotion, circling and rearing behaviors were significantly inhibited by dynorphin A(1-13) (10.0 mg/kg). The inhibitory effects of dynorphin A(1-13)(10.0 mg/kg) were antagonized by the opioid antagonist Mr2266 (5.6 mg/kg). It is thus possible that the systemic administration of dynorphin A(1-13) inhibits different behaviors induced by cocaine through the blood-brain barrier, although the instability of amino acids bonds or the relatively large molecular weight of dynorphin A(1-13) may result in the failure to demonstrate opioid activity by the peptide after systemic administration.

516.5

Behavioral Evidence for Changes in 5-HT$_{1A}$ Receptor Sensitivity During Withdrawal From Continous or Intermittent Cocaine. JOYNER, C. M., KING, G. R., SUNDAR, K. S.*, ELLINWOOD, E.H., JR. Changes in 5-HT neurotransmission may partially mediate the development of sensitization and tolerance to chronic cocaine administration. Rats were pre-treated with 40 mg/kg/day of cocaine for 14 days by either subcutaneous injections or continous infusion by osmotic minipumps. The rats were then withdrawn from the pretreatment regime for 7 days and behaviorally assessed. In Experiment 1, rats received 0, 0.5, 1.0, or 2.0 mg/kg i.p. injections of NAN-190, 1-(2-Methoxyphenyl)-4-[4-(2-phthalimido) butyl]piperazine, a putative 5-HT$_{1A}$ receptor antagonist. In Experiment 2, the rats received the same doses of NAN-190 in combination with a 15 mg/kg i.p. injection of cocaine. The results of Experiment 1 indicate that the continuous infusion group demonstrated a dose dependent supression of locomotor behavior by single doses of NAN-190. NAN-190 had no consistent dose dependent effect on the locomotor behavior of the subjects in the other pretreatment groups. The results of Experiment 2 indicate that NAN-190 generally had a greater suppressive effect on cocaine induced locomotion in the daily injection group, than the saline control group, which is consistent with 5-HT$_{1A}$ receptor supersensitivity. In contrast, NAN-190 had no suppressive effect on cocaine induced locomotion in the continuous infusion group, which is consistent with 5-HT$_{1A}$ receptor subsensitivity. Changes in 5-HT$_{1A}$ receptor sensitivity may mediate the withdrawal symptoms of anxiety and depression exhibited by human cocaine abusers thus providing a potential basis for treatment .This research was supported by NIDA grant SRCD-5P5D-DA05303-02.

516.6

CHRONIC COCAINE TREATMENT ENHANCES SEROTONIN-MEDIATED BEHAVIORS, BUT NOT NEUROENDOCRINE RESPONSES, IN THE RAT. M.H. Baumann* and R.B. Rothman, Lab. Clin. Psychopharmacol., NIDA Addiction Research Center, Baltimore, MD 21224.

There is compelling evidence that acute cocaine alters serotonin (5HT) neurotransmission, but the effects of chronic cocaine on 5HT neurons are not well characterized. In an attempt to assess 5HT function after repeated cocaine injections, we have employed the 5HT-releasing drug *d,l*-fenfluramine as a pharmacological probe *in vivo*. Male rats were fitted with indwelling jugular catheters under metofane anesthesia. Beginning 2 days after surgery, rats were treated twice daily with cocaine (15 mg/kg,ip) or saline for 7 consecutive days. At 42 hr and 8 days after the final chronic injection, rats were challenged with low dose (1.25 mg/kg, iv) or high dose (5.0 mg/kg, iv) fenfluramine. Specific aspects of the 5HT behavioral syndrome, including flat body posture and forepaw treading, were scored on a graded scale at 2, 10, 20 and 30 min post-fenfluramine; serial blood samples (0.5 ml) were removed immediately before and at 15, 30 and 60 min after drug. Plasma was assayed for prolactin (PRL) and corticosterone (CORT) by RIA. Cocaine-treated rats exhibited enhanced behavioral sensitivity to high ($P<0.01$) and low ($P<0.001$) doses of fenfluramine given 42 hr after cessation of chronic treatment. The potentiation of fenfluramine-induced behaviors was still observed at 8 days post-cocaine. PRL responses to fenfluramine were virtually identical in cocaine- and vehicle-treated groups at all times and doses tested. Interestingly, the plasma CORT response to low dose fenfluramine was significantly blunted ($P<0.01$) in cocaine-treated rats at the 42 hr time point, but this effect was normalized by 8 days of cocaine abstinence. These data suggest that chronic cocaine differentially affects 5HT function: 1) while 5HT-mediated behaviors are persistently enhanced, 2) 5HT activation of the HPA axis is transiently impaired. The relevance of these findings to clinical medicine is unclear at present, but the possibility of serotonergic dysfunction in human cocaine addicts should not be discounted.

516.7

5-HT$_3$ ANTAGONIST INHIBITION OF COCAINE-INDUCED BEHAVIOR AND SEROTONERGIC INNERVATION OF DISTINCT ANATOMICAL SITES. A. Svingos* and R. Hitzemann, Departments of Psychiatry and Psychology, SUNY at Stony Brook, NY 11794-8101 and VAMC, Northport, NY 11768.

We have previously reported that endogenous 5-HT is required for 5-HT$_3$ antagonists to attenuate cocaine-induced hyperactivity (Soc. Neurosci. Abs. 348.16, 1991). It has been suggested that both the caudate-putamen (Cp) and nucleus accumbens (Na) are anatomical sites of action for 5-HT$_3$ antagonism of dopamine (DA) mediated behaviors (Blandina et al. 1988, Carboni et al. 1989, Chen et al. 1990). In order 1) to further investigate the requirement of endogenous 5-HT in the 5-HT$_3$ antagonist inhibition of cocaine-induced behavior and 2) to localize possible anatomical sights of action, *p*-chlorophenylalanine (PCPA) treated animals were observed in a longitudinal-recovery study. Animals were pretreated with PCPA (100 mg x 3 days) and then either sacrificed on days 0, 7, 14, 28, and 56 or tested behaviorally for the 5-HT$_3$ antagonist-cocaine interaction. Recovery of 5-HT levels in the Na and Cp and the raphe nuclei was followed by immunohistochemical techniques. There was no apparent differential recovery of 5-HT in the Cp and Na; the rate of recovery was slow with 5-HT levels being only 30 to 50% of normal by day 14. As expected, recovery of 5-HT in the raphe nuclei occurred more rapidly. Data will be presented paralleling 5-HT recovery with 5-HT$_3$ antagonist behavioral efficacy.

516.8

COCAINE SENSITIZATION: RESPONSIVITY OF SEROTONIN (5-HT) NEURONS TO IONTOPHORETIC 5-HT. *K.A. Cunningham* and S.K. Bryan.* **Dept. Pharmacol. Toxicol., Univ Texas Med Branch, Galveston, TX 77550.**

With repeated exposure, rodents become sensitized to the motor-activating properties of cocaine. Cocaine is known to interact with the 5-HT systems in the brain, and cocaine sensitization appears to be associated with an enhanced sensitivity of 5-HT dorsal raphe (DR) neurons to systemic cocaine, fluoxetine and 8-OHDPAT. The present study is designed to compare the sensitivity of somato-dendritic autoreceptors to local application of 5-HT in cocaine-sensitized vs. control rats. Male rats were treated with IP saline (n=6) or cocaine (15 mg/kg; n=5) BID for 7 days. Comparisons of behavioral ratings (Kilbey-Ellinwood) between Day 1 and Day 7 indicated that sensitization developed with this cocaine regimen. Following a 24-hr withdrawal, rats were anesthetized with urethane (1.5 g/kg) and single-unit extracellular recording and iontophoretic studies of DR 5-HT neurons were conducted. Both *'fast'* (>1.2 Hz) and *'slow'* cells (<=1.2 Hz) in controls exhibited similar current-response curves to 5-HT (2.5-10 nA; 0.04 M, pH 4.0); an average inhibition of 52±8% was observed at 7.5 nA. In contrast, the responsiveness of 5-HT neurons from cocaine-sensitized rats appeared to be dependent upon basal firing rates. *Slow* cells in cocaine-sensitized rats were more responsive to 5-HT than *fast* cells from cocaine-sensitized rats as well as both *fast* and *slow* cells from saline-treated rats. For example, the mean suppression observed at 7.5 nA of 5-HT was 79.9 ± 5.7% in *slow* cells and 35.0 ± 6.0% in *fast* cells sampled from cocaine-treated rats. These preliminary studies suggest that at least a subpopulation of 5-HT DR neurons becomes supersensitive to local application of 5-HT after repeated cocaine exposure. Thus, the enhanced sensitivity observed in response to systemic cocaine, fluoxetine and 8-OHDPAT in cocaine-sensitized rats appears to occur in part at the level of the cell body. ***Supported by DA 05708, DA 06511 and NARSAD.***

516.9

SEROTONIN LESIONS AFFECT RESPONDING ON A PROGRESSIVE RATIO SCHEDULE REINFORCED BY EITHER INTRAVENOUS COCAINE OR FOOD. E.A. Loh[1*], G. Baker[2], G. Vickers[1] & D.C.S. Roberts[1]. [1]Dept. of Psychology, Carleton University, Ottawa, [2]Dept. of Psychiatry, University of Alberta, Edmonton, Canada.

We have shown that injections of the serotonergic neurotoxin 5,7-DHT into the amygdala or the medial forebrain bundle of rats will result in an apparent increase in motivation to self-administer cocaine (Psychopharm. 101 (1990) 262). In the present study we examined the effect of intraventricular (icv) injections of 5,7-DHT on cocaine self-administration reinforced under a progressive ratio (PR) schedule of reinforcement. On this schedule, the response ratios escalate following each reinforcement. Rats were implanted with chronically indwelling iv cannulas and trained to self-administer cocaine on a PR schedule. Rats then received icv infusions of either 5,7-DHT (10 μg; n=10) or ascorbic/saline vehicle (n=5) and were tested for an additional 7 days. Lesioned rats demonstrated substantial increases in final ratio attained and decreases in forebrain 5HT levels. In a second study, similar increases in final ratio were found in animals responding for food reward following identical 5,7-DHT lesions. The data indicate that 5,7-DHT lesions may produce a global influence on reinforcement rather than a specific effect on cocaine reinforcement. (Supported by the MRC)

516.10

NORADRENERGIC RESPONSIVITY DURING COCAINE ABSTINENCE. C. J. McDougle*, L. H. Price, T. R. Kosten, J. Black, R. T. Malison, R. C. Zimmermann, H. D. Kleber. Yale Univ. Dept. of Psychiatry, New Haven, CT 06519.

Preclinical and clinical evidence suggests involvement of noradrenergic systems in the neurobiology of cocaine abstinence. Pharmacological challenge with yohimbine hydrochloride, an alpha 2-adrenergic receptor antagonist, was used to investigate noradrenergic responsivity during early and late abstinence from cocaine in human cocaine addicts. METHOD: Ten inpatients (9 men, 1 woman) with DSM-III-R cocaine dependence received 2 mg/kg of intranasal cocaine 3X/day for three consecutive days. Each subject then received randomized, double-blind challenge tests of active or placebo yohimbine (0.4 mg/kg, I.V.) on the two days following cocaine administration (early abstinence) and again two weeks later (late abstinence). Plasma 3-methoxy-4-hydroxyphenethyleneglycol (MHPG) (ng/ml) and cortisol (ng/ml) responses, as well as behavioral and physiological (sitting and standing blood pressure and pulse) measurements were obtained following each challenge. RESULTS: 3-way ANOVA (drug X time X phase of abstinence) with Huynh-Feldt correction revealed a significant interaction for plasma MHPG ($F=2.7$, $df=5,45$, $p<0.05$) (greater during early abstinence), but not for plasma cortisol, behavioral, or physiological variables. Placebo-corrected peak change between abstinence phases showed a significant difference for a measure of "NERVOUS" (30.2±31.9 (early) vs. 5.2±19.9 (late), $df=9$, $t=-2.7$, $p<0.02$), and a trend toward significance for sitting systolic blood pressure. CONCLUSION: These data suggest that early abstinence from cocaine in humans may be associated with increased noradrenergic responsivity which normalizes over time.

516.11

UP-REGULATION OF SIGMA BINDING SITES FOLLOWING REPEATED EXPOSURE OF GUINEA PIGS TO COCAINE. I. Stein* and Y. Itzhak, Dept. of Biochemistry & Molecular Biology, REPSCEND Labs, University of Miami School of Medicine, Miami, FL. 33101

Although it is believed that blockade of the dopamine (DA) transporter by cocaine is primary for its psychotropic effects, the drug interacts with several transporters and CNS receptors. Cocaine, for instance, has similar affinities for the DA transporter and sigma binding sites (ca. 1 uM). The latter are postulated to be involved in the effects of various psychotropic agents, and are down-regulated following exposure to the antipsychotic agent, haloperidol (Itzhak & Stein, Brain Res. 566: 166, 1991), that has similar affinities for both D2 and sigma receptors (ca. 2 nM). Inasmuch as both cocaine and haloperidol interact with sigma binding sites, but affect DA neurotransmission in "opposite directions", we sought to investigate the effects of cocaine-exposure on sigma binding sites. Guinea pigs were treated with either saline or cocaine (40 mg/kg/day; i.p.) for 7 days, sacrificed 24 h following the treatment, and the brain was dissected and prepared for sigma-ligand-receptor binding assays. Binding of (+)[^{3}H]pentazocine and (+)[^{3}H]-3-PPP, two selective sigma ligands, in various brain regions indicated a significant up-regulation (142% of control) of sigma binding sites in the substantia nigra. In other brain regions no significant change in the binding parameters of the sigma-ligands was detected. The specific alteration in sigma binding sites observed in substantia nigra may be related to the behavioral sensitization that usually follows repeated exposure to cocaine. Supported by NIDA DA07589.

516.12

CHRONIC CONTINUOUS VS. INTERMITTENT COCAINE ADMINISTRATION: DIFFERENTIAL ALTERATIONS IN NEUROTENSIN CONCENTRATIONS FOLLOWING A CHALLENGE STIMULATION. S.T.Cain*D.Griff, C. Joyner, and E.H. Ellinwood. Dept. Psychiatry, Duke Univ. Med. Ctr., Durham, NC 27710.

We have previously reported that 14 days of either continuous or intermittent exposure to cocaine yields regimen-specific neuroanatomically-selective changes in basal NT-like immunoreactivity [NT-LI] (Cain et al., Soc. Neurosci. 1991). We have now evaluated residual alterations in NT responsivity in response to a challenge stimulation. Rats were treated with 40 mg/kg cocaine/day for 14 days using continuous or intermittent administration paradigms. After 7 days of withdrawal, the animals were given a 20 mg/kg ip. challenge dose of cocaine and sacrificed 24 hrs. later. In rats treated continuously with cocaine, NT-LI was significantly increased in the nucleus accumbens 24 hrs. following the challenge dose relative to animals treated for 14 days with saline vehicle or intermittent cocaine injection. In contrast, in animals treated with chronic intermittent injections of cocaine, NT-LI in the substantia nigra was significantly increased relative to animals treated for 14 days with saline vehicle or continuous cocaine. These results indicate that differential cocaine dosing protocols induce neuroanatomically-selective changes in the responsivity of CNS NT systems and thus, support the possibility that NT is an important modulator of the behavioral consequences of chronic cocaine exposure. Supported by NIDA DA-05303.

516.13

INFLUENCE OF DIFFERENT TRAINING DOSES OF COCAINE ON GENERALIZATION TO NONCOMPETITIVE NMDA ANTAGONISTS. M.A. Napolitano, K.M. Kantak and R.D. Spealman*. Boston Univ. Boston, MA 02215 and New England Reg. Primate Res. Ctr., Southborough, MA 02215.

Generalization of the discriminative stimulus (DS) effects of cocaine to $MgCl_2$, dizocilpine, and PCP was studied in rats trained to discriminate cocaine (2, 5, or 10 mg/kg) from vehicle. In rats trained to discriminate 2 mg/kg cocaine, $MgCl_2$ (10-100 mg/kg) produced partial generalization (maximum 73%). With higher training doses of cocaine (5 and 10 mg/kg), there was no reliable generalization. There was also partial generalization of cocaine to dizocilpine (0.03-0.3 mg/kg; maximum 75%) and PCP (1.0-3.0 mg/kg; maximum 78%) in 2 mg/kg cocaine-trained rats. Additionally, at the 5 mg/kg training dose of cocaine, partial generalization of cocaine to dizocilpine (maximum 29%) and PCP (maximum 87%) was produced. After pretreatment with $MgCl_2$ (30 mg/kg) in rats trained to discriminate 5 mg/kg cocaine, the DS effects of a low dose of cocaine (1 mg/kg) were enhanced from 12% to 63% and the DS effects of higher doses of cocaine (5 and 10 mg/kg) were attenuated from 100% to 75% and 89%, respectively. Similarly, with pretreatment with dizocilpine (0.178 mg/kg) in rats trained to discriminate 5 mg/kg cocaine, the DS effects of a low dose of cocaine (1 mg/kg) were enhanced from 12% to 32% and the DS effects of higher doses of cocaine (5 and 10 mg/kg) were attenuated from 100% to 79% and 59%, respectively. Pretreatment with PCP (1.78 mg/kg) in rats trained to discriminate 5 mg/kg cocaine produced an enhancement of the DS effects at a low dose of cocaine (1 mg/kg) from 12% to 60%. Pretreatment with this dose of PCP did not alter the DS effects of higher doses of cocaine. These results suggest that noncompetitive NMDA antagonists have characteristics of partial cocaine mimetics, with the degree of generalization dependent upon the training dose of cocaine.

516.14

DISCRIMINATIVE STIMULUS EFFECTS OF MG^{2+} AND GENERALIZATION TO COCAINE AND NONCOMPETITIVE NMDA ANTAGONISTS. K.M. Kantak* and E.S. Kitchell. Lab. of Behav. Neurosci., Dept. Psychol., Boston Univ., Boston, MA 02215.

Six rats were trained to discriminate 100 or 135 mg/kg $MgCl_2$ from saline in a 2-lever FR 10 food-reinforced procedure. After 35 training sessions, rats made 99.9% of the total responses on the drug lever following injections of the training dose. There were dose-dependent decreases in % total drug lever responding following generalization testing with various doses of $MgCl_2$ (3.0-100 mg/kg). There was partial generalization of $MgCl_2$ to cocaine (1.0-10 mg/kg) with a maximum of 82% drug lever responding with 3.0 mg/kg. Partial generalization was also engendered to the noncompetitive NMDA antagonists dizocilpine (0.03-0.178 mg/kg) and PCP (1.0-3.0 mg/kg). There was a maximum of 75% drug lever responding with 0.1 mg/kg dizocilpine and a maximum of 82% drug lever responding with 3.0 mg/kg PCP. These data indicate that $MgCl_2$ can serve as a discriminative stimulus in rats which extends previous findings showing $MgCl_2$ to have reinforcing stimulus properties in a cocaine self-administration substitution procedure (Kantak et al., 1991). Furthermore, these discriminative stimulus effects of $MgCl_2$ partially generalize to cocaine, suggesting that these two compounds share some common effects. That these effects may be mediated through the NMDA receptor complex, which includes recognition sites for Mg^{2+}, PCP, and dizocilpine, is suggested by the findings showing partial generalization of $MgCl_2$ to other noncompetitive NMDA antagonists. Further characterization of the role of the NMDA receptor complex in cocaine pharmacology is warranted.

516.15

EFFECTS OF COCAINE, MORPHINE AND MK-801 ON STRIATAL ACETYLCHOLINE OVERFLOW. A. Zocchi* and A. Pert. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892

Cocaine, morphine, and MK-801 all produce increases in locomotor behavior in rats through different initial receptor interactions. One common link through which these drugs may exert at least part of their behavioral effects is the cholinergic interneuron in the striatum. The purpose of this study was to evaluate the effects of these three compounds on striatal acetylcholine (ACh) following systemic injections. The effects of cocaine on ACh were examined in both anesthetized and awake preparations. For the latter, rats were implanted with CMA guide cannulae aimed for the striatum. One week following surgery, microdialysis probes were introduced into the striatum and perfused with artificial CSF containing 10 μM neostigmine at 0.5 μl/min. The animals in the anesthetized study were injected with chloral hydrate, placed in a stereotaxic frame and then had dialysis probes introduced into the striatum. Dialysates were analyzed for ACh and choline by a reverse phase HPLC system with electrochemical detection.

Cocaine (20 mg/kg i.p.) increased striatal ACh levels approximately 50% during the first three 20-min sampling periods. The increase in ACh was even more pronounced in anesthetized rats (peak effect of approximately 160% increase during the second sampling period). Systemic administration of MK-801 (0.5 mg/kg) or morphine (10 mg/kg) had little if any effect on striatal ACh in anesthetized rats. The effects of cocaine on ACh are unexpected since indirect and direct dopamine agonists have been reported to inhibit ACh function in vitro. Our findings are, however, compatible with those from a recent microdialysis study in which amphetamine was found to increase ACh through a D_1 dopaminergic mechanism. The negative findings with MK-801 and morphine suggest that the ACh interneurons may not have important glutamatergic or opioid inputs.

EPILEPSY: BASIC MECHANISMS IV

517.1

EPILEPTIFORM ACTIVITY ELICITED IN THE RAT DENTATE GYRUS IN VITRO IN SOLUTIONS WITH NORMAL $[Ca^{2+}]_o$ CONTAINING AMINO-ACID RECEPTOR ANTAGONISTS. P.R. Patrylo[1,3]*, J. Schweitzer[2], and F.E. Dudek[1,3]. Mental Retardation Res. Ctr.[1] and Div. of Neurosurgery[2], UCLA Sch. of Med., Los Angeles, CA 90024, and Dept. of Anatomy and Neurobiology, Colorado State University, Fort Collins CO 80523[3]

We have shown that the dentate gyrus can undergo ictal-like seizure activity *in vitro* in response to concurrently lowering $[Ca^{2+}]_o$ from 1.3 mM to "0" or 0.5 mM and elevating $[K^+]_o$ from 3 mM to 7-11 mM (Schweitzer et al., submitted). Addition of DL-AP-5 and DNQX, antagonists to the NMDA and non-NMDA receptors, did not block this seizure-like activity. In these experiments we examined whether seizure-like activity could be induced in the dentate gyrus in solutions containing "normal" $[Ca^{2+}]_o$ (i.e., 1.3 mM) or slightly decreased $[Ca^{2+}]_o$ (i.e., 0.9 mM). Such reduced Ca^{2+} concentrations are observed *in vivo* during high-frequency stimulation (15 Hz) of afferent input. In the presence of "normal" $[Ca^{2+}]_o$ (1.3 mM), $[K^+]_o$ as high as 12 mM failed to produce these epileptiform events (n=6). Epileptiform activity could be elicited in 1.3 mM $[Ca^{2+}]_o$ solutions, however, if the $GABA_A$ receptor antagonist bicuculline (30 μM) and elevated $[K^+]_o$ were used concurrently (n=4). The addition of DL-AP-5 (10-50 μM) and DNQX (10-50 μM) either before (n=6) or after (n=4) raising $[K^+]_o$ did not block this epileptiform activity. Bursts were also elicited in solutions without bicuculline, if the $[Ca^{2+}]_o$ was 0.9 mM and $[K^+]_o$ was 9-11 mM (n=2). These results suggest that dentate granule cells can generate epileptiform activity in normal or slightly reduced $[Ca^{2+}]_o$ when $[K^+]_o$ is elevated, particularly when synaptic inhibition is depressed. Furthermore, this form of seizure activity persists in the presence of antagonists for excitatory amino acid receptors. Supported by NIH grant NS16683 and NRSA fellowship NS08993.

517.2

SIMULATION AND EXPERIMENTAL ANALYSIS OF AN EPILEPTIC OSCILLATION IN THE *IN VITRO* HIPPOCAMPAL SLICE. R.D. Traub*, R. Miles and J.G.R. Jefferys. IBM Watson Res. Ctr., Yorktown Heights, NY 10598 USA, Institut Pasteur, Paris 15, France and St. Mary's Hospital Med. Sch., London W2 1PG, England.

The potent $GABA_A$ blocker picrotoxin, applied to guinea-pig hippocampal slices, induces in the CA3 region an epileptic event called an "afterdischarge" (AD). ADs consist of a long initial (1^e) burst, lasting up to several hundred ms, a number of briefer secondary (2^e) bursts at 50-65 ms intervals, and a long afterhyperpolarization (AHP). All AD components are synchronized between cell body, apical dendrites, different cells, and the local field potential.

We constructed a model of the disinhibited CA3 region with 100 19-compartment pyramidal cells, each with Na, K and Ca currents whose kinetics were determined (where possible) from whole cell patch records in isolated cells (Traub, Wong, Miles & Michelson, *J. Neurophysiol.* 1991, 66: 635-650). Cells were randomly connected by excitatory synapses with "AMPA" (fast, voltage-independent) and "NMDA" (slow, voltage-dependent) synapses. The model generates ADs as follows: AMPA synapses mediate the initial synchrony and depolarize the dendrites, unblocking NMDA receptors. The long 1^e burst results from the delayed onset of the AHP (Lancaster & Adams, *J. Neurophysiol.* 1986, 55: 1268-1282). A prolonged NMDA current generates repeating dendritic Ca spikes, producing the 2^e bursts. 2^e bursts are synchronized in turn by the AMPA synapses. Experimental results supporting this model include (1) effects of NMDA blockers (suppressing the 2^e bursts); (2) effects of dendritic current injection: a rhythmical series of brief bursts with slow action potentials (presumed Ca spikes).

In conclusion, in this type of experimental epilepsy, recurrent excitatory synapses engage intrinsic cellular oscillatory properties. Recurrent excitation also maintains synchrony of the oscillations.

517.3

EXCITATORY SYNAPTIC COUPLING BETWEEN GABAERGIC INTERNEURONS IN THE HIPPOCAMPUS. H.B. Michelson* and R.K.S. Wong, Department of Pharmacology, SUNY Health Science Center, Brooklyn, New York 11203.

We are interested in the interactions between interneurons in the hippocampus. Experiments were carried out using dual intracellular recordings in the guinea pig hippocampus slice preparation, where excitatory glutamate transmission is blocked by CPP and CNQX (both 10 uM). Synchronized discharge of interneurons is recorded in the presence of 4-aminopyridine. At least two glutamate-independent mechanisms exist for the synchronization of inhibiitory interneurons in the hippocampus. One process is mediated by an excitatory action of GABA via depolarizing $GABA_A$ receptors. This process is blocked by picrotoxin (PTX). Another synchronization process persists in the presence of PTX, producing large amplitude slow inhibitory postsynaptic potentials (IPSPs) in all principal cell populations. These events are 7-15 mV in amplitude; they reverse monophasically at -93 mV, and occur at a slower frequency than the $GABA_A$ synchronized event. Only a subpopulation of interneurons participate in the generation of the slow IPSP. Some interneurons which are synchronized by the $GABA_A$ mechanism become silent after PTX; others show the slow IPSP after PTX. In a third group of interneurons, PTX abolishes the depolarizing envelope, but the interneurons continue to fire bursts of action potentials during the slow IPSP. These bursts show properties suggesting that they are sustained by electrotonic transmission. Thus, interneurons can be coupled by depolarizing $GABA_A$ receptors or by electrotonic junctions. (Supported in part by grants from the N.I.H and American Epilepsy Foundation)

517.4

SYNAPTIC MODIFICATION UNDERLYING IN VITRO EPILEPTOGENESIS. L.R. Merlin* and R.K.S. Wong. Depts. of Neurology and Pharmacology, SUNY/Health Science Center, Brooklyn, NY 11203

We are interested in the modification of synaptic events leading to electrically induced epileptiform discharges. Intracellular recordings were obtained from CA3 pyramidal cells in guinea pig hippocampal slices; stimuli (bipolar, .35-3mA, 140μs) were applied to the stratum radiatum at the border between CA1 and CA2. These stimuli presumably activated the recurrent synapses between the CA3 pyramidal cells. The typical response to a single (test) shock consisted of an EPSP followed by a compound IPSP. Successive tetanization (60Hz, 2s, intensity 2x test stim) progressively altered these synaptic events, ultimately resulting in evoked burst discharges. Specifically, we noted that tetanization induced long-lasting depression of the early IPSP. This could result from either a reduction in the IPSP conductance (g) or an increase in the concomitant gEPSP, or both. To determine whether gIPSP is reduced, we examined the test response at different membrane potentials. The IPSP amplitude varied linearly with the membrane potential. Following tetanization of adequate intensity, the slope of this relationship decreased, suggesting a decrease in IPSP conductance (cf. Stelzer et al, *Nature* 1987). This decrease was cumulative with successive tetani. With sufficient suppression of inhibition, delayed EPSPs first emerged most typically between the peaks of the early and late IPSPs. Additional tetani often induced evoked (and occasionally spontaneous) synchronized bursting consisting of an initial component lasting up to 110ms, followed by phasic bursting at approximately 15Hz. These results suggest that the reduction of inhibitory conductance following tetanization allowed recurrent excitatory synapses to sustain epileptiform activity in the CA3 region.

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517.5

HIGH POTASSIUM INDUCED SEIZURES IN ORGANOTYPIC CULTURES OF RAT HIPPOCAMPUS DEPEND UPON TEMPERATURE DURING GROWTH. Y. Tamaki*, J. Wilson, and C. Shin. Epilepsy Research Laboratory, Duke and VA Med. Ctr., Durham, N.C.27705

We had reported that modest elevation of extracellular K^+ (8.3 mM) induced seizures (SZs) in organotypic hippocampal(HPC) cultures more reliably than in acute adult HPC slices. The literature suggests that HPC organotypic cultures grown in higher temperature show more spontaneous epileptiform discharges. We examined the effect of temperature during the culture period on high K^+ induced SZs in organotypic HPC cultures.

Organotypic cultures were prepared from 6-day-old rat HPC according to the methods of Gähwiler (1988, TINS). Cultures were maintained in a 35°C or a 37°C incubator. At 9-14 DIV, extracellular field recordings were made from CA1 pyramidal layer in standard aCSF (95/5, O_2/CO_2) at 36°C.

Only 6 of 24 cultures grown at 35°C displayed SZs in high K^+ (8.3 mM) media, while 38 of 43 grown at 37°C exhibited SZs ($p < 0.0001$, Fisher's Exact). The SZ frequency and duration were not significantly different between these two groups (35°C [n=5] vs 37°C [n=33]; 1.7 ± 0.6 vs 2.9 ± 0.3 SZs/10min; 30.6 ± 6.1 vs 34.4 ± 3.2 sec/SZ)

Cultures grown at higher temperature were more likely to develop SZs in high K^+ medium. This suggests that temperature may play an important role during growth and maturation of pyramidal neurons in the organotypic culture in determining the intrinsic properties and/or the synaptic responses of the neurons.

517.6

Chronic Epileptogenesis *in vitro*. S.N. Hoffman, P.A. Salin, K.L. Chow*, D.A. Prince, Department of Neurology & Neurological Sciences, Stanford University School of Medicine, Stanford, CA 94305.

We used an *in vitro* model of chronic epileptogenesis in partially isolated neocortical slabs (Prince and Tseng Neurosci. Abstr. 1991) to explore critical aspects of the epileptogenic lesion. Guinea pig neocortical slices were prepared from areas of previous *in vivo* lesions. Recordings from sensorimotor and lateral cingulate cortex were made using extracellular arrays and patch electrodes. Lesions consisting of a white matter undercut and /or surrounding multiple transcortical linear incisions were made at P7-P34 and examined *in vitro* 18-103 days later (N=16). Long latency "interictal" or "ictal" cellular and field potential activities occurred most prominently in layers II-III within 1-2 mm of the transcortical lesion. Normal field potentials were evoked in homotopic cortex and >2-3 mm from the transcortical injury. Transcortical lesions alone induced abnormal activities, however a contiguous white matter lesion resulted in the most hyperexcitable tissue which had spontaneous interictal discharges. Conduction velocities of evoked epileptiform events in layer II-III ranged from 37mm/sec near the transcortical injury to 6-7mm/sec away from this site. APV (100 μM, bath) abolished all epileptiform activity. Epileptiform responses were also blocked by increasing stimulus intensities, suggesting that inhibition was present and recruitable. Spontaneous and evoked IPSCs were present in identified filled layer III and V pyramidal cells in slices showing abnormal discharges. Intracellular events during "ictal" field potentials consisted of complex prolonged (5-10 sec) depolarizations which triggered high frequency spikes. Undercut lesions in white matter or layer VI, without surrounding transcortical incisions, did not result in development of hyperexcitability. This model will allow a detailed assessment of factors contributing to the development of epileptogenesis following cortical injury. Supported by NIH grants NS12151, NS07280 from the NINDS and Dana and Pimley Postdoctoral Fellowships.

517.7

4-AMINOPYRIDINE-INDUCED SPONTANEOUS ACTIVITY IN THE IMMATURE RAT HIPPOCAMPUS: DEVELOPMENTAL AND PHARMACOLOGICAL CHARACTERISTICS. C. Psarropoulou* and M. Avoli. MNI, McGill University, Montreal, QC, Canada H3A 2B4.

Perfusion with 4-aminopyridine (4-AP, 50μM) induced the appearance of spontaneous activity in the CA3 and CA1 areas of immature (2-30 days postnatally) rat hippocampal slices. These potentials were ictal-like and interictal-like epileptiform discharges, a GABA-mediated depolarizing potential and spreading depression (SD) episodes (in 30% of the slices). The overall pattern of activity and the characteristics of each type of potential changed with maturation (their frequency increased, their duration decreased), and in addition the changes took place at different stage of development for each potential. SDs and ictal discharges were rarely seen after 30 days postnatally. In CA1 area, interictal discharges had significantly smaller amplitude than in CA3 and SDs had larger amplitude and duration and also, occurred more frequently.

The NMDA receptor antagonist CPP (1-5μM) blocked SD (n = 15 slices), but had no effect on the other types of activity (n = 16). The non-NMDA receptor antagonist CNQX (2-5μM) did not block SD (n = 11), but blocked the ictal and interictal discharges (n = 20). Sensitivity to CNQX application was greater in the first 10 days postnatally. The $GABA_A$ receptor antagonist bicuculline (3-10μM) blocked SD (11/14 slices) and the GABA-mediated depolarization and increased the frequency of epileptiform discharges (n = 13). The non-uniform temporal profile of the maturational changes of the various 4-AP-induced spontaneous potentials and their pharmacological profiles suggest different mechanisms of generation and possibly different impact on the overall neuronal activity.

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517.8

TETRAETHYLAMMONIUM-INDUCED EPILEPTIFORM ACTIVITY IN YOUNG AND ADULT RAT HIPPOCAMPUS. Y. Fueta* and M. Avoli. MNI and McGill University, Montreal, QC, Canada H3A 2B4

Extracellular field potential recordings were used to study in vitro the epileptiform activity evoked by tetraethylammonium (TEA, 3-10mM) in the CA3 subfield of hippocampal slices obtained from young (12-18 day-old) and adult (>60 day-old) rats. TEA induced ictal- and interictal-like activity in young slices. By contrast, in adult slices only interictal-like discharges were observed. Both the N-methyl-D-aspartate (NMDA) receptor antagonist CPP (5-10μm) and the non-NMDA receptor antagonist CNQX (5-10μM) were necessary to suppress ictal- and interictal-like discharges in young slices. As opposed to this, interictal-like activity in adult slices was reduced and eventually blocked by CNQX (0.5-3μM) only. Furthermore, epileptiform activity recorded in young slices was modified by CPP (i.e. decrease in the rate of occurrence of ictal-like events and reduction in duration of interictal-like discharges) while that observed in adult slices was resistant to this NMDA antagonist.

Our data indicate that the epileptiform discharges induced by TEA in the CA3 subfield of the rat hippocampal slice display patterns of activity that are dependent upon age. Furthermore, the participation of non-NMDA and NMDA receptors in the TEA-induced activity undergoes age-dependent changes.

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517.9

GABA INCREASES CA1 NEURONAL SYNCHRONY DURING ZERO CA^{2+}-INDUCED BURSTING IN THE HIPPOCAMPAL SLICE. P.L. Watson* and R.D. Andrew. Anatomy Department, Queen's University, Kingston, Ontario K7L 3N6.

Hippocampal slices (400 μm) superfused with ≤ 0.2 mM $CaCl_2$ saline exhibit spontaneous epileptiform bursts which can be recorded extracellularly across the CA1 region despite the loss of synaptic transmission. Application of 100-500 μM GABA in "low" Ca^{2+} saline (0.2 mM $CaCl_2$) reversibly arrested bursting (n=13). The effect was blocked by co-application of 50-100 μM bicuculline methiodide (BMI), a GABAa receptor antagonist (n=3). GABA in "zero" Ca^{2+} saline (ϕ $CaCl_2$, 1 mM EGTA) increased the interburst interval, but paradoxically also increased burst duration and enhanced population spike amplitude and frequency during each burst (n=11). This dramatic increase in neuronal synchronization was also eliminated by co-application of 50-100 μM BMI (n=3).

The results suggest that in the absence of extracellular Ca^{2+}, activation of the GABAa receptor promotes non-synaptic synchronization of neuronal firing. One possible mechanism is an enhancement of a Ca^{2+} channel-mediated Na^+ conductance which only operates at less than micromolar concentrations of extracellular Ca^{2+} (Almers and McCleskey, 1984, J. Physiol. 353, 585). The data also suggest that the "low" and "zero" calcium models are more physiologically distinct than previously suggested.

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517.10

OPTICAL REAL-TIME MAPPING OF SPONTANEOUS EPILEPTIFORM ACTIVITY IN HIPPOCAMPAL BRAIN SLICES. L.V. Colom* and P. Saggau. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

The site of origin and propagation pathways of spontaneous epileptiform discharges have not been clearly demonstrated for the different *in vitro* models of epilepsy. The sites of generation and their correlation to local circuits are critical for the understanding of the phenomena of epilepsy. While multichannel electrical recording techniques harm the tissue, optical techniques are non-invasive and thus well suited for this study. Voltage-sensitive dyes were used to monitor spatio-temporal pattern of epileptiform activity in transverse hippocampal slices of both guinea pigs and mice. Two *in vitro* models of epilepsy have been established in hippocampal slices, employing increased recurrent excitation or decreased synaptic inhibition. These conditions were reproduced in our experiments by either raising the bath potassium concentration to 8-10 mM or adding $GABA_A$ antagonists to the bath (bicuculline and picrotoxine, 20 μM and 50 μM respectively). Neural activity was optically monitored from a region including *cornu ammonis* (CA) and *gyrus dentatus*. Changes in extrinsic fluorescence from an area up to 2 x 2 mm were recorded by a photodiode matrix with 100 detectors, connected to a specially designed multichannel amplifier. Individual signals, each proportional to local neural activity, were topographically monitored in real-time by using a video camera and superimposing them on a videomicrograph of the preparation in use. A latency analysis of the spontaneous discharges resulted in two major findings. In both models of epilepsy the site of origin remained the same within each of the studied species. However, in the mouse the epileptiform activity was originated in the CA3c region, while in the guinea pig the site of origin was in the CA2-CA3a region. These results suggest that CA2-CA3a and CA3c are the areas with the lowest threshold for the generation of epileptiform activity in guinea pig and mouse respectively.

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517.11

CNQX-INSENSITIVE SPONTANEOUS EPSPS AND EPILEPTIC BURSTS IN LOW Mg^{2+} IN RAT HIPPOCAMPAL SLICES

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Low concentrations of Mg^{2+} in cerebrospinal fluid have been shown to elicit spontaneous epileptic burst discharges. Here we examined the relationship between spontaneous epsps and epileptic bursts in this model.

Hippocampal slices in vitro were prepared from adult male Sprague-Dawley rats. Intra- and extracellular recordings were made of spontaneous activity in area CA3 in the presence of 1 mM or 0 mM Mg^{2+}. The slow, APV-sensitive component of glutamatergic excitation was isolated from the fast component by bath application of 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). In the presence of CNQX no fast epsp could be evoked at either 0 or 1 mM Mg^{2+}. Spontaneous epileptic bursts occurred in 0 mM Mg^{2+} (mean ± SEM frequency 7.1 ± 1.0 Hz, n=7) and persisted in the presence of CNQX at a reduced rate (0.2 ± 0.1 Hz, n=5). In the absence of Mg^{2+} two distinct populations of spontaneous epsps were seen with modal half-widths of 6 ms and 14 ms. The short duration epsps were seen alone in the presence of 1 mM Mg^{2+} and were blocked by CNQX. The longer epsps persisted in the presence of CNQX at a reduced frequency.

These observations show that APV-sensitive glutamatergic excitation alone was sufficient to generate epileptic bursts. The occurrence of bursting correlated with long-duration spontaneous epsps suggesting that the initial trigger for these epileptiform events was synaptic activation of the NMDA-subtype of glutamate receptor. *Supported by the Wellcome Trust*

517.12

CHOLERA TOXIN MODEL OF EPILEPSY IN RAT HIPPOCAMPAL SLICES EX VIVO: POSTSYNAPTIC POTENTIALS

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Injecting cholera toxin into rat hippocampus induces an epileptic syndrome lasting 7-10 days. Epileptic activity is preserved in the hippocampal slice in vitro. We have attributed the epileptogenesis to a depression of intrinsic potassium currents responsible for accommodation and for AHPs. Here we measure both spontaneous and evoked postsynaptic potentials (psp's) to assess synaptic excitation and inhibition.

Cholera toxin (0.5-1.0 μg) was injected bilaterally into dorsal hippocampus of anaesthetised adult male Wistar rats, which were then allowed to recover. Transverse hippocampal slices prepared 3-4 days later, at the peak of the syndrome, exhibited both evoked and spontaneous epileptic discharges. When these all-or-none bursts were blocked by raising Ca^{2+} in the bathing medium from 2 to 6 or 8 mM we were able to evoke epsp - ipsp sequences. These consisted of a fast epsp followed by both a fast and a slow ipsp. The times to peak of the epsp and slow ipsp did not differ from controls ($p>0.05$), although the fast ipsp peaked slightly later ($p=0.02$). Reversal potentials did not differ significantly from controls. No changes were apparent in the conductance underlying the evoked psp's. Initial analysis of spontaneous epsp's indicated a slight increase in epsp amplitude but no change in half-width.

Models of epilepsy where inhibition is intact, such as that induced by cholera toxin, may provide new insights into mechanisms of the epilepsies. *Supported by the Wellcome Trust.*

517.13

EPILEPTIFORM ACTIVITY INDUCED BY VERATRIDINE IN CA1 NEURONS IN RAT HIPPOCAMPAL SLICES.

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Epileptiform activity can be generated in individual neurons without synaptic involvement. The intrinsic mechanism for this is unclear. Using conventional intracellular recording techniques, we report here results showing that abnormality of Na^+ channels may lead to epileptiform discharges or even seizure-like events in rat hippocampal CA1 pyramidal neurons *in vitro*. Veratridine-induced Na^+ channel abnormality is utilized as a model.

In the presence of veratridine (30-100 nM) the action potential induced by a brief depolarizing current pulse is followed by a slow depolarizing plateau (SDP, 6 neurons). Tetrodotoxin (TTX, 10 nM) markedly blocks the SDP amplitude with no significant effects on the amplitude and duration of the initial action potentials (4 neurons). When Ca^{2+} concentration is doubled in the superfusate, the SDP amplitude decreases. The amplitude and duration of SDP decrease when the membrane is hyperpolarized and increase when depolarized.

A burst of action potentials riding on the SDP appears when concentrations of veratridine over 100 nM are used (20 neurons). This burst is paroxysmal and can be blocked by TTX (30 nM) as well as by high Ca^{2+} concentrations, apparently, by decreasing the SDP. These results suggest that an abnormality of Na^+ channels could be an intrinsic mechanism for epileptogenesis.

517.14

TRANSPUTER MODELLING OF EPILEPTIFORM ACTIVITY IN THE CA1 REGION OF THE HIPPOCAMPUS

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The activity of individual cells in the hippocampus has been modelled using a similar approach to that of Traub (1991, *J. Neurophy.*, 66, 635-650). This model includes a description of the cellular channel currents I_{Na}, $I_{K(DR)}$, $I_{K(C)}$, $I_{K(A)}$, I_{Ca} and I_{AHP}. However, the kinetics of the processes were empirically tuned to fit bursting behaviour of CA3 and CA1 pyramidal cells.

The pyramidal cells together with two populations of interneurons in CA1 have been incorporated into a 3D model of the synaptic connectivity of the CA3 and CA1 areas. Mechanisms of non-NMDA and NMDA EPSCs, as well as IPSCs mediated by $GABA_A$ and $GABA_B$ receptors, are included. Bursting in CA1 driven by CA3, or bursting in CA1 following the synaptic plasticity reported following KA lesion (Wheal, 1989, *Comp. Biochem. Physiol.*, 93A, 211-220) has been investigated.

The simulations of the hippocampal network are performed on transputers using software that is both fast and interactive. A general purpose numerical integration routine and an efficient communication scheme have been developed to allow the model to be run on toroidally connected transputer arrays of different sizes. For example, 100ms of activity on a network of 6400 neurones can be simulated in 8 minutes using a Parsytec Multicluster with 19 Inmos T800 transputers.

Preliminary studies indicate that this anatomically based model can be used to study the dynamic properties of neurones in the hippocampus, including monitoring the behaviour of subpopulation of cells that contribute to epileptiform activity.

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517.15

EFFECTS OF ESTRONE-3-SULFATE ON SYNAPTIC CURRENTS IN CA1 PYRAMIDAL CELLS IN THE RAT HIPPOCAMPUS.

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Previous work has demonstrated the convulsant nature of the estradiol metabolite, Estrone-3-Sulfate (ES). In this study, we used whole-cell, thick slice, patch clamp techniques to observe the action of Estrone-3-Sulfate on synaptic currents in hippocampal CA1 neurons of 18-25 day old male rats. Synaptic currents were evoked orthodromically by stimulating the Schaffer Collateral pathway. Patch clamp recordings were made using 3-6 MΩ electrodes filled with 125 mM potassium gluconate. Evoked currents were observed at holding potentials ranging from -70 to -55 mV. Stimulation of the Schaffer Collaterals elicited the typical EPSC (inward current)/IPSC (outward current) response. Perfusion of Estrone-3-Sulfate, at a pharmacological dose (450 μM), produced an increase in the amplitude of the inward current and a decrease in the outward current. In some cases, however, there was a more sudden transition to a prolonged, multiphasic inward current (EPSCs) that was not preceded by a decline of the IPSC. In addition, pyramidal cells exposed to ES exhibited both evoked and spontaneous bursting activity. Bursts were associated with a large amplitude inward current similar to currents associated with other models of epilepsy. These data suggest that Estrone-3-Sulfate may either facilitate excitatory synaptic transmission or depress inhibitory transmission. However, further experiments need to be performed to clarify the mechanism by which Estrone-3-Sulfate produces epileptic activity.

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517.16

EXCITABILITY OF CA1 REGION OF RAT HIPPOCAMPUS CHANGES POSTNATALLY TO ELEVATION OF $[K^+]_o$.

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Seizure susceptibility in rats peaks during the second postnatal week and declines thereafter toward adult levels. We hypothesized that this may be due to age-related differences in hippocampal sensitivity to elevations in $[K^+]_o$. Hippocampal slices (400-500 μm thick) were prepared from rats of four different age groups; 9-12, 18-22, 28-32, and 60-80 days old (d.o.) and maintained in vitro at 31-33°C. Field potentials were recorded in the CA1 pyramidal cell layer in response to stimulation of the Schaffer collaterals with constant current pulses (10 msec; 50-250 μA). $[K^+]_o$ was increased stepwise from 3.5 mM to 6 mM, 8.5 mM, and 11 mM. Both the amplitude and number of evoked population spikes increased in rats ≥18 d.o. as $[K^+]_o$ increased, with a peak response at 8.5 mM K^+. In contrast, spike amplitude decreased in 9-12 d.o. rats, or was blocked completely in association with a large negative extracellular potential. However, evoked responses recovered more readily in slices from younger rats upon washing with 3.5 mM $[K^+]_o$. Spontaneous epileptiform activity occurred in elevated $[K^+]_o$ in 50-83% of slices from rats ≥18 d.o. but not in 9-12 d.o. rats. Our results show that regulation of $[K^+]_o$ is less effective in the hippocampal CA1 region of 9-12 d.o. rats than in adults, probably because of poorly developed mechanisms for transporting and buffering K^+. However, 18-22 d.o. rats appear to be most susceptible to develop epileptiform activity in response to rises in $[K^+]_o$.

517.17

THE METABOTROPIC GLUTAMATE RECEPTOR CONTRIBUTES TO THE KINDLING OF EPILEPTIFORM EVENTS IN RAT HIPPOCAMPAL SLICES. S. M. Bawin*, W. M. Satmary, A. R. Sheppard and W. R. Adey. Loma Linda University and VA Medical Center, Loma Linda, CA 92357.

We previously demonstrated that repeated sine wave stimulation (SW, 60 Hz, 100-600 μA_{p-p}, 2-4s, every 5 min) in the CA2/3 stratum radiatum kindled afterdischarges (ADs) and electrographic seizures (EGSs) in CA1 and CA2/3. Spontaneous interictal bursts (ISs) developed 2-4 min following SW (induction phase). SW-induced ADs and EGSs required activation of the NMDA receptor, while the expression of kindled ISs required activation of non-NMDA ionotropic glutamate receptors.

We used the same kindling techniques to study the role of the metabotropic receptor (mGluR) in SW-induced epileptiform events. We compared kindling in control medium (ACSF) with kindling during bath applications of the mGluR agonist t-ACPD (50-100 μM), the antagonist DL-AP3 (100 μM), and a Ca^{2+}-dependent K^+ current blocker (TEA, 50-100 μM).

T-ACPD slightly facilitated ADs and EGSs. However, the initial IS frequency, measured during the first 10 min of spontaneous bursting, was twice as high as in ACSF (0.08-0.12 Hz), stabilized at about 0.1 Hz, and persisted for hours following the last SW. TEA also facilitated SW-induced ADs and EGSs. The initial frequency of ISs was 20% higher than in ACSF and remained unchanged for 120-180 min. Addition of AP3 to ACSF or TEA prior to kindling did not alter ADs and EGSs. However, AP3 reduced the initial frequency of ISs by about 40% and the bursts disappeared in 60-70 min. By contrast, addition of AP3 following kindling did not alter the IS frequency.

These results suggest that mGluR 1) does not significantly contribute to the short term effects of SW (depolarizations, ADs, EGSs), 2) is not required for the maintenance of established bursts, but 3) activates long-lasting bursting mechanisms during the induction phase of the ISs.

517.18

DIFFERENTIAL SENSITIVITY OF HIPPOCAMPAL CA1 REGION IN THE SPRAGUE-DAWLEY AND WISTAR RATS TO EVOKED EPILEPTIFORM ACTIVITY IN HIGH POTASSIUM MEDIUM. T.E.Nelson, D.L. Quinn*, R.A. Anderson. Biology Department, Muskingum College, New Concord, OH 43762.

Brain slices from Sprague-Dawley rats were found to be more prone to electrically-induced epileptiform activity in the hippocampal CA1 region than in Wistar rats. The stimulating electrode was placed in the afferent pathway to the CA1 (including Schaffer collateral and commissural fibers) in hippocampal slices bathed in high potassium medium (8.0 mM) in a static pool system. Morphological classification of evoked extracellular field potentials was similar to the methods described by Gilbert et al (Brain Res 361:389-391, 1985). Evoked interictal discharges, patterns of repetitive discharge, in the CA1 and CA3 (mossy fibers were stimulated to activate the CA3) regions of the hippocampal slices were collected. Frequencies of spontaneous bursting were also noted in these regions. In the CA1 only, Sprague-Dawley rats showed a significantly higher number of spikes (Chi square, $p<.01$) following electrical stimulation of the afferent pathways. The CA3 evoked spike patterns were similar in both Sprague-Dawley and Wistar rats. There were no significant differences between the two rat strains with regard to spontaneous burst frequency.

517.19

A GABA-WITHDRAWAL SYNDROME IN HIPPOCAMPAL SLICES. García-Ugalde G. , Galarraga E.*, Bargas J. and Brailowsky S. Instituto de Fisiología Celular. UNAM. México 04510, D. F.

The interruption of intracortical infusion of GABA induces electrographic seizures in rats (Brain Res. 442:175, 1988). This phenomenon was named "GABA withdrawal syndrome" (GWS). We now describe this phenomenon *in vitro*. Field potentials were taken from the CA_1 subfield of the rat hippocampal slice to study the effects induced by the interruption of GABA superfusion. Also, GABAergic inhibition was examined using the paired-pulse paradigm. Saggital hippocampal slices were incubated in GABA (1-5 mM) for 1 or 2 hours. In the washing period, the "intensity vs response amplitude" relationship was analyzed. In some experiments the whole procedure was performed in the recording chamber, that is, the same slice was used before, during and after GABA superfusion (1-5 mM).
With the stimulation parameters used (0.2 Hz, 200 µsec), activation of the Schaffer afferents produced one population spike in control conditions (n=9). In contrast, multiple population spikes were observed in 70% of the slices previously incubated in GABA (5mM). Also, we recorded an increase (210 %) in the amplitude of the population spike with respect to its control value (n=3). This effect was even more evident after 1 hour of GABA washout. A marked reduction of the paired-pulse inhibition was recorded in the slices previously incubated in GABA (1 mM) for 2 hours (n=4).
The results indicated that the interruption of GABA superfusion induces hypersynchronic activity in hippocampal slices. This effect may be due to a dysfunction in GABAergic neurotransmission.

This work was partially supported by DGAPA-UNAM.

517.20

Evidence of Epileptiform Activity in Slices of Piriform Cortex in Response to Stimulation of the Putative Focal Seizure Generation Region, Area Tempestas (AT). J. Doherty* and D.A. Eagles. Department of Biology, Georgetown University, Washington, D.C. 20057.

Generalized, bilateral focal motor seizures can be evoked in rats in response to injection of picomolar amounts of the GABAa receptor antagonist, bicuculline, in a highly discrete region in the anterior extent of the deep endopiriform nucleus (Piredda and Gale, 1985). This region has been termed the area tempestas (AT). This work has prompted the hypothesis that the area tempestas may be a critical site that is somehow involved in the generation of limbic seizures. Using the patch slice technique, we have generated a model system in which the neuronal circuitry which underlies this AT mediated epileptogenicity can be examined. We have focally stimulated area tempestas with bicuculline methiodide and have evoked epileptiform-like activity in the pyramidal cells of the overlying piriform cortex. This model system should allow characterization of the processes which underlie the epileptogenicity of area AT.

Using both single cell whole-cell patch and field potential recordings, we have shown evidence for AT mediated activation of the overlying piriform cortex. Bicuculline methiodide, focally delivered over AT through a pressure driven micropipette potentiates the response of afferent input into the piriform cortex in single cell recordings and generates epileptiform-like responses in populations of layer II pyramidal cells. This epileptiform-like activity is characterized in single cell recordings by paroxysmal depolarizing shifts (PDSs) and long latency depolarizing potentials and in field potential recordings with rhythmic, oscillatory depolarizing waves.

DEGENERATIVE DISEASE: ALZHEIMER'S—NEUROPHARMACOLOGY AND NEUROTRANSMITTERS III

518.1

CORRELATIONS BETWEEN COGNITIVE IMPROVEMENTS AND PHARMACOKINETICS DURING TREATMENT WITH BMY-21502 IN PATIENTS WITH DEMENTIA OF THE ALZHEIMER TYPE (DAT). A. Berardi*, S. Asthana, K.C. Raffaele, J.V. Haxby, R. Shrotriya, K. Dandekar, T.T. Soncrant. Lab. of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892 and Bristol-Myers Squibb Pharm. Res. Inst., Wallingford, CT 06492-7660.

We have reported that treatment with BMY-21502 in DAT leads to significant improvements on visuomotor and attentional tasks, namely on a simple reaction time task ($p < .05$), on the Symbol Digit ($p < .05$), Digit Span ($p < .05$), Stroop Naming ($p < .01$), and Stroop Interference ($p < .005$) tests. The objective of the present study was to determine the relation of significant cognitive improvements after treatment with BMY-21502 in DAT patients to plasma concentrations of BMY-21502 and its two major active metabolites, BMY-42191 and BMY-40440. Nine DAT patients (mean MMS = 21.4 ± 4.03, range = 15-26; 5 probable and 4 possible DAT) underwent psychometrics at various doses of BMY-21502 (200-900 mg/day); all doses were combined for correlation analyses. One subject with kinetic values > 3SD over the group mean was excluded. Improvement on simple reaction time was significantly correlated with BMY-21502 ($r = -.39$, $p < .05$); improvements on the Symbol Digit and Stroop Color tests were significantly correlated with both BMY-21502 (Symbol Digit: $r = .43$, $p < .05$; Stroop Color: $r = .42$, $p < .05$), and BMY-40440 (Symbol Digit: $r = .42$, $p < .05$; Stroop Color: $r = .45$, $p < .05$). Because high positive correlations were found between levels of BMY-21502 and both major metabolites (all $p = .0001$), second order partial correlations were used to determine whether significant cognitive improvements could be accounted for by BMY-21502 or one of its metabolites separately, after the contribution of the other two had been partialled out. Significant positive partial correlations were found between improvement on the Symbol Digit test and the plasma concentrations of both BMY-21502 ($r = .38$, $p < .05$) and BMY-40440 ($r = .48$, $p < .01$). A significant negative partial correlation was found between improvement on the Symbol Digit test and the plasma concentration of BMY-42191 ($r = -.56$, $p < .005$). These results suggest that cognitive improvement during treatment with BMY-21502 in DAT patients correlates with kinetic measures and that it may result from the combined effect of BMY-21502 and its two major metabolites.

518.2

TOXICITY DURING CONTINUOUS INTRAVENOUS ADMINISTRATION OF PHYSOSTIGMINE IN PATIENTS WITH DEMENTIA OF THE ALZHEIMER TYPE
S. Asthana*, K.C. Raffaele, A. Berardi, M.B. Schapiro, T.T. Soncrant. Unit on Pharmacology and Pharmacokinetics, Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, MD.

Alzheimer's disease (AD) is accompanied by a depletion of presynaptic cholinergic markers in the neocortex that has been implicated in cognitive impairments. In an attempt to improve cognition in AD, physostigmine, a reversible cholinesterase inhibitor, was administered by continuous intravenous infusion to 9 patients with possible or probable Alzheimer's disease. Escalating doses (0.5-25 mg/day) were administered over a 2-week period. A dose was identified that optimized cognition and then was administered in a randomized, double-blind, placebo-controlled, cross-over design . Five subjects developed nausea, vomiting, headache, nightmares, sweating, generalized malaise or dizziness during the escalating or double-blind phase. Two of these patients could not tolerate the higher range of doses and the other three developed toxicity when their optimum dose was administered during the double-blind phase. These results suggest that steady-state administration of physostigmine is associated in some subjects with toxicity that may limit its use as a potential therapeutic agent.

518.3

COMPARISON OF MEMORY PERFORMANCE DURING PHYSOSTIGMINE AND ARECOLINE TREATMENT IN PATIENTS WITH DEMENTIA OF THE ALZHEIMER TYPE. K.C. Raffaele,* A. Berardi, S. Asthana, M.B. Schapiro, J.V. Haxby, and T.T. Soncrant. Laboratory of Neurosciences, National Institute on Aging, Bethesda, MD 20892.

Since the cholinergic system is compromised in Alzheimer's disease, we have compared the responses of 6 patients with mild or moderate dementia of the Alzheimer type (DAT) during treatment with continuous intravenous infusions of physostigmine (a cholinesterase inhibitor) or arecoline (a direct cholinergic agonist). During separate inpatient stays, patients received infusions of escalating doses of arecoline or physostigmine. Response was tested during infusions of 2, 6, 12, 18, and 25 mg/day of physostigmine or at 1, 4, 16, 28, and 40 mg/day of arecoline. As measured by changes in verbal memory (Buschke selective reminding test), patients' responses to the two drugs were not always consistent. Two subjects improved their performance with at least one dose of arecoline but not with any dose of physostigmine; two subjects improved with at least one dose of physostigmine but not with any dose of arecoline; one subject improved with both drugs; one subject did not improve with either drug. Verbal memory improvement following arecoline tended to occur at low doses (4 or 16 mg/day), while improvement following physostigmine was more likely to occur at high doses (25 mg) which also were likely to cause adverse side effects. Differences in the therapeutic/toxic dose ratio may account for some differences in response to the two drugs.

518.4

DIHYDROPYRIDINE AND PHENYLALKYLAMINE BINDING TO Ca^{++} CHANNELS IN HUMAN NEUROLOGICAL DISORDERS AND AGE-IMPAIRED ANIMALS. A. Sen, T. V. Dam*, D. Cécyre, W. Rowe, P. Boksa and R. Quirion. Douglas Hospital Research Centre, and Dept. of Psychiatry, Faculty of Medicine, McGill University, Montréal, Québec, Canada H4H 1R3.

Alterations in Ca^{++} availability and metabolism have been suggested as possible mechanisms of cellular aging. Particularly pertinent to brain Ca^{++} availability is the recent demonstration that L-type Ca^{++} channel blockers, such as nimodipine, can facilitate learning and memory in animals. We have investigated the status of this class of Ca^{++} channels in human neurodegenerative disorders associated with memory deficits, including Alzheimer's (AD), Parkinson's (PD) and Huntington's (HD) diseases. Additionally, comparison was made with data obtained in age-impaired and unimpaired (Morris-maze tested) 24 month old rats. [^{3}H]PN200-110, (dihydropyridine) and [^{3}H]D-888 (phenylalkylamine) were used as radioligands to evaluate binding parameters to L-type Ca^{++} channels using either membrane binding homogenate (human) or quantitative receptor autoradiography (rat). Rather surprisingly, and despite neuronal cell losses, it appears that both [^{3}H]PN200-110 and [^{3}H]D-888 binding are well preserved in AD, PD and HD brain tissues, except for a significant decrement in B_{max} values in the basal ganglia of HD patients. Specific [^{3}H]PN200-110 binding parameters are also rather similar in various regions of the age-impaired and unimpaired rat brain. Taken together, these results suggest that the L-type Ca^{++} channel binding protein is well preserved in various neurodegenerative disorders. This also indicates that clinical trials with related drugs should not be hampered by losses of the relevant Ca^{++} channel receptor protein. Sponsored by Miles/Bayer.

518.5

SUT-8701, CCK8 ANALOG, HAS POTENTIAL AS AN ANTIDEMENTIA DRUG FOR ALZHEIMER'S DISEASE. K. Sugaya*, M. Takahashi, K. Kojima, T. Katoh[2], M. Ueki[2] and K. Kubota[3] Aging and Intractable Diseases Area, Res. Inst. for Biosciences, [2]Dept. of Applied Chem., Faculty of Science and [3]Dept. of Pharmacol., Faculty of Pharmaceutical Sciences, Science Univ. of Tokyo, 2669 Noda, Chiba 278, JAPAN.

Alzheimer's disease (AD) patients have severe degenerations of cholinergic systems in their cerebral corticies. We reported that cholecystokinin octapeptide (CCK8) prevented the decline of cerebral cholinergic markers in the basal forebrain (BF) lesion rat as a model animal of AD. In this study, we compared the action of CCK8 and SUT-8701 in the several experiments. Continuously s.c. administered SUT-8701 dose dependently preserved the K^+ stimulated ACh release and choline acetyltransferase (ChAT) activity in the rat cerebral cortex micro punches 2 weeks after BF lesion. These effects were more potent than those of CCK8 and they could be seen even when SUT-8701 was administerd with a few days interval after the lesion. Learning and memory experiments were performed with using Morris water maze. We used young and aged rats. Several days after the pre-training (3 trial/day for 5 days), half of the young rats were lesioned. After 2 weeks drug treatment, we examined the each group again in the same paradigm. The goal latency of aged or lesioned rats was longer than the young control rats. With the SUT-8701 treatment, the goal latency of aged or lesioned rats was decreased as the young control rat level. The affinity to the CCK receptor of SUT-8701 compared to that of CCK8 was 80 times less in the guinea-pig puncreus, but only half in the mouse cerebral cortex. SUT-8701 had about 100 times less effect on the suppression of liquid food intake, which supposed to be a maijer peripheral type side effect, than CCK8. These results suggest that SUT-8701 has more potent and selective preventing effect of the degenerations of cholinergic systems compared to CCK8 and potential as an antidementia drug for AD.

518.6

ENHANCED HYPOTHALAMIC-PITUITARY-ADRENAL AXIS RESPONSE TO PHYSOSTIGMINE IN AGING AND ALZHEIMER'S DISEASE. M.A. Raskind*, E.R. Peskind, D. Wingerson, M. Pascualy, D.J. Dobie, R.C. Veith, D.M. Dorsa, and C.W. Wilkinson. Dept. of Psychiatry and Behavioral Sciences, University of Washington School of Medicine, and Seattle and American Lake VA GRECC, Seattle, WA 98195.

Increased hypothalamic-pituitary-adrenocortical (HPA) axis responsivity has been documented in aged rodents and glucocorticoids have been implicated in age-associated neuronal degeneration. We asked if enhanced HPA axis responsivity occurs in normal human aging and in Alzheimer's disease (AD). The plasma ACTH, beta-endorphin (βE), and cortisol responses to the cholinesterase inhibitor physostigmine were determined in older normals (n=10, age 71 ± 2 yrs.), AD patients (n=11, age 72 ± 2 yrs.), and young normals (n=7, age 27 ± 2 yrs.). Expressed as area under the curve, older normals and AD patients had significantly higher ACTH, βE, and cortisol responses ($p<0.01$) than did young normals. Plasma physostigmine concentrations were similar among groups. Unexpectedly, at peak response, the ratio of βE to ACTH was significantly lower in the AD subjects (.39 ± .03) than in the older normal (1.24 ± .73) or young normal (1.09 ±.36) subjects. These results suggest that the age-associated enhanced HPA axis responsivity observed in rodents also occurs in humans and that processing, secretion, or metabolism of POMC products may be altered in AD. Supported by AGO5136, AGO8419, and the Dept. of Veterans Affairs.

518.7

CHRONIC INFUSION OF MUSCARINIC AGONISTS MAY BE NECESSARY TO ATTENUATE AF64A COGNITIVE IMPAIRMENTS IN RATS. H. Morris*, S. A. Maurer, F. E. Storch, and C. A. Boast. Wyeth-Ayerst Research, Princeton, NJ 08543-8000.

In rats, AF64A selectively and irreversibly reduces cholinergic markers resulting in cognitive deficits (Walsh et al., Br. Res., p. 91, 1984), which are considered a useful model of SDAT. Replication of reductions of AF64A-induced cognitive deficits in rats after acute administration of cholinomimetics (Nakahara et al., Soc. Neuro. Abs., p. 837, 1987) has not been reported. Acute administration of arecoline is ineffective on SDAT memory impairments (Raffaele et al., Psychopharm Bull., p. 315, 1991). Chronic administration of cholinomimetics in animals (Nakahara ibid) and humans (Raffaele ibid) was of greater benefit. We now report that acute administration of various cholinomimetics to AF64A-treated rats did not improve performance deficits on an 8-arm radial maze task. Five daily treatments with oxotremorine (0.1 mg/kg, i.p.) also did not improve maze performance; however, two-week infusion of arecoline (10 mg/kg/day) or oxotremorine (1 mg/kg/day) via osmotic mini-pumps produced significant transient improvements. Two-week infusion of AF102B or pilocarpine did not improve performance. Thus, in the AF64A model, as in SDAT, acute administration of cholinomimetics may be inadequate to reduce cognitive impairments. Chronic treatment with cholinomimetics in this model, as in SDAT, appears to be of greater benefit in attenuating cognitive deficits.

518.8

ANIMAL MODEL OF WANDERING IN ALZHEIMER'S DISEASE: DOPAMINERGIC-CHOLINERGIC INVOLVEMENT, ACTIVITY, AND LONG TERM CHANGES. J.P. Ryan*, M. Markham, A. Woods, & K. Metroka. Department of Psychology, State University of New York at Plattsburgh, Plattsburgh, New York 12901.

Ryan and colleagues (1990, 1991) have developed an animal model of wandering using bilateral injections of 15 ug/uL colchicine into the rat dentate gryus. The present set of experiments elaborated on the model by investigating neurotransmitter changes, activity levels and duration of lesion effects. Experiment I examined cholinergic and dopaminergic interactions through the use of several agonists and antagonists: Mecamylamine, Pimozide, Spiperone, SCH 23390, SKF 38393, and Quinpirole. The results indicate that the D1 and D2 receptor antagonists administered simultaneously ameliorate spatial learning and memory deficits. The beneficial effects are significantly enhanced by the additional administration of the nicotinic antagonist. The nicotinic antagonists administered alone worsened circumference swimming. Dopamine receptor agonists have no effect on observed behavior. Experiment II investigated the differential affect of administering 7, 15 and 25 ug/uL colchicine on activity and spatial learning. The results indicate that the highest dose of colchicine consistently induces circumference swimming and this appears to be associated with an increase in activity in the Wahmann wheel. Experiment III examined behavior 60 and 120 days postlesion. The results indicated that behavioral deficits are stable by 60 days. By 120 day nonlesioned animals exhibit age related behavioral deficits. The studies confirm the viability of the animal model and have implications for treatment.

518.9

Neuropathological and Behavioral Effects of Intraventricular Immunotoxin to the Nerve Growth Factor Receptor. R.G. Wiley*, T. Berbos, M. Ward and D.A. Lappi. Neurology Service, DVAMC, Nashville, TN 37212-2637.

Cholinergic neurons of the basal forebrain have been implicated in learning and memory. In Alzheimer's disease degeneration of these neurons may cause some of the behavioral manifestations of the disease. These are the only neurons in the forebrain that normally express the low affinity nerve growth factor receptor ($p75^{NGFr}$) abundantly. In the present study, we sought to determine the histopathological and behavioral consequences of intraventricular injection of immunotoxin directed at $p75^{NGFr}$. The immunotoxin consisted of the monoclonal antibody, 192 IgG, disulfide coupled to a ribosome inactivating protein, saporin. 192 IgG binds to rat $p75^{NGFr}$. Anesthetized, adult male Sprague-Dawley rats were stereotactically microinjected with either 4 μg of 192 IgG-saporin or 7 μg of OKT1-saporin into the lateral ventricle. After 4 weeks, they were tested on a step-down passive avoidance task. On the first and ninth trials, footshock was administered when the animal stepped down off a platform. 192 IgG-saporin rats were clumsy, more reactive to being handled but less reactive to the footshock than either OKT1-saporin, sham-operated or naive control rats. Also, the 192 IgG-saporin rats continued to step down more rapidly than the other three groups ($p<0.001$). These doses of 192 IgG-saporin produce major cell loss in the medial septum, diagonal band of Broca and nucleus basalis as shown by immunocytochemical staining for choline acetyltransferase and $p75^{NGFr}$. Purkinje cells of the cerebellum are also damaged. We conclude that immunotoxin-mediated destruction of the cholinergic forebrain caused behavioral changes consistent with impaired new memory formation. However, diminished pain sensitivity, decreased ability to inhibit the punished behavior, hyperactivity or damage to other neural systems are alternatives that require consideration.

518.10

CHRONIC CYTOCHROME OXIDASE INHIBITION BY SODIUM AZIDE INFUSION ALTERS HIPPOCAMPAL PROTEIN KINASE C ACTIVITY M. Catherine Bennett[1]*, Lea Waltrip[2], David M. Diamond[1,3], Gregory M. Rose[1,3] and Jeanne Wehner[2]. [1]Dept. of Pharmacology, UCHSC, [2]Inst. Behav. Genetics, UC Boulder and [3]Medical Research, VAMC, Denver, CO 80220

Several investigators report that PKC activity is decreased in the particulate fraction and increased in the cytosolic fraction of brain tissue from Alzheimer's disease (AD) patients (*Brain Res., 453*, 165-169, 1988; *J. Neur. Trans., 30*, 69-78). We investigated PKC activity in rats with chronic cytochrome oxidase inhibition induced by azide infusion. A dysfunction of the mitochondrial enzyme cytochrome oxidase is a concomitant of AD (*Neurol., 40*, 1302-1303). In previous work, we reported that azide treatment causes deficits of learning and hippocampal plasticity (*JGPN, 5*, 93-101, 1992). We now report that chronic azide treatment alters PKC activity.

Adult male S-D rats (350-425g) were implanted (SC) each with an Alzet osmotic minipump (2ML4) containing 0.9% saline or sodium azide (160 mg/ml, in saline). Two weeks later, rats were sacrificed and the hippocampi were assayed for PKC activity (*Brain Res., 523*, 181-187, 1990). PKC activity was decreased in the particulate fraction, which includes mitochondria, and increased in the cytosolic fraction. Thus, the alterations in PKC activity induced by azide treatment parallel those found in AD. These data support the hypothesis that a defect in mitochondrial metabolism may be pathogenic in AD.

518.11

EFFECTS OF AMIRIDIN ON ACETYLCHOLINE RECEPTOR-GATED ION CHANNELS. J. F. Roper*, W.-C. Chau and R. J. Bradley. Dept. of Psychiatry and Behav. Neurobiol., Univ. of Ala. at Birmingham, Birmingham AL 35294.

Amiridin (9-amino-2,3,5,6,7,8-hexahydro-1H-cyclopenta(b)quinoline monohydrate hydrochloride) (NIK-247) has been patented as a learning-stimulation and memory-improvement drug (U.S. Pat. No. 4,735,953, Lavretskaya *et al.*,1988). It was developed in the former USSR, where it is prescribed for symptomatic treatment of senile dementia of Alzheimer type. Its mechanism of action may include regulation of cell membrane lipid content (Burov *et al.*,1991) or modulation of neuronal K^+ channels (Y. Burov, pers. comm.). Amiridin is structurally similar to tacrine (9-amino-1,2,3,4-tetrahydroacridine), which is thought to improve cholinergic transmission by inhibiting brain acetylcholinesterase (AChE) (Summers *et al.*,1986, 89). AChE inhibitors can interact directly with acetylcholine receptors (AChR) (Wachtel, 1990). The objective of this study was to investigate the effects of amiridin on AChR single channel kinetics. Nicotinic AChR-gated ion channels in clonal BC_3H1 mouse tumor cells were studied using the cell-attached patch clamp technique. 200 nM acetylcholine (ACh) and .1 to 40 μM amiridin were applied at transmembrane potentials of -70 to -140 mV. Amiridin reversibly blocked open AChR-gated ion channels in a concentration-dependent manner. In the presence of drug, brief closed periods, or gaps, appeared in ACh-activated single channel currents, transforming them into a burst configuration (Neher and Steinbach, 1978). The mean channel open time decreased with increasing drug concentration. The mean burst duration and mean gap duration increased with increasing drug concentration. The amplitude of single-channel currents did not appear to be altered by the drug. Channel blocking may antagonize the cholinergic activating effects of AChE inhibition or of K^+ channel-mediated restoration of a more normal resting membrane potential in pathologically depolarized neurons. The physiological significance of this antagonism would depend on the relative efficacies of the drug at its various sites of action.

518.12

Effects of Linopirdine (DuP 996) on KCl and $CaCl_2$ Dose Response of Potassium Evoked Release of [^{3}H]Acetylcholine from Superfused Hippocampal Slices. W. J.Tinker, C. Maciag, S. W. Tam and R. Zaczek*. The DuPont Merck Pharmaceutical Co., Wilmington, Delaware.

Linopirdine, a drug which improves the performance of rodents in several learning paradigms, has been shown to enhance the K^+-stimulated release of [^{3}H]acetylcholine ([[^{3}H]ACh), [^{3}H]dopamine and [^{3}H]serotonin from rat brain slices without affecting their basal efflux. To futher elucidate the mechanism of action of linopridine we examined K^+ and Ca^{++} dose response curves for [^{3}H]ACh release from superfused rat hippocamal slices in the presence and absence of the drug. We found that linopirdine shifted the K^+ dose response curve of [^{3}H]ACh release to the left suggesting that the drug lowers the depolarization threshold for release. However, maximal release realized at saturating concentrations of K^+ (60 mM) was not increased in the presence of the drug. Linopirdine also affected the alterations in K^+ evoked [^{3}H]ACh release brought about by changes in Ca^{++} concentrations . There was substantial release of [^{3}H]ACh in the presence of very low (0.1 mM) extracellular calcium when linopirdine was present in the superfusion medium, indicating a shift in the dose response curve of this ion to the left . In addition, the maximal release of [^{3}H]ACh reached at saturating concnetrations of Ca^{++} was increased in the presence of the drug. The present study suggests that linopirdine exerts its effects by increasing the sensivity of neuronal membranes to K^+, Ca^{++} or to both ions. The complexity of linopirdine actions will require futher study in order to fully understand the mechanism through which the drug enhances neurotransmitter release as well as cognitive performance.

518.13

DEXAMETHASONE AND INTERLEUKIN-1 INDUCE THE EXPRESSION OF ALPHA-1-ANTICHYMOTRYPSIN IN HUMAN CORTICAL, BUT NOT CEREBELLAR OR BRAIN STEM ASTROCYTES: IMPLICATIONS FOR ALZHEIMER'S DISEASE. S. Das and H. Potter*. Department of Neurobiology, Harvard Medical School, Boston, MA.

The acute phase protein α_1-antichymotrypsin (ACT) is a primary component of the mature senile plaques characteristic of Alzheimer's disease (AD), where it is found bound to β/A4 protein, a proteolytic fragment of the amyloid precursor protein (APP). Immunocytochemistry and *in situ* hybridization indicates that astrocytes are a source of the increased amounts of ACT in the AD brain. We have previously shown that dexamethasone, and perhaps some other unknown cytokines, can induce the expression of ACT mRNA in cultured rat astrocytes. Since the acute phase mediators IL-1α and dexamethasone have been shown to induce ACT in HepG-2 cultures in vitro, we asked whether these factors could induce ACT expression in human astrocyte cultures from different brain regions. We found that dexamethasone and IL-1 could cooperatively induce ACT message 20-fold in subconfluent astrocytes from the frontal cortex. Confluent cortical astocytes expressed a high level of ACT message constitutively and no further induction could be detected after addition of dexamethasone and IL-1. However, astrocyte cultures from the brain stem and cerebellum had a negligible baseline expression of ACT message (even in the confluent stage), and the induction by dexamethasone and IL-1 in these cultures was minor compared to the induction seen in the cortical subconfluent cultures. These preliminary results indicate that the differential response of astrocytes from distinct brain regions may underlie the localization of pathology seen in AD: mature plaques are seen in cortical regions where the response of the astrocytes to acute phase mediators is more robust, whereas in the cerebellum, where diffuse plaques never progress to mature ones, the astrocytes are only weakly induced by the same factors. We postulate that the pathogenesis of AD involves an initial insult—the deposition of β/A4 and formation of diffuse plaques—followed by progression to mature plaques and neuronal cell death in those regions of the brain where the local glial environment is more likely to develop an inflammatory response.

518.14

'IN VIVO' PHARMACOLOGY OF PD 142676, A NOVEL CHOLINESTERASE INHIBITOR. M. J. Callahan*, W. J. Lipinski, C. J. Moore, M. R. Emmerling, V. E. Gregor and R. E. Davis. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

PD 142676, a chemically novel cholinesterase inhibitor, was assessed for effects on gastrointestinal motility, core body temperature, quantitative electroencephalography (EEG), brain acetylcholine (Ach) levels measured by microdialysis, performance in a water maze task, and short-term memory in a delayed match-to-sample test. Gastrointestinal motility in rats was decreased when PD 142676 was administered acutely or chronically. PD 142676 decreased core body temperature and produced a pattern of EEG activity characterized by a predominance of low voltage, desynchronized activity. These changes in EEG activity are consistent with an increase in electrophysiological arousal in rats and rhesus monkeys. In rats this electrophysiological arousal was maintained over 14 days of repeated administration. In anesthetized rats, PD 142676 increased Ach overflow in dialysates from frontal cortex to a level equivalent to that produced by other cholinesterase inhibitors. Water maze performance was improved on day 1 and day 2 when tested in the C57/B10j mouse. In aged rhesus monkeys, match-to-sample performance was improved on long delay trials (5-15 sec) to a level equivalent to performance on short delay trials (1 sec). These data suggest that PD 142676 is a potent, orally active cholinesterase inhibitor that improves cognitive performance in rodents and aged rhesus monkeys.

518.15

"IN VITRO" PHARMACOLOGY OF PD 142676, A NOVEL CHOLINESTERASE INHIBITOR. M.R. Emmerling, R.D. Schwarz, V.E. Gregor, C. Lee, C. Raby*, J.D. Scholten, T.A. Pugsley, and R.E. Davis. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48106.

Acetylcholinesterase (AChE) inhibitors are potential palliative therapies for the treatment of Alzheimer's disease (AD). The present report describes the *in vitro* properties of a "*second generation* " inhibitor, PD 142676. The new compound is equipotent with tacrine in inhibiting human AChE (IC50 of 40 nM) and similar in potency to other AChE inhibitors being considered for the treatment of AD. The new compound is a poor inhibitor of butyrylcholinesterase (IC50 of 20 μM compared to 5 nM for tacrine). PD 142676, like tacrine, is a reversible, mixed inhibitor of AChE (Kis=45 nM, Kii=81nM) that binds in the active site of the enzyme, as determined by protection from irreversible inhibitors. PD 142676 binds to all subtypes of muscarinic receptors with nanomolar affinity and may act as an antagonist based on its ability to reverse arecoline-induced decreases in the release of 3H-ACh from rat cortical slices. The compound also blocks high affinity choline uptake by rat hippocampal synaptosomes with an IC50 of 2.5 μM compared to 9.59 μM for tacrine. These results show that PD 142676 possesses *in vitro* pharmacological properties similar to tacrine despite its novel chemical structure. The accompanying abstract shows that PD 142676 also has *in vivo* central cholinomimetic activity.

518.16

INHIBITION OF ACETYLCHOLINESTERASE (AChE) BY CHLORO-SUBSTITUTED TACRINE ANALOGUES. C.J. Moore*, V.E. Gregor, C. Lee, and M.R. Emmerling. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48106.

Tacrine is an anticholinesterase that is a potential palliative treatment for Alzheimer's disease. The IC50 of tacrine against human AChE is 30 nM, making it one of the more potent compounds under investigation. The presence of side effects limits the dose of tacrine that can be given without affecting the patient's well being. Thus, it is desirable to obtain improved forms of tacrine with fewer side effects. In an effort to obtain such compounds, we evaluated a number of substituted 4-aminoquinoline and 9-aminoacridine compounds from the company files. We noted that compounds having chlorosubstituents on the benzene ring, in particular in the 6-position of tacrine, were more potent than other analogues. Based on these findings, we synthesized and characterized a group of benzene-ring chlorosubstituted tacrine derivatives. Of these, PD 142012 is the most potent (IC50 = 1.8 nM). It is equipotent with the benzylpiperidine derivative E2020 and 34 times more potent than physostigmine. PD 142012, like tacrine, is a mixed inhibitor of AChE that binds reversibly to the active site of the enzyme. These results indicate that it is possible to make an analogue that is more potent than tacrine itself and that still retains the mechanism of tacrine's inhibition of AChE.

518.17

PURIFICATION OF ACETYLCHOLINESTERASE USING A TACRINE-SEPHAROSE AFFINITY COLUMN. J.L. Grimm, T.W. Hepburn, R.T. Carroll*, and M.R. Emmerling. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48106.

Tacrine is currently under consideration for the palliative treatment of Alzheimer's disease. A method has been established to search for proteins with which tacrine may interact in an effort to understand fully its mechanism of action. A tacrine-sepharose affinity gel was synthesized in a single step by coupling it to epoxy activated sepharose through its primary amine. This affinity gel was tested for its ability to isolate acetylcholinesterase (AChE) from both bovine serum and Torpedo electric organ. AChE is purified to near homogeneity in a single step from these sources. In addition, several other proteins are purified from these sources and are currently under investigation. In summary, other methods used in the purification of AChE are often complicated by lengthy isolation protocols and/or by multiple organic synthetic steps needed to produce the affinity gels. The tacrine affinity gel provides a simple method for creating an affinity column for AChE purification and holds the potential to identify other proteins with which tacrine interacts.

518.18

TRYPSIN ACTIVITY IS NOT INTRINSIC TO ACETYLCHOLINESTERASE (AChE). R.T. Carroll and M.R. Emmerling*. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48106.

Our previous results (BBRC, 1991, 181, 858-862) indicate that the trypsin-like activity associated with purified eel AChE may result from contamination with bovine pancreatic trypsin. In the present study, we tested purified *Torpedo Californica* AChE solubilized by trypsin or collagenase for protease activity. Purified AChE solubilized by trypsinization has a trypsin-like activity that digests BAM-22P but not Substance P. However, velocity sedimentation centrifugation reveals that the trypsin activity, detected by digestion of S-2288, fails to cosediment with the AChE activity. We also observed that the peak of trypsin activity in Sigma (type V-S) electric eel AChE also failed to co-sediment with the peak of AChE activity before or after AChE purification. Purified, collagenase-solubilized Torpedo AChE digests neither Substance P, BAM-22P nor S-2288, even though collagenase itself digested BAM-22P. The sedimentation value and the molecular weight of the AChE solubilized by collagenase were the same as AChE solubilized by trypsinization. Finally, AChE purified from fetal bovine serum has neither trypsin-like nor Substance P degrading activity. These results suggest that the trypsin activity detected in purified AChE results from protease contamination and is not intrinsic to AChE.

518.19

ESTROGEN DEPRIVATION AND REPLACEMENT AFFECTS ACTIVE AVOIDANCE LEARNING IN FEMALE SPRAGUE-DAWLEY RATS. M. Singh*, F.S. Huang, E.M. Meyer and J.W. Simpkins Departments of Pharmacodynamics and Pharmacology, Univ. of Florida, 32610.

The combined endocrine and neurotransmitter deficit hypotheses for the etiology of AD, serve as the basis of our study. The central hypothesis is that ovarian steroids, specifically estradiol (E2), serve a neurotrophomodulatory role in the function of the basal forebrain cholinergic system. We studied the effects of ovarian steroid deprivation (by ovariectomy) and estrogen replacement on learning, using the 2-way active avoidance paradigm, at two separate time points. The short term ovariectomized (OVX) and steroid-replaced (E2) groups were ovariectomized for 3 weeks. The E2 group received estradiol replacement for 2 weeks prior to behavioral testing. These same animals were tested for learning 21 weeks later, with estrogen replacement maintained both during the testing period and between short and long term testing points. Our data show that the short term OVX group were learning-impaired and maintained their inability to learn 21 weeks later. At the long term testing stage, a significant effect of ovariectomy was observed on total avoidances (or correct responses) made over the testing period when compared to intact controls (34 ± 7.8 vs. 88 ± 24.8). Estrogen replacement prevented this deficit. Furthermore, the short term E2 group learned the task by reaching a desired criteria in 9.5 ± 2.1 days. Maintenance on estradiol replacement and subsequent retesting 21 weeks later showed that the animals reached criteria in a much shorter time (in 1.3 ± 0.3 days), suggesting a significant retention of the previously learned task. In contrast, OVX animals never attained the desired criteria. The method employed in restoring estrogen produced plasma levels that were in the physiological range (26 - 43 pg/ml, with one exception (137 pg/ml)). Our interpretation of the results is that physiological levels of estradiol play an important role in cognition and memory. This research may provide a novel animal model for the study of AD and supports a role for estrogens in memory and cognition (Supported by NIH AG 10485).

518.20

COMPARISON OF INTRACRANIAL INFUSIONS OF COLCHICINE AND IBOTENIC ACID AS MODELS OF NEURODEGENERATION IN THE BASAL FOREBRAIN. L. W. Shaughnessy*[1], S. Barone Jr.[2], W. R. Mundy[3] & H. A. Tilson[3]. [1]Curriculum in Neurobiology, UNC, Chapel Hill [2] METI & [3] NTD, U.S. EPA, RTP, NC 27711.

Intracranial infusion of various toxicants has been used to experimentally produce an animal model of neuro-degeneration. The goal of the present experiment was to make direct comparisons of ibotenic acid and colchicine in terms of damage to basal forebrain cholinergic neurons. Bilateral infusions of either colchicine (3.0μg/0.5μl/site), ibotenic acid (6.0μg/0.5μl/site) or vehicle (0.5μl/site) were made in the nucleus basalis magnocellularis (NBM) of male Long Evans rats (n=10/group). Four weeks post-lesion, behavioral assessments were made. The toxicants produced equivalent decreases in step-through latencies in passive avoidance behavior without causing any change in locomotor activity. Five weeks post-lesion, rats were sacrificed for neurochemistry or histochemistry. ChAT and AChE activity were measured in several brain regions of half of the rats. Within the frontal and parietal cortices, a significant decrease in activity (25%) was seen in the lesioned rats. The brains of the remaining rats in each group were sectioned and stained using ChAT immunohistochemistry and AChE histochemistry. Analysis of the number of ChAT-immunoreactive cells in the NBM showed a significant decrease (70%) following infusion of either toxicant. Qualitative examination of the AChE histo-chemical staining in the NBM and cortex showed a similar decrease following infusion of either toxicant. In summary, similar levels of cholinergic hypofunction were achieved for both toxicants and further comparisons of "extracholinergic" markers are planned.

519.1

POTENTIAL BIOACTIVATED NEUROTOXINS, N-METHYLATED ß-CARBOLINIUM IONS, ARE PRESENT IN HUMAN BRAIN. K. Matsubara*, M.A. Collins[2], S. Fukushima, J. Ikebuchi[1], A. Akane, S. Takahashi, E.J. Neafsey[2], and H. Shiono, Dept. of Legal Med., Shimane Med. Univ., Izumo 693, [1]Tottori Univ. Med. Sch., Yonago 683, Japan and [2]Biochem. & Anatomy Depts., Loyola Univ. Med. Center, Maywood, IL 60153.

Potential bioactivated neurotoxins, 2-N-methyl-ß-carbolines and 2, 9-N,N'-dimethyl-ß-carbolines, were analyzed in the parietal association cortex of human brain using GC/MS. The brains (n=9) were taken from fresh corpses with no history of abnormal neuropathology, during forensic autopsies. 2-Methyl-norharman existed in all samples (0.16 ± 0.05 pmol/g, mean ± SD) and 2,9-dimethyl-norharman was detected in 8 out of 9 samples (0.10 ± 0.04 pmol/g). 2-Methyl-harman and 2,9-dimethyl-harman were detectable in only two samples (0.03 ± 0.07 and 0.05 ± 0.10 pmol/g, respectively). Norharman and harman were also measured using HPLC/fluorescence detection. Norharman was present in all samples (0.59 ± 0.45 pmol/g), whereas harman was detected in 7 out of 9 samples (0.21 ± 0.07 pmol/g). Generally, the levels of N-methylated ß-carbolines in the brain were lower than those of their non-methylated forms. Recent studies show that N-methylated ß-carbolines resemble the synthetic parkinsonian toxicant, MPP+, with respect to structure and neurotoxic activity (Brain Res. 570, 154, 1992). Such "bioactivated" carbolinium ions could be endogenous causative factors in Parkinson's disease. Supported by the Japanese Ministry of Education, Science and Culture.

519.2

IDENTIFICATION OF C3 ADRENALINE NEURONS IN HUMAN MEDULLA; LOSS OF C1 AND C3 NEURONS IN THE MEDULLA OBLONGATA IN PARKINSON'S DISEASE

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Phenylethanolamine N-methyltransferase (PNMT) neurons were mapped in the medulla oblongata from 7 patients with idiopathic Parkinson's disease (PD) and 8 age-matched controls. Brains were removed and fixed by perfusing aldehyde through carotid and vertebral arteries. Serial transverse sections (50µm) through the brainstem were divided into 15 series. Neuropathological examination of the substantia nigra and locus coeruleus were performed to confirm PD, using conventional criteria. One series of sections was incubated with a rabbit antibody to bovine PNMT, diluted 1 in 10000, and processed using the avidin-biotin-peroxidase procedure. PNMT cells in each section were counted using the Magellan program and a Macintosh IIcx computer. The total number of cells was estimated by multiplying the number per section by 15. In the ventrolateral medulla, from the level of the obex to 11 mm rostral to the obex, there were 7631±844 C1 neurons in normals. This number was significantly reduced in PD (3604±1051, P<0.01, 53% loss) and many PNMT neurons contained Lewy bodies. We observed a midline C3 group of PNMT neurons in normal brains and this group was also severely affected (88% loss) in PD. Neither the C2 group nor the small PNMT neurons in the nucleus tractus solitarius was significantly reduced. Our results demonstrate a selective loss of C1 and C3 cells in PD, providing the first quantitative evidence for damage to brainstem sympathetic premotor neurons. These changes may underlie some of the autonomic symptoms of PD.

519.3

EXPRESSION OF CORTICAL NICOTINIC CHOLINOCEPTORS IN PARKINSON'S DEMENTIA COMPARED TO ALZHEIMER'S DISEASE H. Schröder*¶,♦, E. Giacobini¶, R.G. Struble+ A. Maelicke♦ Depts. Pharmacology¶ and Psychiatry+, Southern Illinois Univ. Sch. of Med., Springfield, IL 62794, ♦Dept. Physiological Chemistry and Pathobiochemistry, Univ. of Mainz, F.R.G.

In Alzheimer's disease (AD) as well as in Parkinson's dementia (PD) cerebrocortical nicotinic binding sites are markedly reduced. In AD frontal cortex the neuronal expression of nicotinic cholinoceptors (nAChR) is significantly decreased (Schröder et al., *Neurobiol. Aging*, 12:259, 1991) whereas no data are available on nAChR expression in PD cortices. Using the monoclonal nAChR-antibody WF 6 (Fels et al., *J. Biol. Chem.*, 261: 15746, 1986) autopsy samples of human frontal cortex were studied immunohistochemically in: (1) PD patients [n=6; 78±4yrs] and (2) age-matched controls [n=4; 75±9yrs]. Densities of WF6-immunoreactive [(1) 2677±697 neurons/mm^3 (mean±s.e.m) (2) 5605±1357] and of cresylviolet-stained neurons did not show statistically significant differences (p>0.05). Although ranges of nicotinic binding site reductions in homogenate binding studies are similar in AD and PD (Whitehouse et al., *Arch. Neurol.*, 45:722, 1988), in contrast to AD, neuronal nAChR expression appears to be only slightly reduced in PD. This speaks in favour of a qualitative alteration of nAChR binding sites in PD rather than for a shortage of nAChR protein supply. Supported by the Deutsche Forschungsgemeinschaft (Schr 283/8-1, 11-1), Southern Ill. Univ. Central Res. Committee award and R.J. Reynolds Tobacco Co..

519.4

STRIATAL DOPAMINE, TYROSINE HYDROXYLASE, AND DOPAMINE TRANSPORTER ARE MARKEDLY REDUCED IN A PATIENT WITH DOPA-RESPONSIVE DYSTONIA. X.H. Zhong*, A.H. Rajput, O. Hornykiewicz, and S.J. Kish. Clarke Institute of Psychiatry, Toronto, and Univ. of Saskatchewan, Saskatoon, Canada

Dopa-responsive dystonia (DRD) is a variant of childhood-onset idiopathic torsion dystonia which shows a therapeutic response to L-dopa. To date, little is known regarding the biochemical changes underlying the disease.

We have measured the striatal dopamine (DA) level, tyrosine hydroxylase (TH) activity and protein level, and DA uptake sites (by specific GBR 12935-binding) in the caudate and putamen of one DRD patient (aged 19, first described by Rajput et al., 2nd Intl. Cong. Movement Disorders, Munich, June 24-26, 1992) and four controls matched with respect to age and postmortem time. A marked DA loss (-97%) accompanied by reduction of TH activity (-88%) and TH protein concentration (-79%), and reduction of DA uptake sites (-68) in putamen was found in our patient, with somewhat less severe changes in caudate (-83%, -45%, -42% and -9% respectively). The extent of reduction was in the pre-parkinsonian range, being less severe than that in Parkinson's disease (PD). However, the subregional pattern of the reduction was similar to that in idiopathic PD. Our findings are consistent with the excellent clinical response of DRD patients to L-dopa therapy and suggest a cause-effect relationship between the striatal DA loss and the DRD syndrome.

519.5

SUPEROXIDE DISMUTASE EXPRESSION IN THE NIGRO-STRIATAL PATHWAY OF PATIENTS WITH PARKINSON'S DISEASE. A. Baccichet*, C. Thiffault, N. Aumont, D. Dea and J. Poirier. Douglas Hospital Research Centre, Department of Psychiatry and Centre for Studies in Aging, McGill University, Montreal, Quebec, Canada.

Several recent animal and human studies have provided evidences suggesting a role for the excessive formation of destructive hydrogen peroxide and/or the lack of antioxidant protection in the progressive loss of dopaminergic neurons of the substantia nigra of parkinsonian patients. The most interesting and consistent finding related to the antioxidant status in idiopathic parkinson's disease (IPD) has come recently from different laboratories showing a significant increase in the activity of the superoxide dismutase (SOD) in the striatum and substantia nigra. While SOD activity has been widely associated with superoxide detoxification, the end product of the reaction, namely the hydrogen peroxide, has received little attention. Accordingly, we have begun a systematic analysis of the two main forms of superoxide dismutase (cupper/zinc and manganese) in term of activity, protein levels and mRNA prevalence in the substantia nigra and striatum of control and IPD individuals. Results obtained so far indicate that the major portion of the cupper-zinc SOD mRNA and protein is restricted to neuromelanin-containing neurons of the substantia nigra. In the striatum, the distribution of the cupper/zinc SOD is more homogenous and appears to be mostly restricted to neuronal population. A significant increase in the activity of the manganese form of SOD (but not of the cupper/zinc form) was found in the striatum of parkinsonian patients. Supported by the Parkinson Foundation of Canada and by a Scholarship from the Medical Research Council of Canada.

519.6

THE EFFECT OF L-DEPRENYL AND MPTP ON SUPEROXIDE DISMUTASE ACTIVITY IN THE STRIATUM OF C57BL/6 MICE

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Douglas Hospital Research Centre, Department of Pharmacology and Therapeutics, Department of Psychiatry and Centre for Studies in Aging, McGill University. Montreal, Quebec, Canada, H4H 1R3.

L-deprenyl, a potent monoamine oxidase B inhibitor is currently used in the treatment of Parkinson's disease (PD). L-deprenyl appears to delay the necessity of l-dopa therapy in the early stages of the disease although it does not halt the progression of PD. The beneficial effect of deprenyl in PD seems to be of short duration (6-12 months). Recently, it has been demonstrated that administration of l-deprenyl to rats in vivo increases the superoxide dismutase (SOD) activity in the striatum. SOD is a key enzyme involved in the detoxification of superoxide radicals and is principally expressed in neurons. Results obtained in our laboratory indicate that acute administration of l-deprenyl (10 mg/kg, every 2 days) and deprenyl/1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; 3 X 20mg/kg, every 2 hrs) over 15 days period induces an increase in the activity of SOD in the striatum of C57BL/6 mice by more than 2 fold. It is not clear at this time whether the induction of SOD is beneficial to dopaminergic neurons. The MPTP regimen which destroyed 40-60% of tyrosine hydroxylase immunopositive neurons causes a marked decrease in the striatal SOD activity in C57BL/6 mice. These latest results contrast with the increase of the SOD activity as observed in the striatum of PD patients.

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519.7

LOSS OF BASIC FIBROBLAST GROWTH FACTOR IN SUBSTANTIA NIGRA NEURONS IN PARKINSON DISEASE. P.L. McGeer*, I. Tooyama, T. Kawamata, D. Walker, T. Yamada, K. Hanai[1], H. Kimura[1], M. Iwane[2], K. Igarashi[2], and E. G. McGeer. Kinsmen Lab. of Neurol. Res. and the Neurodegenerative Disorders Centre, U. of British Columbia, Vancouver, Canada, V6T1Z3; [1]Institute of Mol. Neurobiology, Shiga U. of Med. Sci., Otsu, Shiga, Japan; and [2]Takeda Biology Res. Lab., Takeda Chem. Ind., Osaka, Japan

Basic fibroblast growth factor (bFGF) has been shown to occur in mesencephalic dopaminergic neurons and to have a neurotrophic effect on these neurons in vitro and in vivo. We examined the substantia nigra (SN) of 6 cases of Parkinson's disease (PD) and 8 controls immunohistochemically using a monoclonal antibody to bFGF. The mean number of melanin-positive neurons in sections of PD SN was 30.3% of the control mean, but the number of bFGF-immunopositive neurons was only 4.7% of the control mean. BFGF-immunoreactivity was observed in only 8.2% of PD, but in 93.7% of control melanin-positive neurons. These results suggest a profound depletion of bFGF in surviving dopaminergic neurons of the SN in PD. This depletion may be related to the disease process. (Supported by the Japan Found. for Aging & Health, the MRC, and the the Parkinson Found. of Canada)

519.8

CSF STUDIES IN UNMEDICATED PARKINSON DISEASE: DOPAMINE METABOLISM AND XANTHINE. P. LeWitt[1], M. Galloway[1], W. Matson[2], P. Milbury[2], M. McDermott[3], D. Oakes[3], I. Shoulson[4], and The Parkinson Study Group (DATATOP Study)[4]. [1]Clin. Cell. Neurosci. Progr., Detroit, MI 48207, [2]ESA, Inc., Bedford, MA 01730, [3]Div. Biostat. and [4]Dept. Neurology, Univ. Rochester Sch. Med., Rochester NY 14642.

Striatal HVA is a major component of CSF HVA. Despite this, its CSF concentration did not correlate with a variety of Parkinson Disease (PD) indices such as bradykinesia and disability scores. Our studies also found sub-stantial overlap of CSF HVA concentration between PD and control subjects. In PD, concentrations of CSF dopamine, DOPAC, dopamine-3-sulfate, 3-methoxytyramine, homovanillol, levodopa, and 3-O-methyldopa were 12.6% of HVA. None of these substances correlated to Parkinsonian severity or differentiated PD from controls.

CSF xanthine and HVA concentrations were highly correlated ($r = 0.82$, $p < 0.0001$). PD and control subjects differed when CSF xanthine concentrations were indexed against HVA: PD xanthine/HVA: 15.44 ± 6.50; controls: 10.91 ± 4.48 ($p < 0.002$). Though dopaminergic neurotransmission interacts with purines, the basis for the relationship is unknown.

519.9

EFFECT OF PHENYLALANINE CHALLENGE ON LEVODOPA TRANSPORT INTO CSF IN PARKINSONIAN PATIENTS. R.M.Beckner, W.R.Woodward, C.W.Olanow, J.G.Nutt*. Depts. of Neurology, Oregon Health Sci. Univ., Portland, OR and Univ. of S. Florida, Tampa, FL.

Levodopa crosses the blood-brain barrier via the large neutral amino acid (LNAA) transporter. LNAA's have been shown to reduce the clinical efficacy of levodopa in fluctuating parkinsonian patients, possibly by competitive inhibition of levodopa transport into brain. To determine whether CSF levodopa is an accurate index of brain levels, we administered phenylalanine challenges during two-hour, constant-rate levodopa infusions and monitored plasma and CSF drug levels in two parkinsonian patients with Omaya reservoir implants in their lateral ventricles. CSF levodopa levels were not reduced by phenylalanine when compared to levels achieved during infusions without an amino acid challenge. In contrast, the duration of the clinical response to levodopa was markedly reduced from over 100 minutes in the absence of a challenge to less than 20 minutes in the presence of phenylalanine. Phenylalanine levels rose 16-fold in plasma and 10-fold in CSF in one patient, while DOPAC and HVA levels in CSF were not appreciably altered. The plasma and CSF levodopa kinetics with and without phenylalanine were similar to those observed in monkeys, with levodopa entry into CSF lagging behind that of plasma. These results suggest that CSF levodopa is not in equilibrium with brain extracellular or neuronal concentrations and that CSF levodopa levels are not an accurate indicator of clinical response. Levodopa is probably transported into CSF at the choroid plexus, and this transport must differ from that at the capillary endothelium. Supported by NIH Grant NS21062.

519.10

EFFECT OF INHIBITION OF CATECHOL-O-METHYLTRANSFERASE IN LEVODOPA-TREATED PARKINSONIAN PATIENTS. J.G.Nutt, R.M.Beckner, W.R.Woodward, J.H.Carter, S.T.Gancher, J.P.Hammerstad*, A.Gordin. Dept. of Neurology, Oregon Health Sci. Univ., Portland, OR 97201, and Orion Pharmaceutica, Finland.

A major route for disposition of levodopa (L-DOPA) in the presence of carbidopa is O-methylation by catechol-O-methyltransferase (COMT). Inhibition of COMT could augment the clinical effects of L-DOPA by decreasing elimination and increasing bioavailability of L-DOPA, and by reducing accumulation of 3-O-methyldopa (3OMD). The acute effects of Entacapone (OR-611), a competitive inhibitor of systemic, and to a lesser extent, brain COMT, on the pharmacokinetics and pharmacodynamics of levodopa has been examined in 7 parkinsonian patients with fluctuating motor responses to levodopa.

OR-611 did not affect maximum concentrations of plasma L-DOPA achieved after oral or intravenous administration, nor did it affect the time to maximum concentrations after oral administration. The area under the time-concentration curve was increased by 58% ($p<0.04$, $n=6$) after oral administration and by 45% ($p<0.01$, $n=7$) after intravenous administration. The elimination constants were reduced by 44% and 34% respectively. The duration of clinical response to L-DOPA was increased by 50% ($p<0.06$, $n=6$) with oral doses and 47% ($p<0.02$, $n=7$) with intravenous infusions. These results indicate that OR-611 may help in the treatment of parkinsonism by prolonging the clinical response to L-DOPA. This effect is achieved without increasing maximum plasma drug concentrations, which may reduce adverse effects. Supported by NIH Grants NS21062 and RR00334, and Orion Pharmaceutica.

519.11

CHANGES IN DOPAMINERGIC RESPONSE TO ACUTE AND CHRONIC APOMORPHINE IN PARKINSONISM. S.T. Gancher, J.G. Nutt, W.R. Woodward, E.A. Zimmerman*, Department of Neurology, Oregon Health Sciences University, Portland, OR 97201.

The dopaminergic agonist, apomorphine (APO), is effective in the treatment of parkinsonism. We sought to determine whether tolerance develops to the antiparkinsonian effects of APO, and if so, what temporal factors influence its development. In an acute study, 7 patients with motor fluctuations received test doses of APO (50 μg/kg), preceding and following 6 and 22-31 hour APO infusions. The responses to both preinfusion test doses were similar. The duration of response to test APO doses that followed either infusion were reduced, by 35% following the short infusion and by 68% following the long infusion. In addition, the duration of effect after discontinuing the long infusion was briefer than after discontinuing the short infusion. In a chronic study, 7 patients received test APO doses (12.5-100 μg/kg) before and after a 3 month period of subcutaneous APO infusions administered during waking hours. No overall change in APO dose-response or in APO kinetics was observed over the 3 month period. However, 3 patients were able to lower their APO infusion rate, and these patients achieved higher plasma APO levels and AUCs following APO test doses at the end of the 3 month period. Our data suggest that tolerance develops rapidly to APO (hours), the magnitude of which is duration-dependent. This tolerance, however, disappears after an overnight period without medications, and cumulative tolerance to chronic diurnal APO infusion does not occur. Supported by NIH Grants NSO1539 and NS21062, and the American Parkinson's Disease Association.

519.12

CHARACTERIZATION OF THE ROTENONE BINDING SITE OF MITOCHONDRIAL COMPLEX I IN AN AUTORADIOGRAPHIC ASSAY. JT Greenamyre, DS Higgins and RV Eller, University of Rochester, Rochester, NY 14642.

Complex I (NADH dehydrogenase) is the proximal enzyme of the mitochondrial electron transport chain and is inhibited with high affinity by rotenone. Defective complex I activity has been implicated in the pathogenesis of Parkinson's disease, but little is known of the characteristics or distribution of this enzyme in brain. We have custom-synthesized and radiolabeled a rotenone analog, [^{3}H]dihydrorotenone (DHR), and have used it to develop a quantitative autoradiographic assay for complex I in brain. Using 100 μM rotenone to define nonspecific binding, DHR binding to rat brain is saturable with a K_D of about 10 nM; the B_{Max} varies more than 20-fold across brain regions. At DHR concentrations below 10 nM, virtually all binding is specific. Scatchard analysis suggests that DHR binds to a single class of sites. The specificity of DHR binding for complex I is indicated by *(i)* displacement of binding by rotenone and MPP^+, *(ii)* loss of binding after lipid extraction, and *(iii)* marked enhancement of binding by NADH. We conclude that DHR binding provides a quantitative method to assess the biochemical characteristics of the rotenone binding site of complex I with a high degree of anatomical resolution. *(Supported by the Hope Geoghegan Fund, the American Academy of Neurology, the United Parkinson Foundation, and USPHS Grant NS01487)*

519.13

QUANTIFICATION OF TYROSINE HYDROXYLASE IN THE DOPAMINERGIC MESENCEPHALIC NEURONS OF CONTROL SUBJECTS AND PATIENTS WITH PARKINSON'S DISEASE AND ALZHEIMER'S DISEASE. A. KASTNER, E.C. HIRSCH*, F. JAVOY-AGID and Y. AGID. INSERM U289, Hôp. de la Salpêtrière, 75013 PARIS.

Parkinson's disease (PD) is characterized by massive degeneration of the dopaminergic neurons in the substantia nigra (SN). Moreover, the functional capacity of the surviving nigral neurons is affected, as indicated by a reduction in tyrosine hydroxylase (TH) mRNA in these neurons. Thus, to test the ability of the remaining neurons to express TH protein, a semi-quantitative immunocytochemical method was developed and was used to determine the relative amount of TH per neuron on mesencephalic sections of 9 control subjects, 6 patients with PD and 3 with Alzheimer's disease (AD). A second set of experiments was performed on 5 other controls and 5 PD patients for which the neuronal content of TH mRNA has been analysed previously. Proportionality between the densitometric values obtained in the operative conditions and the tissular concentrations of TH was established using bovine adrenal medulla homogenates as standards. Variable amounts of TH were detected in the dopaminergic neurons from one subject to another, and between the different cell groups of the mesencephalon in the same subjects. Reduced content of TH protein was found in the neurons of the parkinsonian SN, but not in those of the central grey substance, which are spared by the disease. Similarly, in patients with AD, the amount of TH per cell was specifically reduced in the ventral tegmental area, a region in which catecholaminergic neurons degenerate during the disease. Besides, in the SN of PD patients, a correlation was observed between the reduction of the cellular content of TH protein and that of TH mRNA, suggesting that the efficiency of TH gene expression is altered in the remaining dopaminergic neurons.

DEGENERATIVE DISEASE: OTHER

520.1

GABAERGIC LOCAL CIRCUIT NEURONS DEGENERATE IN THE MOTOR CORTEX OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS. Kuninobu Nihei , Ann C. McKee and Neil W. Kowall*. Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Recent evidence suggests that neuronal degeneration in amyotrophic lateral sclerosis (ALS) may be mediated by excessive glutamate receptor activation. In the cerebral cortex, GABAergic local circuit neurons resist NMDA-type glutamate receptor mediated excitotoxicity but are disproportionately sensitive to non-NMDA receptor agonists. In Huntington's disease, a distinctive subset of GABAergic local circuit neurons containing parvalbumin are relatively spared, consistent with NMDA receptor-mediated toxicity. We evaluated GABAergic parvalbumin positive cortical interneurons in the rolandic cortex of six patients with sporadic ALS and six age-matched controls to determine if the pattern of neuronal loss in ALS is consistent with glutamate receptor mediated neuronal injury. In ALS motor cortex, the density of parvalbumin immunoreactive neurons (per mm^2) was significantly decreased compared to control values (31.4±3 vs. 52.2±4, p=0.003, unpaired t-test). The depletion of parvalbumin immunoreactive neurons in ALS motor cortex contrasts with the pattern of sparing found in Huntington's disease and is consistent with non-NMDA glutamate receptor mediated neurotoxicity.

520.2

RODENT BRAIN METABOLISM OF CYCASIN AND DNA ALKYLATION BY METHYLAZOXYMETHANOL (MAM). J.F. Kabel, G.E. Kisby*, †S.C. Mako, †R.H. Glew, P.S. Spencer. Center for Research on Occupational and Environmental Toxicology, Oregon Health Sci. Univ., Portland, OR. 97201 and †Dept. of Biochem., Univ. of New Mexico, School of Medicine, Albuquerque, NM 87131.

Cycasin, the β-D-glucoside of methylazoxymethanol (MAM), is a toxic agent in cycad seed which is under study as a possible etiological factor for western Pacific amyotrophic lateral sclerosis (ALS). Presently, there are 3 mammalian β-glucosidases: a lysosomal (β-glucocerebroside, **I**), membrane (TX-100 soluble, **II**) and cytosolic (broad specificity, **III**). Studies with guinea pigs demonstrated that whole-brain tissue cytosolic β-glucosidase activity is 500 x higher than activity in peripheral organs (e.g. liver, kidney). Cycasin hydrolysis by guinea pig liver β-glucosidase (**II**) was 50% of total *p*-nitrophenylglucopyranoside activity. Sprague-Dawley (SD) rat brain tissue enzyme activity differed among the β-glucosidases (**II** >> **I** > **III**). Approximately 5% of a single intracarotid bolus injection of cycasin (6 mg/kg; 100 µl over 60 sec) was detected in the CNS of a SD rat, predominately in the form of MAM. MAM reacts *in vitro* with calf thymus DNA to produce 7-methylguanine and O^6-methylguanine. Taken together, these studies demonstrate that brain tissue is able to rapidly hydrolyze cycasin to MAM, an agent known to alkylate DNA. Cycasin, which is neuronotoxic at low (1 µM) concentrations (Kisby *et al.*, 1992), may be acting as a 'slow protoxin' by permanently altering post-mitotic neural tissue DNA. Further study is needed to characterize the role of cycasin in western Pacific ALS. [Supported by a grant from NS19611]

520.3

ASTROGLIOSIS IN GUAM AMYOTROPHIC LATERAL SCLEROSIS (ALS) D. Munoz-O'Regan, C. Bergeron, R.M. Garruto, D.P. Perl, S. Wright* and P.D. Kushner, ALS Research Foundation, California Pacific Medical Center, San Francisco, CA 94115

Guam amyotrophic lateral sclerosis (GALS) has three salient features which distinguish it from ALS, (1) high incidence rate, (2) dementia, and (3) particular histopathological features common to Alzheimer's disease and Parkinsonism-dementia complex (PDC). Nineteen cases (mid-frontal and temporal cortices), eight GALS, two GALS presenting with PDC (GALS/PDC), five Guam PDC (GPDC) and four neurologically normal Guam cases comprised of persons of Chamorro descent (because of the incidence of neurofibrillary tangles within the population even amongst non-ALS, non-PDC cases), were examined as cryosections stained with immunoperoxidase technique for glial fibrillary acidic protein (GFAP).

Astrogliosis in GALS reveals itself in similar fashion to ALS within the subcortical white matter. Results indicate the morphological parameters of an on-going, "reactive" astrogliotic process within the superficial subcortical white matter in GALS in which stained astrocytes have two distinct morphologies; these profiles are located in different areas. The astrocytes of the gray/white matter junction display very numerous, elongated processes; in contrast, regressive astrocytes are evident just deep to this junction. Gray matter astrocytes have smaller cell bodies, shorter processes, and staining is not prominent. Peculiar to GALS is the presence of gliosomes, bead-like structures found on many linear processes of astrocytes, descending from the glial limitans. GALS/PDC cases show more consistency in gliotic response and similarity to GALS cases; conversely, GPDC cases display either numerous intensely stained astrocytes (4 cases) or no gliosis (3 cases). GALS has clinical and pathological differences from ALS and yet the parallel of superficial subcortical white matter astrogliosis implicates a special region of involvement in the pathology of all forms of ALS.

520.4

GFAP AND BETA AMYLOID STAINING IN THE CORTICAL GRAY MATTER IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) D. Nagy, T. Kato, D. T. Stephenson, P. D. Kushner* ALS Research Foundation, California Pacific Medical Center, San Francisco, CA 94115

We examined cryosections of the midfrontal, inferior parietal, cingulate, temporal, and occipital cortices, as well as the primary motor cortex from 15 cases of adult-onset amyotrophic lateral sclerosis (ALS) with immunocytochemistry using anti-glial fibrillary acidic protein (GFAP) and anti-beta amyloid protein.

By GFAP staining there was found an increased subpial gliosis, "patchy" astrocytosis in LII-III, occasionally a few patches in the LIV, and a "finger-like" astrocytosis in LV-VI. The patchy astrocytosis was characterized by multiple clusters of astrocytes, each containing 2-8 glial cells, and it occured in 9 out of 15 cases. The "finger-formation" of the gray/white matter interface seemed to be an extension of the previously described widespread subcortical astrogliosis (J Neuropath Exp Neurol 50:263-277, 1991) and was found in 11 out of 15 cases.

By beta amyloid immunostaining we observed primitive plaques in 5 out of 15 cases. There were no classic plaques. Total number of ALS cases with both astrocyte-patches and amyloid-plaques was 3 out of 15. Our study demonstrates a ubiquitous patchy astrocytosis in widespread cortical brain regions, suggesting a potential involvement of the whole brain in the pathomechanism of the ALS. We cannot account for these patches as classic or primitive plaques, although they may indicate imminent future beta amyloid production. (Supported by M.D.A.)

520.5

PROCESSING OF TAU PROTEIN IN PICK'S DISEASE. S. Murayama*, Y. Higashi+ and H. Mori. Department of Neuropathology, University of Tokyo, Tokyo, 113 and +Kawasaki Medical School, Kurashiki, 701-01, Japan

In Alzheimer's neurofibrillary tangles (ADNFT), tau protein is cleaved in the N-terminus and only its carboxyl third is left (Kondo et al, Neuron 1988;1:827). We adopted an immunocytochemical approach whether similar process is observed in Pick bodies (PB) that share immunocytochemical properties with ADNFT. Formalin-fixed, paraffin-embedded hippocampi that were abundant with PB from four cases of Pick's disease (PD) were employed for this study. Ultrastructurally, PB in these cases contained 15nm straight filaments with occasional twisted profiles. PB-type filaments were also observed in ballooned neurons (BN) and neuropil around PB. Immuno-electron microscopically, phosphorylated epitope of the carboxyl third of tau protein was localized on these filaments (Murayama et al, Ann Neurol 1991;27:394). Employed antibodies and recognized sequence of tau protein were as follows: Alz 50 (2-16); anti-human tau (45-61); tau 1 (191-204); P2 (299-385); tau 0 (354-369); and tau 6 (420-429). Antibodies against both N- and C-termini of tau protein visualized PB, BN and neuritic change around PB. However, the staining pattern with anti-N or anti-C-terminus was different in: 1) anti-C-terminus stained PB more intensely than anti-N-terminus; 2) C-terminus-positive neurites were thicker and more curled than N-terminus-positive neurites; and 3) C-terminus-positive area in BN was smaller in size and more crisp than N-terminus-positive area. The most intense staining was obtained by P2 that recognized a sequence that was tightly bound to the core of paired helical filaments (PHF). These data suggest: 1) first abnormal deposition of tau protein and then cleavage in N-terminus occur in PD as well as in Alzheimer's disease; and 2) the core of PB-type filament and PHF may share the same sequence of tau protein.

520.6

PHF POSITIVE NEURITES IN A SEVERE CASE OF PICK'S DISEASE. E.J. Cochran, R.P. Zimmerman*, E.J. Mufson. Department of Neurological Sciences and Pathology, Rush Alzheimer's Disease Center, Rush-Presbyterian St. Luke's Medical Center, Chicago, IL 60612.

Pick's disease (PD) may present with a clinical picture which is indistinguishable from Alzheimer's disease (AD), although the neuropathological characteristics are significantly different. We report a case of PD with classic pathology, as well as extensive paired helical filament (PHF) containing neurites, without the AD-like senile plaques or neurofibrillary tangles. Gross evaluation of the brain of this 69 year old woman with a 11 year history of progressive dementia revealed severe atrophy involving the frontal, temporal, parasagittal parietal and occipital lobes. Paraformaldehyde (4%) immersion fixed tissue was either paraffin-embedded or cut on a freezing microtome. Numerous argyrophilic Pick Bodies (PB), predominantly located in neocortical and limbic cortical layers 2,3, and 5, were immunoreactive to ALZ 50, and antibodies against PHF and ubiquitin. In addition, numerous ALZ-50 positive neurons were seen in the hippocampus. Double immunostaining with ALZ 50 and anti-PHF showed colocalization in many PB. Strikingly, PHF immunostaining revealed extensive neuritic degeneration in neocortex and hippocampal complex similar to that observed primarily in AD. Within the hippocampus, not only were numerous PHF positive PB seen in the dentate granule cell layer, but also extensive neuritic PHF staining was found within the hilar region of the dentate gyrus. An occasional neurofibrillary tangle-like structure similar to that seen in AD was observed in the outer layers of the inferior temporal cortex. No senile plaques were identified with any of these cytoskeletal markers or A4 antibodies. The neuritic abnormalities described in this Pick's case suggests that neuritic degeneration primarily associated with AD occurs in a subpopulation of Pick's Disease. (Supported by AG09466 and AG10161.)

520.7

AUTOIMMUNITY TO GLUTAMATE DECARBOXYLASES GAD65 AND GAD67. D.L. Kaufman*, M. A. Atkinson, G. Ting, P. Robinson, J.D. Tian, D. Newman, M.G. Erlander, N. Kim, T. Phan, N. K. Maclaren, A.J. Tobin and M. Clare-Salzler. University of California, Los Angeles and University of Florida, Gainesville.

Immune responses to the GABA synthetic enzyme glutamate decarboxylase (GAD) may play an important role in the development of insulin-dependent diabetes mellitus (IDDM), Stiff-man syndrome, and IDDM-associated neuropathy. Years before the clinical onset of IDDM, we can detect autoantibodies to one or both forms of GAD (GAD65 and GAD67), synthesized from their respective cDNAs in recombinant expression systems. Although autoantibodies to GAD generally decline after IDDM onset (with the loss of the antigen containing β-cells), IDDM patients who develop acute neuropathies have high levels of GAD antibodies. These autoantibodies may reflect the continued stimulation of the immune system by GAD released from damaged peripheral nerves.

To study the role of GAD in the pathogenesis of IDDM, we have examined the ontogeny of T-cell responses to known IDDM autoantigens in a murine model of IDDM, the NOD mouse. Of the autoantigens tested (GAD, HSP65, CPH and insulin), the T-cell response to GAD is the earliest, suggesting that GAD may be a primary autoantigen in IDDM. (supported by grants from NIH, JDF and the ADA)

520.8

CHANGES IN BRAIN LEVELS OF CARNITINES IN THE DIABETIC RAT: EFFECT OF ACETYL-L-CARNITINE. F. Maccari, P. Chiodi, M.T. Ramacci, M. Calvani*, L. Angelucci[1]. Sigma-Tau, Institute for Research on Senescence and Dept. of Neurol. Res., Pomezia, Rome; [1]Institute of Pharmacology II, La Sapienza Univ. of Rome, Italy.

Many biochemical and functional alterations have been observed in a number of diabetic rat organs, such as, among others, the variations in the content of carnitines related to the metabolism of glucose alternative substrates. However, the brain as the only one organ that maintains a normal glucose metabolism in diabetes seems to contradict this relation: in fact, in diabetic rats brain carnitine levels are dramatically reduced (-32%). Recently, acetyl-L-carnitine (ALCAR) has shown to be beneficial in modifying biochemical, electrophysiological and motor alterations in the diabetic rat. Thence, an experiment was set on to verify whether ALCAR was able to compensate for the loss of brain carnitines. Male Sprague-Dawley rats, weighing 300 g, either normal or diabetic (diabetes was induced with 50mg/kg i.v. streptozotocine) were used. Diabetic rats were treated daily for 14 weeks with i.p. 10 ml/kg vehicle or 50mg/10ml/kg ALCAR. At the end of treatment, 24 h after the last administration and 48 h of fasting, carnitine levels were determined. In diabetic rats, statistically significant decreases in the brain levels of free carnitine (-25%), acetylcarnitine (-26%) and long-chain acylcarnitines (-25%) were found. Treatment with ALCAR totally impeded the loss of brain carnitines due to diabetes. Besides confirming the loss of brain carnitines in diabetes, these results indicate that such a loss occurs in all three main classes of carnitines. They also indicate that ALCAR corrects both qualitatively and quantitatively the reduction in brain carnitines and are in accordance with the beneficial effects exerted by ALCAR in diabetes-induced neuropathies.

520.9

DECREASED NA PUMP ALPHA-1 ISOFORM IN SCIATIC NERVE OF SPONTANEOUSLY DIABETIC BB/WOR RATS. S.C. Specht* and Rosa Figueroa-Nieves. Institute of Neurobiology and Department of Pharmacology, Univ. P. R. Sch. Med., San Juan, P.R. 00901.

Sciatic nerves of diabetic and control female BB/Wor rats were analyzed by immunoprecipitation to determine their content of the α1 and α2 isoforms of the catalytic subunit of Na,K-ATPase. Immunobeads were prepared with the McK1 and McB2 antibodies developed by Sweadner and associates (JBC **264**:8271, 1989) and employed according to the protocol of Fambrough and Bayne (JBC **258**: 3923, 1983) with modifications. The eluted antigens were separated by SDS-PAGE, silver-stained, scanned and quantified with the 2D-ANALST program. Only α1 was found in detectable quantities. The average amount in control nerves was 1.6 pmol per mg protein (microsomal pellet) vs 1.12 in nerves of age-matched rats after 16 weeks of insulin-dependent diabetes, corresponding to a 30% reduction. The generally higher levels of Na pump in axons as compared to glia, suggests a neuronal deficit. [Supported by JDF grant 190919 and NIGMS grant SS RR 08224].

520.10

SHORT AND LONG TERM EFFECTS OF STREPTOZOTOCIN-INDUCED DIABETES ON THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS OF THE RAT. S.T. Dheen, S.S.W. Tay* and W.C. Wong. Department of Anatomy, National University of Singapore, Singapore 0511.

Streptozotocin-induced diabetic and age-matched saline-injected control rats were perfused at 3 and 7 days, 1,3,6,9 and 12 months post-induction. At 3-7 days, degenerating axon terminals showed a vacuolated electron lucent cytoplasm and clustering of small spherical agranular vesicles. The affected dendrites were characterised by a vacuolated electron lucent cytoplasm containing swollen mitochondria and disoriented neurofilaments. At 1-6 months, degenerating dendrites were hypertrophied and contained numerous vacuoles, tubulovesicular elements and unidentifiable electron dense residua. These degenerating dendrites were postsynaptic to either normal or affected axon terminals. Some myelinated axons showed vacuolation and displacement of their axoplasm. Degenerating somata contained a condensed nucleus and numerous vacuoles of various sizes, probably formed from dilated rER. At 9-12 months, degenerating dendrites showed an electron dense cytoplasm containing swollen mitochondria and dilated rER. Degenerating axon terminals were characterised by an electron dense or lucent cytoplasm containing swollen mitochondria and small spherical agranular vesicles. These degenerating axon terminals were presynaptic to either normal or affected dendrites. A few myelinated axons also showed electron dense degeneration. However, the somata appeared to be normal. We suggest that various metabolic disorders in the experimentally-induced diabetic rats may have contributed to these structural changes which may affect the synthesis of neurohormones in the paraventricular nucleus of the rat.

520.11

TREATMENT OF EXPERIMENTAL DIABETIC NEUROPATHY WITH ACETYL-l-CARNITINE. A.M.Di Giulio*, B.Tenconi, A.Bertelli, P.Mantegazza, +S.De Biasi, M.T.Ramacci and A.Gorio. Dept. of Medical Pharmacol., +Dept. of Physiol. and Biochem., Univ. of Milano; *Inst. for Res. on Senescence, Pomezia, Italy.

We have previously shown that acetyl-l-carnitine (ALCAR) treatment prevents the decline of nerve conduction velocity as well as the establishment of autonomic neuropathy in diabetic rats. We now report that in the sciatic nerve of alloxan treated rats a 50% reduction of substance P (SP) accumulation occurs at a ligature point, indicating a marked impairment of both anterograde and retrograde peptide transport. In the same animals we have also examined at the electron microscope the basal lamina of blood vessels and axons of the sciatic nerve. In diabetic animals the basal lamina is thicker than normal and made up of multiple layers. In ALCAR treated diabetic rats the basal lamina is normal and the SP axonal transport is at control values as well. These data further support the potential value of ALCAR treatment in diabetic neuropathy.

520.12

THREE-DIMENSIONAL MODELS DEMONSTRATE THE CHANGES IN CRF- AND VP-IMMUNOSTAINED NEURONS IN 139H-INFECTED HAMSTERS. X. Ye, R.I. Carp, Y. Yu, R. Kozielski, P. Kozlowski. NYS Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, New York, 10314.

Scrapie is a neurodegenerative disease of sheep and goats. Previous studies have shown that the 139H-infected hamsters causes marked lesions in the cells of the islets of Langerhans, and pituitaries. In the current studies, we used three-dimensional modeling to demonstrate the changes of hypothalamic immunostained corticotropin-releasing factor(ir-CRF) and vasopressin(ir-VP) neurons in 139H-infected hamsters. By using the immunostained technique, we observed the following: 1) A significantly higher number of ir-CRF neurons in hypothalamus in 139H-infected hamsters than in control hamsters. 2) A significantly lower number of ir-VP neurons in the lateral hypothalamus in 139H-infected hamsters than in control hamsters. 3) No significant difference between the number of ir-VP neurons in the dorsalmedial hypothalamus(DMH) or the supraoptic nuclei in 139H-infected hamsters and the number in control hamsters. However, the population of ir-VP neurons in DMH shifted to the anterior hypothalamus in 139H-infected hamsters.

520.13

AMYLOID-LIKE FIBRILS FORMED IN VITRO FROM PRION PROTEIN SEGMENTS. F Tagliavini F Prelli, L Verga, G Giaccone, M Salmona, F Passerini, T Wisniewski, B Ghetti, O Bugiani, B Frangione* Ist Neurol Besta, Milan, NYU Med Ctr, New York, Ist Farmacol Negri, Milan, Indiana U Sch Med, Indianapolis

Brain amyloid deposition is a pathologic hallmark of Gerstmann-Sträussler-Scheinker disease (GSS). The major component of amyloid fibrils isolated from patients of the Indiana family with GSS is an 11 kDa fragment of PrP spanning residues 58 to ~150. This family carries a missense mutation of PRNP gene, causing Ser for Phe substitution at codon 198. We studied fibrillogenesis *in vitro* using synthetic peptides homologous to residues 57-64, 106-126, 127-147, 181-205 wild-type and 181-205 with mutant Ser 198. Peptide 57-64, corresponding to the repetitive octopeptide of the PrP molecule, did not produce fibrils. Peptides 106-126 and 127-147 readily formed 8 nm fibrils; the latter also aggregated into paired helical-like filaments. Peptide 181-205 showed only a moderate tendency to produce fibrils, which was not influenced by the 198 mutation. These findings suggest that fragment 106-147 plays a major role in fibrillogenesis, whereas octopeptide repeats in the 11 kDa amyloid protein do not. Finding low fibrillogenic properties of peptide 181-205 agrees with the absence of this PrP segment in amyloid fractions extracted from patients of the Indiana kindred with GSS. Thus it appears that PrP codon 198 mutation does not directly influence amyloid fibril formation.

520.14

WITHDRAWN

520.15

NORMAL NUMBER OF LOCUS COERULEUS CELLS IN FRONTAL LOBE DEMENTIA. K.F. Manaye*, D.D. McIntire, W.K. Smith, D.M.A. Mann, K. Woodward and D.C. German. Dept. of Psychiatry and Academic Computing Service, UT Southwestern Med. Cntr., Dallas, TX 75235, and Dept. of Pathology, Univ. of Manchester, UK.

In several neurodegenerative diseases, which have an accompanying dementia, including Alzheimer's disease (AD), Down's syndrome (DS), Pick's disease and Parkinson's disease (PD), there is a significant loss of locus coeruleus (LC) noradrenergic neurons. In AD, DS and PD we have shown disease-specific patterns of LC cell loss across the rostral-caudal extent of the nucleus. The present study sought to determine whether there are changes in the distribution of LC cells across the rostral-caudal extent of the nucleus in cases of frontal lobe dementia (FLD). Six FLD cases (mean age - 65 years; disease duration 1-17 years), were compared with seven normal controls (mean age - 67 years). The normal LC spans a rostral-caudal distance of 11-14 mm, and contains 16,773 ± 1,133 cells (mean ± SEM) on one side of the brain. There was no significant difference in the length or number of LC cells in the FLD cases. These data indicate that FLD is like multi-infarct dementia in that there is no degeneration of the LC neurons. Supported by AG-08013.

520.16

EARLY PATHOLOGICAL LESIONS IN THE PERIAQUEDUCTAL GRAY IN CHRONIC RECURRENT EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS P.O.Gerrits[1]*, E.J.'s-Gravenmade[2], D.H.Croon[1], and G.Holstege[1]. Dept. Anatomy[1], and Neurology[2], University of Groningen, The Netherlands.

Multiple Sclerosis (MS) is characterized by demyelination and plaque formation in the brain and spinal cord. Early stages of MS are often associated with changes of emotional behaviour and visuomotor complaints. The area of the mesencephalic periaqueductal gray (PAG) seems to play an important role in mediating responses to emotional stimuli. The question arises whether histopathological changes in the PAG play a role in the development of MS. Chronic recurrent experimental allergic encephalomyelitis (Cr-EAE) represents a well established animal model for MS. To determine the occurrence and the development of histo-pathological changes in the PAG, Cr-EAE-rats were investigated.

Using a new GMA-resin embedding technique for high resolution light microscopy, a cytoarchitectonic map of the normal rat PAG was made, which precisely defines myelin division patterns. Histopathological changes within these myelinated areas were studied histochemically using Sudan Black B, Cresyl Fast Violet and PAS and precisely mapped. Results clearly show that in Cr-EAE in rats, early histopathological changes such as inflammation, demyelination and gliosis are abundantly present within these areas. Pathology occurs predominantly around the small and larger blood vessels in the ventro- and ventrolateral areas of the caudal, and to a lesser extent of the rostral PAG.

A preliminary studie in human MS material revealed complete demyelination of the PAG, medial longitudinal fasciculus and parts of the tegmentum adjacent to the PAG, indicating that the PAG may be associated with changes of emotional behaviour.

520.17

BEHAVIORAL DEFICITS OBSERVED IN THE VARIOUS STAGES OF THIOACETAMIDE INDUCED HEPATIC ENCEPHALOPATHY IN MALE RATS. N.S. Norton[1]*, J.R. McConnell[2], and J.F. Rodriguez-Sierra[1]. Depts. of Cell Biology and Anatomy[1] and Radiology[2], University of Nebraska Medical Center, Omaha, NE 68198.

Hepatic Encephalopathy (HE) can be described as a collection of neuropsychiatric disturbances that occur following significant liver dysfunction in patients. HE is clearly associated with progressive complications in motor function such as rigidity, ataxia, and in the terminal phases, coma, eventually leading to death. To further elucidate the various stages of acute HE in an animal model, we examined the effects of acute HE using a series of behavioral tests. Male Sprague-Dawley rats were habituated to test conditions for 5-7 days prior to any treatment. Rats were treated with thioacetamide (600 mg/kg) dissolved in normal saline or in a saline control series. The HE syndrome was classified into 4 stages based on behavioral alterations. To assess the progression of HE, the animals were monitored every hour until reaching the appropriate stage of HE upon which they were euthanized. Blood (1 ml) was removed for liver enzyme function tests. Our results demonstrate a decrease in behavioral responses of thioacetamide-treated rats as compared to saline treated controls. In stages I and II, the rats exhibit decreased behavioral abilities and a gradual increase in liver enzymes. Upon reaching stage III, the behavioral impairments and the elevated liver enzymes are greatly augmented. This difference continued until the animals reached coma during stage IV. Our results provide an objective behavioral model for the study of acute HE in animals. This model could provide a source for uncovering factors responsible for the behavioral deficits in HE (Supported in part by the Department of Radiology, UNMC).

520.18

MITOCHONDRIAL mRNAs ENCODING FOR CYTOCHROME OXIDASE SUBUNITS CHANGE IN RESPONSE TO DISRUPTION OF DOPAMINE. A. C. Grobin*, P. S. Dannies, and A. Y. Deutch. Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06508, and VA Medical Center, West Haven, CT 06516.

Recent data have indicated that there is a decrease in mitochondrial complex I activity in tissue from Parkinson's Disease (PD) patients. This appears to result from a change in mitochondrial DNA in PD. We have therefore examined changes in mitochondrial specific cytochrome oxidase (CO) subunit gene expression.

Administration of 3-acetylpyridine (3-AP) to rats results in a slowly progressive loss of the dopamine (DA) innervation of the dorsolateral striatal (CP_{dl}); the DA innervations of the medial striatum (CP_m) and nucleus accumbens (NAS) are spared. 3-AP rapidly and completely lesions the climbing fiber innervation of the cerebellum. Both CO I and II mRNAs increased in the CP_{dl} of 3-AP-treated rats. The changes in CO mRNAs progressively increased up to eight days after 3-AP treatment. The mitochondrial specific 16S ribosomal RNA also increased in the CP_{dl}. No changes in CO I or II mRNAs in the CP_m or NAS were observed. In contrast, 3-AP treatment resulted in a decrease in CO gene expression in the cerebellar vermis. Haloperidol treatment for 21 days did not alter CO I and II. The present data suggest that DA neurons undergoing degeneration exhibit a compensatory increase in CO gene expression, consistent with the increased physiological activity of these neurons, while neurons that have degenerated (cerebellar climbing fibers) exhibit decreased mitochondrial gene expression. Supported by the National Parkinson Foundation Center, MH-45124, and TG GM-07324.

ISCHEMIA: DRUG TREATMENT I

521.1

Effects of the 21-Aminosteroid Tirilazad Mesylate (U-74006F) on the Eicosanoid Levels in the Gerbil Brain Following Ischemia and Reperfusion. P.K. Andrus*, E.D. Hall, B.M. Taylor,[1] L.M. Sam[1] and F.F. Sun[1]. CNS Dis. Res. and Hypersens. Dis. Res.[1], The Upjohn Co., Kalamazoo, MI 49001.

The present study measured the early production of eicosanoids in the gerbil brain during early reperfusion, following either a 3 hr unilateral carotid occlusion (UCO - model of focal ischemia) or a 10 min bilateral carotid occlusion (BCO - model of global ischemia). Arachidonic acid (AA) metabolites were examined to determine if pretreatment with U-74006F could influence brain synthesis of eicosanoids. In the severe UCO ischemia model, there was an early (5 min) post-reperfusion elevation in brain levels of $PGF_{2\alpha}$, TxB_2 and LTC_4. In contrast, PGE_2 and 6-keto-$PGF_{1\alpha}$ were decreased. Pretreatment with known neuroprotective doses of U-74006F did not affect the increase in $PGF_{2\alpha}$ or TxB_2, but blunted the rise in LTC_4. The decreases in PGE_2 and 6-keto-$PGF_{1\alpha}$ were also attenuated. In the less severe BCO model, there was a post-ischemic increase in all of the measured eicosanoids. U-74006F decreased the rise in TxB_2 and LTC_4, but did not affect the other eicosanoids. The results of these studies suggest U-74006F can selectively decrease TxB_2 levels in the less severe model and it selectively inhibits leukotriene synthesis in both models. The inhibition of leukotriene formation is believed to be a manifestation of the lipid antioxidant properties of U-74006F, as lipid peroxides are potent activators of 5-lipoxygenase.

521.2

LAZAROID PROTECTION AGAINST NEUROTOXICITY IN CEREBELLAR GRANULE CELL MODELS. G.J. Fici, J.S. Althaus, D.E. Decker, S.E. Buxser and P.F. VonVoigtlander.* The Upjohn Company, Kalamazoo, MI 49001.

Novel *in vitro* methods for testing lipid peroxidation inhibitors (lazaroids) as protectants of neurotoxic insults in cerebellar granule cells were developed. We showed that these cells are susceptible to injury induced by buthionine sulfoximine (BSO), an inhibitor of γ-glutamylcystein synthetase, and ferrous ammonium sulfate (FAS), an initiator of lipid peroxidation. This study revealed that 30-1000 μM BSO after 24 hrs produced significant reduction (~15-60% of control) in cell viability, which corresponded to a decrease in glutathione (GSH) levels of 80% or greater. A FAS toxicity model was established as well and it produced 95-98% cell death relative to control.

The 21-aminosteroids, U-74006 and U-74500E, that were delivered to cells in a microemulsion demonstrated differing effects. U-74006 (0.1-100 μM) was unable to protect in either toxicity model. U-74500E (10 μM) significantly protected across the entire toxic range of BSO. In addition, it was completely protective in the FAS toxicity model at 1 μM. At this concentration, amount of actual drug delivered to cells was ~0.5 mole%. Measurements of GSH in the BSO-treated cells showed no sparing or protection of GSH with drug and GSH declined 35%-90% across the BSO dose range of 1-1000 μM. The ability of U-74500E to protect in these models may be a function of greater antioxidant activity (measured by cyclic voltammetry). It is hoped that these cell models of neurotoxicity will further reveal mechanisms of lazaroid mediated neuronal protection.

521.3

NBQX DOES NOT ALTER THE ISCHEMIA-INDUCED REDUCTION OF KAINATE/AMPA RECEPTOR GENE EXPRESSION IN RATS

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We reported (see Pellegrini et al, these proceedings) that transient forebrain ischemia in rats selectively reduces expression of the GluR2 subunit that controls Ca^{++} permeability in CA1 hippocampal neurons. This reduction of GluR2 message may be causally related to Ca^{++} mediated injury to CA1 neurons. NBQX, a selective AMPA receptor antagonist, protects CA1 neurons against ischemia. In this study we examined whether NBQX altered the ischemia-induced changes in kainate/AMPA receptor message. Sham-operated rats and rats subjected to 10 min of forebrain ischemia were treated immediately after reperfusion with either vehicle, NBQX (30 mg/kg i.p. x 3), or MK-801 (2.5 mg/kg i.p.). Twenty-four hrs later glutamate receptor gene expression in CA1 hippocampus was examined by in situ hybridization. The results confirmed that GluR2 message in CA1, but not in CA3 or dentate gyrus, was markedly reduced. Message for GluR1 and NMDAR1 were little affected. However, neither NBQX nor MK-801 altered the ischemia-induced changes in glutamate receptor expression. The neuroprotective response of NBQX may be due to blockade of kainate/AMPA receptors, rather than blockade of the mechanism causing the downregulation of GluR2 expression.

521.4

GUANIDINOETHANE SULFATE IS NEUROPROTECTIVE TOWARD DELAYED CA1 NEURONAL DEATH IN THE GERBIL MODEL OF FOREBRAIN ISCHEMIA. H. Igarashi, I. L. Kwee, and T. Nakada*. Neurochem Res Lab, VA Res Service, Martinez, CA 94553 and Dept of Neurology, Univ of Calif, Davis, CA 95616.

Guanidinoethane sulfate (GES) is a taurine analogue originally introduced as a competitive transport inhibitor of taurine. Recent studies indicated that GES in brain cytosol may function as additional alkali which in turn protects brain pH against intracellular lactic acidosis. Indeed, GES has been shown to enhance the survival rate of mice exposed to anoxia. In this study, we investigated the protective effects of GES on delayed CA1 neuronal death using a gerbil model of forebrain ischemia. Pretreatment with GES (675 mg/kg/day IP for 2 weeks) showed significant neuroprotection on CA1 neurons studied 7 days after 5 minutes bilateral carotid occlusion. The result indicates that intracellular acidosis likely plays a role in mediating the harmful effects of ischemia, perhaps in the early steps of a deleterious cascade leading to excitatory amino-acid excess.

521.5

VOLTAGE SENSITIVE AND RECEPTOR OPERATED CALCIUM CHANNEL DENSITIES IN FOCAL CEREBRAL ISCHEMIA. M.J.Hogan, S.Takizawa, A.Gjedde*, A.M.Hakim. Cerebrovascular Research Unit, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4

We have measured the number of L-type voltage sensitive calcium channels (VSCC) and *N*-methyl-D-aspartate (NMDA) receptors in acutely ischemic rat brain using in-vitro binding of the L-type VSCC antagonist [^{3}H]nimodipine and the competitive NMDA receptor antagonist [^{3}H]CGS-19755. Focal cerebral ischemia was produced in male Sprague Dawley rats under halothane anesthesia. After four hours of ischemia the rats were decapitated, the brains removed, frozen and sectioned at 20 μm onto glass slides. These were then dipped in incubation baths containing either [^{3}H]nimodipine or [^{3}H]CGS-19755. Washed and dried sections were then exposed to tritium sensitive photographic film and regional ligand binding determined autoradiographically. The maximal number of binding sites (B_{MAX}) for nimodipine in ischemic and contralateral non-ischemic frontal cortex were 16.3 ± 1.6 and 24.4 ± 1.1 pmol/g respectively. For the CGS-19755 these values were 217 ± 5 and 228 ± 14 pmol/g. In-vitro [^{3}H]nimodipine binding is comparable to in-vivo binding in ischemic tissue only and does not demonstrate focal VSCC activation. This can only be demonstrated in-vivo. Similarly, focal increases in NMDA receptors are not observed in-vitro after 4 hours of ischemia. Since NMDA receptor sites are 10 fold more common than VSCC's they may be more important to calcium influx during ischemia.

521.6

LACK OF PROTECTION BY THE ALPHA-2 ADRENORECEPTOR AGONIST DEXMEDETOMIDINE IN A FOCAL MODEL OF CEREBRAL ISCHEMIA C. M. Maier, D. M. Kunis*, G.H. Sun, T. Z. Guo, M. Maze, and G. K. Steinberg. Depts. of Neurosurgery and Anesthesia, Stanford University Medical Center, Stanford, CA 94305.

Dexmedetomidine, a highly selective alpha-2 adrenoreceptor agonist, decreases central sympathetic activity and reduces the anesthetic requirement of halothane. Preliminary studies show that dexmedetomidine improves the outcome from ischemic injury and thus may have potential therapeutic value. We studied eleven rabbits that underwent two hour occlusion of the left internal carotid, anterior cerebral, and middle cerebral arteries followed by four hours of reperfusion. Ten minutes after occlusion the animals were treated with either normal saline (n=5) or dexmedetomidine (n=6) using a computer controlled infusion rate set to maintain a steady state plasma concentration. Halothane concentration was reduced by 50% for drug treated rabbits in order to maintain a comparable level of anesthesia. Somatosensory evoked potentials were used to confirm adequate ischemia and injury was assesed with histopathology. There were no significant differences in the area of ischemic neuronal damage between the groups in either cortex (control 45.8 ± 8.8 % vs drug 41.4 ± 11.5 %) or striatum (control 72.1 ± 18.4 % vs drug 57.5 ± 18.6 %), or in physiological parameters. Drug plasma levels obtained every 90 minutes showed a mean of 4.0 ± .15 ng/ml. Our study demonstrates that dexmedetomidine, in the dose given, does not have a neuroprotective effect in this model of focal cerebral ischemia.

521.7

DELAYED TREATMENT WITH A SELECTIVE NEURONAL CALCIUM CHANNEL ANTAGONIST PROTECTS AGAINST NEURONAL DEATH AFTER GLOBAL CEREBRAL ISCHEMIA. K. Valentino*, S. Bowersox, M.L. Smith, B.K. Siesjo, T. Singh, T. Gadbois, A. Justice, J. Ramachandran and B.B. Hoffman. NEUREX Corporation, Menlo Park, CA, USA and University of Lund, Lund, Sweden

We previously reported that the synthetic omega-conopeptide SNX-111, a blocker of calcium flux through voltage-sensitive neuronal calcium channels, protected hippocampal CA1 pyramidal cells from ischemic damage in the rat 4-vessel occlusion model of reversible forebrain ischemia when administered by IV bolus injection either immediately or 1 hour post-occlusion [1]. We now report that (1) 5mg/kg SNX-111 infused IV over 15 minutes at 6 hours post-occlusion significantly decreases neocortical damage ($p<0.008$) in rats subjected to either 10 or 15 minutes of forebrain ischemia produced by common carotid occlusion (2-VO) plus hypotension, and (2) neuroprotection is provided when a single IV bolus injection of 5mg/kg SNX-111 is administered up to 24 hours after the ischemic insult in 4-VO (CA1 damage relative to untreated controls: 6 hr = 19%, 12 hr = 26%, 24 hr = 19%). Animals receiving SNX-111 under the latter conditions show comparable levels of neuroprotection when sacrificed at 5 or 12 days post-treatment, indicating that SNX-111 prevents, and not simply delays, cell loss after the ischemic insult. These findings (1) provide the first demonstration of neuroprotection in the rat 4-VO model by delayed treatment with a neuronal calcium channel blocker and (2) confirm that SNX-111 is neuroprotective in two different rat models of global forebrain ischemia.

[1] Valentino, K., et al. *Soc Neurosci. Abstr.* 17, 1079 (1991).

521.8

EFFECTS OF DISTINCT ω-CONOPEPTIDES ON THE RELEASE OF GLUTAMATE AND GABA IN-VIVO. R. Newcomb* and A. Palma. NEUREX Corporation, Menlo Park, CA 94025.

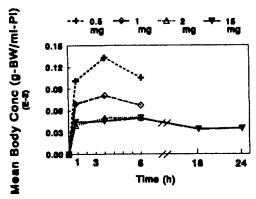
Two synthetic ω-conopeptides, SNX-111 and SNX-183, originally isolated from the cone snails, *C. magus* and *C. striatus*, respectively, have revealed a diversity of voltage sensitive calcium channels (Miljanich, et al., Soc. Neurosci. Abstr., 17, 1161, 1991). We used in-vivo dialysis to define the actions of these conopeptides on the release of amino acid transmitters in the dorsal hippocampus of the rat. The release evoked by potassium perfusion was suitable for quantitative comparison across experiments, and release of glu and GABA could be blocked with 10mM magnesium. SNX-183 and the structurally similar peptide SNX-230 (M-VIIC from *C. magus*; see abstracts by Hillyard, et al. and Gaur, et al.) blocked the release of glu and GABA at much lower concentrations than those required for SNX-111: The probe concentration of SNX-230 giving 50% of maximal inhibition was 0.2 uM, as compared to 200uM for SNX-111. Dose response curves for glu and GABA were similar in the hippocampus, while GABA release in the thalamus was comparatively resistant to the conopeptides. Chromatographic analysis showed that differences in potency were not due to differential degradation.

521.9

EARLY AND CONSTANT RELEASE OF NIMODIPINE FROM IMPLANTED PELLETS IN CONSCIOUS RATS FOR 24 HOURS A.D. Perez-Trepichio* and S.C. Jones Cerebrovascular Research Laboratory, Cleveland Clinic Foundation, Cleveland OH 44195

Experimental and clinical studies have shown potential benefit of nimodipine (NP) in cerebral ischemia. Therefore, it is important to evaluate methodologies for continuous administration of NP that may reduce the complexity of experimental therapy.

Sprague-Dawley rats were briefly (5 min) anesthetized during pellet implantation and assigned into two groups: Pellet (PE) and Placebo (PL). PE rats (n=14) received either 0.5, 1, 2 or 15 mg pellets and PL rats (n = 5) received placebo. Pellets were implanted in the nape of the neck. Plasma levels of NP were measured at 1, 3, 6, 18, and 24 h.



The plasma levels obtained with the 15 mg pellets are known to have a therapeutic effect on brain ischemia and can be rapidly achieved within one hour and maintained for 24 h. As shown in the figure, NP levels resulting from 2 and 15 mg pellets did not achieve the same mean body concentration (plasma concentration normalized to body weight and administered dose) as did lower doses.

These results indicate that pellets may be suitable for further evaluation of NP in experimental cerebral ischemia. Also it is important to emphasize that there was not a significant drop in mean arterial pressure ($p > 0.05$). (Supported by AHA-NEO F244, National AHA 91013150 and Miles, Inc.)

521.10

EFFECTS OF BERAPROST Na (BPS), A PROSTACYCLIN ANALOGUE, ON PROGRESSIVE CEREBRAL ATROPHY IN GERBILS.

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We have already reported that BPS, a new prostacyclin derivative, has protective effects against various ischemic models. In the present study, we investigated the effects of BPS on progressive cerebral atrophy in gerbils. The unilateral carotid artery of gerbil was repeatedly occluded. At the first ischemic procedure, we selected the symptomatic animals according to their stroke index score (K.Ohno, Brain Res, 297: 151, 1984), its score greater than 10. The selected animals were administered BPS at a dose of 1 to 100 μg/kg, p.o. twice a day for 4 weeks. We measured area ratio of ischemic hemisphere to opposite one with image processing system (Olympus, Japan). In the cerebral cortex of ischemic hemisphere, neuronal loss, acidophilic neurons and progressive atrophy were observed with increasing time of reperfusion after ischemic insult. On 4 weeks after the first ischemic episode, area ratio was approximately 90% in the control animals. BPS was found to inhibit the atrophy of ischemic hemisphere dose-dependently. Our results suggest that prostacyclin has a protective effect on the ischemia induced progressive atrophy.

522.1

INHIBITION OF NITRIC OXIDE SYNTHASE DOES NOT PROTECT AGAINST ISCHAEMIA-INDUCED HIPPOCAMPAL DAMAGE. E.M. McGowan* and P.C. Emson. MRC Group, Dept. of Neurobiology., A.F.R.C., I.A.P.G.R. Babraham, Cambridge, U.K.

Activation of NMDA receptors results in a Ca^{++} dependent release of Nitric Oxide (NO). Thus a role for NO has been postulated in NMDA receptor mediated excitotoxicity. Sustained NMDA receptor activation after an ischaemic insult and the subsequent accumulation of intracellular Ca^{++} is thought to be responsible for the selective pattern of delayed neuronal death seen in the CA1 region of the gerbil hippocampus. In an attempt to attenuate hippocampal ischaemic damage gerbils were treated twice daily with Nω-nitro-L-arginine (NArg, 5 or 10 mg/kg, i.p.), a competitive inhibitor of NO synthase (NOS). NArg was administered for 4 days prior to a single 5 min. period of global forebrain ischaemia, induced by occlusion of both common carotid arteries. NArg was also given throughout the survival period. Four days after reperfusion animals were sacrificed and the extent of neuronal damage determined histologically. No protection against ischaemic damage was observed at either dose used. To verify the degree of enzyme inhibition caused by NArg, NOS activity was assayed in crude enzyme extracts of forebrain by measuring the conversion of (^{3}H)-L-Arginine to (^{3}H)-citrulline. At the doses used NArg inhibited NOS activity by more than 85% when compared to saline treated animals. Contrary to *in vitro* studies on hippocampal slice preparations our data suggest that inhibition of NOS activity does not have a neuroprotective role in ischaemia.

522.2

BLOCKADE OF NITRIC OXIDE SYNTHETASE BY NITRO-ARGININE FAILED TO PROTECT THE BRAIN AGAINST TRANSIENT FOCAL ISCHEMIA IN TWO RAT MCA MODELS. D. Xue*, Z.G. Huang, S.Z. Gertler and A.M. Buchan. Neuroscience Unit, Ottawa Civic Hospital, Ottawa, Canada, K1Y 4E9.

NMDA and AMPA antagonists have been shown to reduce cortical injury following focal ischemia. A positive link between nitric oxide (NO) production and glutamate receptor activation on cyclic GMP has been proposed through in vitro culture studies. The possible effectiveness of NO synthetase inhibition by nitro-arginine in treating ischemic brain injury associated with an over-stimulation of glutamate receptors has been suggested. We studied this by using two focal stroke models. Transient focal ischemia was achieved by temporary clamping of the right middle cerebral artery for 2 hrs followed by 22 hrs of recovery in both Wistar (n=14) and SHR (n=21). Treatment with either saline (1 mL) or nitro-arginine (2 or 10 mg/kg) was given IV 40 minutes prior to ischemia. Regional cerebral blood flow was monitored at the time of drug infusion, the onset of ischemia, arterial reperfusion, and sacrifice (24 hrs). The volume of cortical infarction was then quantimented with frozen-sectioned brain slices. One-way analysis of variance was used.

Groups (n=7)		Cortical Infarction (mm^3 ± SE)	Reduction (%)
Wistar:	Saline	198±25	
	Drug (10 mg/kg)	199±35	
SHR:	Saline	164±9	
	Drug (2 mg/kg)	151±20	8 NS
	Drug (10 mg/kg)	145±11	12 NS

Pre-treatment with nitro-arginine, unlike glutamate antagonists, failed to protect the brain against transient focal ischemia.

522.3

FAILURE TO PROTECT HIPPOCAMPAL CA1 NEURONS BY NITRIC OXIDASE SYNTHETASE. H. Li*, S.Z. Gertler and A.M. Buchan. Neuroscience Unit, Ottawa Civic Hospital, Ottawa, Canada, K1Y 4E9.

Nitric oxide, a free radical, has recently been postulated to play an important role in a number of biological processes. Its proposed role as a mediator of ischemia-induced neuronal cytotoxicity was studied in a 4-vessel occlusion (4-VO) rat model which induces selective neuronal death. We have previously shown that this process is linked to AMPA, but not NMDA, glutamate receptors.

Adult male Wistar rats were subjected to 10 min. of severe transient forebrain ischemia using a modified 4-VO model. Nitro-arginine (10 mg/kg), a potent nitric oxide synthetase inhibitor, was infused intravenously 1 hour prior to ischemia. Saline-injected animals served as controls. Criteria rats were sacrificed 7 days later. The number of damaged hippocampal CA1 neurons were counted and calculated as a percentage. A Mann-Whitney U test was employed.

	% of Hippocampal CA1 Injury
Group (n)	Mean ± SD
Saline (8)	77.6 ± 10.4
N-arginine(8)	78.4 ± 11.3 NS

In this experiment, inhibition of nitric oxide synthetase failed to change the outcome of hippocampal CA1 neurons from ischemic injury.

522.4

BRAIN NITRIC OXIDE PRODUCTION IN FOCAL ISCHEMIA. Rosario R. Trifiletti *,Abraham Kader and Robert A. Solomon. Departments of Neurology and Neurosurgery,The Neurological Institute of New York, Columbia University College of Physicians and Surgeons.New York, NY 10032.

Nitric oxide (NO) has recently been implicated as a key mediator of N-methyl-D-aspartate (NMDA) receptor-associated glutamate neurotoxicity in primary neuronal cell culture (V.Dawson et al., PNAS 88: 6368-71(1991)) NMDA antagonists are potently neuroprotective in several adult rat models of focal ischemia . We sought to obtain evidence for NO production in cerebral ischemia by examining brain levels of "markers" of NO production (NO_2^- and cGMP) following varying periods of focal ischemia. Adult Wistar rats were subjected to bilateral common carotid ligation followed by cauterization of the (R) middle cerebral artery (MCA). Such treatment reliably produces a (R) cerebral infarct with no histologic damage to the contralateral hemisphere.

We find a marked transient increase (ipsi- vs. contralateral) in brain nitrite of 194 ± 112 % (n=6), 136 ± 102 % (n=7) and 91 ± 32 % (n=4) after 5,10 and 20 min of ischemia, respectively, but -6 ± 8% (n=4) after 60 min of ischemia. Less marked, but more sustained, increases in cGMP are observed as early as 5 min of ischemia. On a rat by rat basis, increases in NO_2^- are closely correlated those in cGMP (r^2 = 0.957 at 20min of ischemia) and can be abolished by pre-treatment with the NO synthase inhibitor N^G-nitro-L-arginine.

We propose that there is a transient activation of NO synthase in focal ischemia which leads to an increase in NO production, activation of guanylate cylcase, and cGMP production. These data support the hypothesis that NO may be a key mediator of focal ischemic damage *in vivo*.

522.5

POST-TREATMENT WITH THE KAPPA OPIOID PD117302 ATTENUATES ISCHEMIC DAMAGE TO CA1 NEURONS. F. Tortella*, L. Robles, M. DeCoster, J. Petras, C. Wingfield and +J. Moreton. Walter Reed Army Inst. Res., Washington, DC 20307 and +U. of Maryland, Sch. of Pharmacy, Baltimore, MD 21201.

Treatment of cultured rat neurons with novel kappa opioids derived from the arylacetamide series, including PD117302 (PD) and CI-977, provides significant protection from glutamate-induced cell injury. Systemic treatment with these compounds has also been reported to provide neuroprotection from focal ischemia and to improve functional outcome following global cerebral ischemia in rats. We now report neuroprotection with PD to rats subjected to 15 minutes of severe forebrain ischemia (4-vessel occlusion). PD (0.2, 1 and 5 mg/kg, i.v.) was given at reperfusion and at 2, 4, and 6 h post occlusion. Animals were perfusion fixed 72 h later. Ischemic injury was quantified in the CA1 of the hippocampus. In saline-treated rats ischemia caused extensive and consistent loss of pyramidal CA1 neurons. PD produced significant protection of CA1 neurons which was dose-dependent and nearly complete at the 5 mg/kg dose. Thus, kappa opioids from this series may be therapeutically effective in the treatment of ischemic neuronal injury.

522.6

EFFECT OF CI-977 ON ISCHEMIC BRAIN DAMAGE AND CEREBRAL BLOOD FLOW AFTER MIDDLE CEREBRAL ARTERY (MCA) OCCLUSION IN THE RAT. K.B. Mackay, K. Kusumoto, D.I. Graham & J. McCulloch*, Wellcome Neuroscience Group, University of Glasgow, G61 1QH, U.K.

Selective kappa agonists (such as CI-977) are putatively beneficial in some animal models of cerebral ischemia. The mechanistic basis for such neuroprotective effects is unknown. We have investigated the effect of CI-977 on ischemic brain damage and cerebral blood flow (CBF) in a rat model of focal cerebral ischemia. The left MCA was permanently occluded under halothane anesthesia in male Sprague-Dawley rats. Four hr later, animals were sacrificed by FAM fixation for histological examination. Local CBF was determined 30 min post-occlusion using [^{14}C]-iodoantipyrine autoradiography. Pre-administration of CI-977 (0.3mg/kg, s.c.) significantly reduced the volume of ischemic brain damage in cerebral hemisphere (by 27%) and cerebral cortex (by 32%) when compared to controls. CI-977 had no significant effect on either local CBF in any of the 24 regions examined or on the cumulative CBF volume in the hemispheres ipsilateral and contralateral to the occluded MCA when compared to controls. These results indicate that the neuroprotective effects of CI-977 following permanent MCA occlusion in the rat cannot be attributed to improved blood flow to ischemic tissue.

522.7

U50488 REDUCES THE SEVERITY OF TISSUE DAMAGE IN A RABBIT MODEL OF FOCAL CEREBRAL ISCHEMIA.

M.A. Widmayer, J.L. Browning and D.S. Baskin*. Dept. of Neurosurgery, Baylor College of Medicine and Research Service, Houston VAMC, Houston, TX 77030.

Following successful investigation of kappa agonist treatment of focal cerebral ischemia in the cat, we have broadened our research to include a more readily available and less costly species. Fourteen male, NZ white rabbits underwent occlusion of the right middle cerebral, anterior cerebral, and internal carotid arteries, beginning at their trifurcation and extending 1mm distally along each vessel. Six hours later half of the rabbits received an injection of 0.5 mg/kg U50488 (U50) and s.c. osmotic pump infusion of 0.4 mg/hr U50, and half received saline (SAL). Rabbits were sacrificed on the eighth post-op day and their brains stained with TTC. Abnormally stained tissue was categorized into infarct (colorless) and ischemic (lightly stained). Generally, abnormally stained tissue comprised 45% of brain tissue regardless of treatment. However, compared to saline treated rabbits, rabbits treated with the kappa agonist U50 had smaller infarcts (mean ± sem = 35.3 ± 5.9% vs 18.1 ± 4.5%; $p < 0.05$) and larger areas of ischemic tissue (12.4 ± 3.8% vs 24.1 ± 3.7%; $p < 0.05$) than SAL treated rabbits.

Corroborating research in cats, the kappa selective compound U50 reduced the severity of tissue damage secondary to focal cerebral ischemia in the rabbit. This research lends further support for clinical use of kappa agonists in the treatment of stroke. Additionally, the three vessel occlusion model shows promise in a species less costly and more available than cats.

522.8

EFFECT OF OPIATE ANTAGONISTS ON HYPOXIA AND CEREBRAL ISCHEMIA.

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In human and animal, the opiates have been shown to have significant physiologic effects on respiratory system and to be implicated in hypoxia and cerebral ischemia.

To determine the role of opiate in these mechanisms, we have studied opiate antagonists : on hypoxia (tail flick method on rat), and on cerebral ischemia by unilateral carotid ligation in gerbil (Delbarre, G., Stroke, 19:126, 1988).

Naloxone (0.5 mg.kg^{-1}, Per Os) significantly antagonizes the increase of reaction time of tail flick test induced by hypoxia ($p < 0.001$). Naltrexone (2.5 mg.kg^{-1}, Per Os) and WIN 44.441, a kappa antagonist (5 mg.kg^{-1}, Per Os), significantly improve the Stroke Index in gerbil cerebral ischemia ($p < 0.05$).

	Percentage of protection
Tail Flick after hypoxia	
(Naloxone 0.5 mg.kg^{-1})	54.93 ***
Ischemia	
- WIN 44.441 (5 mg.kg^{-1})	51.68 *
- Naltrexone (2.5 mg.kg^{-1})	39.71 *

Unpaired Student t test versus control -p < 0.05 * -p < 0.001 ***

These results demonstrate that opiate antagonists might play a therapeutic role in hypoxia and cerebral ischemia.

522.9

THE SELECTIVE GLYCINE ANTAGONIST 7-CHLORO-THIO-KYNURENIC ACID ATTENUATES FOCAL ISCHEMIA INJURY IN RATS.

J. Chen, S.H. Graham, F. Moroni and R.P. Simon*. Department of Neurology, U. California San Francisco, CA 94121.

Ischemic brain injury is mediated, at least in part, by the release of glutamate and other endogenous excitatory amino acid neurotransmitters activating a receptor gated Ca channel. Occupation of the strychnine insensitive glycine potentiation site is required for opening of the NMDA receptor gated Ca channel. Blockade of glycine binding might therefore be expected to attenuate ischemic neuronal injury in the brain.

We examined the effect of the selective glycine antagonist 7-chloro-thio-kynurenic acid (20mg/kg iv) given either 5min prior to or 15min or 60min after permanent middle cerebral artery occlusion in rats (n=6 in each drug treated group with n=9 saline controls). Stroke size was measured by computerized image analysis on serial 20μm cresyl violet stained sections 24 hrs following MCA occlusion. Infarct volume was smaller ($p<0.05$) in the 5 min pretreatment (114.2±13.5mm3) and 15 min posttreatment groups (148.1±21.2 mm3), but not in the 60 min posttreatment group (195.9±15.2 mm3) compared to controls (215.6±10.2 mm3). 5-chloro-thio-kynurenic acid (20mg/kg 5 min pretreatment), which does not bind to the glycine site but has similar antioxidant effects as 7-chloro-thio-kynurenic acid, was not effective (183.0±16.2 mm3).

These data support a potential role for glycine site antagonists in the treatment of stroke.

522.10

THIOKYNURENATES ANTAGONIZE L-GLUTAMATE-INDUCED NEURONAL DEATH BY ACTING AS GLYCINE ANTAGONISTS AND AS RADICAL SCAVENGERS.

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Previous experiments have shown that a series of thioderivatives of kynurenic acid (KYNA) displaced ^{3}H-glycine (GLY) from its strychnine insensitive binding sites in cortical membranes (Eur. J. Pharmacol.199; 227, 1991) and antagonized L-GLU-induced neuronal death in cerebellar granule cells in culture (Eur. J. Pharmacol. in press). Among the thio-KYNA derivatives the most potent were 7-Cl-thioKYNA (its IC50 in displacing ^{3}H-GLY was 0.4 μM) and 5,7-diClthioKYNA (its IC50 was 0.6 μM). Other compounds of the series were less potent and none of the tested molecules (up to 100 μM) displaced ^{3}H-CPP, ^{3}H-KA or ^{3}H-AMPA from the respective recognition sites in cortical membranes, suggesting that these kynurenate derivatives were selective GLY antagonists. When 7-ClthioKYNA or 5,7-diClthioKYNA were compared with the corresponding oxoderivatives it was observed that they had similar potency in displacing ^{3}H-GLY from cortical membranes, but were 5 to 10 times more potent in preventing L-GLU-induced neuronal death. In an attempt to explain this particular effectiveness against L-GLU-toxicity we studied 7Cl-KYNA, 5,7diClKYNA and the corresponding oxoKYNA derivatives as lipid peroxidation inhibitors in a simple "in vitro" model (Neurochem. Res. 16, 43, 1991). 7-ClthioKYNA and 5,7-diClthioKYNA (1-50 uM)inhibited in a dose dependent manner the formation of thiobarbituric acid reacting substances in brain homogenates and their potency was significantly higher than that of vitamin E. Thus 7-ClthioKYNA and 5,7diClthioKYNA may reduce excitotoxic neuronal death by acting both as GLY antagonists and as radical "scavengers".

522.11

GM1 TREATMENT PROTECTS MEMBRANE INTEGRITY AND REDUCES LDH RELEASE FROM NEURONS EXPOSED TO GLUTAMATE; GM1 MEMBRANE SURFACE DISTRIBUTION IS MAINTAINED.

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As an *in vitro* model of ischemic damage, cortical neurons were challenged with glutamate (0.5 and 10mM), and the levels of released LDH [*lactate dehydrogenase*] were monitored at 1hr, 1,2, 4 and 7days. Analysis of neuronal plasma membrane disorganization (damage) was determined by the distribution of endogenous GM1 ganglioside using cholera toxin anti-toxin immunohistochemistry. The effects of GM1 ganglioside on LDH release due to membrane damage were also studied to further elucidate the mechanisms underlying this lipid's neuroprotective effects. Neuronal cortical cultures derived from 15 day fetuses and/or 0-1 day pups were subjected to glutamate (0.5 or 10mM) for 30min. Parallel cultures were treated with GM1: either a 90min pre- or I hr, 1, 2, 4,7 day post-treatment with 80uM of GM1. Exposure to 10mM glutamate caused over a 70-90% increase in LDH release at 1-48hrs. Pre-treatment with GM1 reduced the release to 50-60% while post treatment reduced the LDH release to 45%. The membrane structure changes observed by the GM1 immunohistochemistry paralleled the LDH release data. At 7 days only post-GM1 treated cultures showed significant structural integrity as evidenced by continuous staining of GM1 along the perykariya and processes. These data further support our hypothesis that GM1 treatment (both pre- and post) reduces plasma membrane damage.

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522.12

GENETIC MODEL FOR ISCHEMIA - FEMALE STROKE PRONE SPONTANEOUSLY HYPERTENSIVE RATS [SHRSP]: GM1 TREATMENT EFFECTS

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Stroke Prone Spontaneously Hypertensive Rats [SHRSP] may provide an advantageous model to study CNS ischemia. This genetic strain of rat develops pathological conditions that are associated with stroke. These include hypertension, arterial thickening, fibrinoid necrosis, infarcts, hemorrhage and hemorrhagic infarcts. The focus of this study was to detail mortality rates and occurrences of related stroke symptoms using the **female SHRSP**, and, to assess the neuroprotective effects of GM1 ganglioside treatment. Forty-six female SHRSP rats were randomly assigned to GM1 or saline treatment. Blood pressure was measured weekly beginning at 7wks. At 7.5 weeks all rats were fed Japanese Stroke-Prone Rodent Diet (Zeigler Bros., Gardner, PA) and their drinking water was replaced with 1% NaCl. Chronic GM1 treatment (10mg/kg i.m. daily: FIDIA Research Labs) began at 8.5wks. Spontaneous activity, sensorimotor and neurological performance on a tail-hang task was assessed 3 times weekly beginning at 10wks. At this time the rats were observed daily for *overt* stroke symptoms (>10% weight loss, limb paralysis, stereotypic movement). The female SHRSP rats exhibited 100% mortality within 92 days of being placed on the diet. GM1 reduced hyperactivity observed 1-2wks prior to the onset of overt stroke symptoms (supra) in control (saline) rats. There was no marked effect on functional measures, weight loss and increased blood pressure. GM1 did not alter the underlying pathologies, but did ameliorate the initial indications of CNS damage. The use and inclusion of female SHRSP in ischemia research may be helpful to better detail the pathology and treatment of stroke in patients with similar underlying pathologies.

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522.13

GANGLIOSIDE TREATMENT FAILS TO ATTENUATE COGNITIVE DEFICIT FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA. R.L. Port*, P.W. Parsons, J.L. Briggs, R.N. Gandee and K.S. Seybold. Department of Psychology and Gerontology Program, Slippery Rock University, Slippery Rock, PA 16057 and Grove City College, Grove City, PA 16127.

Systemic administration of gangliosides has been shown to have significant beneficial effects on the central nervous system following various forms of insult. In the present study, adult male rats underwent bilateral carotid occlusion for ten minutes while anesthetized with sodium pentobarbital. Animals were treated at 10 min after occlusion and again at 24 hrs post-surgery with either GM_1 gangliosides (20 mg/kg i.p.) or vehicle-alone (control). A third group of nonoperated controls was included in the behavioral analyses. Animals were permitted two weeks to recover and trained daily in an eight-arm radial maze for four weeks. Analysis of behavioral data revealed that ganglioside- and vehicle-treated occluded animals made significantly more reference errors over the last week of training than did unoperated control animals. Differences in working memory errors were not statistically significant.

522.14

DELAY OF ANOXIC DEPOLARIZATION BY CREATINE, SPHINGOSINE DERIVATIVES, OR MANNITOL. M. Balestrino*[1], I. Cogliolo[1], G. Lunardi[1], A. Leon[2], S. Mazzari[2]. [1]Dept. of Neurology, University of Genoa, Italy, and [2]Fidia Research Labs, Abano Terme, Italy.

Delay of anoxic depolarization (AD) protects neurons from subsequent death (Stroke 21-SIII, 179-183, 1990). In the region of CA1 of rat hippocampal slices the following AD latencies were found (seconds, means±SD): control, 51±9 (N=9); creatine (25mM), 152±40 (N=4, $p<0.01$); Liga4 or PKS3 (sphingosine derivatives from Fidia; 5uM), 67±15 (N=7) and 85±27 (N=5), respectively ($p<0.03$ and $p<0.01$). Additional experiments with a "cross-over" design using mannitol (100mM) increased AD latency (25±9%), compared to paired controls (N=3, $p<0.05$). The effect of creatine (which delays ATP depletion during hypoxia: J. Physiol. 325: 51-65, 1982) supports the hypothesis (Soc. Neurosci. Abstr. 16: 277, 1990) that block of (Na^+, K^+)ATPase is the determining factor in AD. The efficacy of Liga4, PKS3 and mannitol suggests a role for both protein kinase C translocation and cell swelling in the delay of AD, and, possibly, in neuronal protection.

522.15

MK801 FAILED TO REDUCE INFARCT SIZE IN THROMBOTIC MIDDLE CEREBRAL ARTERY OCCLUSION IN RATS. H.Yao, M.D.Ginsberg*, B.D.Watson, R.Prado, W.D.Dietrich, S.Kraydieh and R.Busto. Cerebral Vascular Disease Research Center, University of Miami School of Medicine, Miami, FL 33101.

In animal models of focal cerebral ischemia, almost all studies have demonstrated significant reductions of infarct volume by the noncompetitive NMDA antagonist MK801.However, this drug failed to attenuate focal infarct in our present study.Male Sprague-Dawley rats were randomly assigned to receive either MK801 (1mg/kg) or saline ip 30 min before distal middle cerebral artery occlusion (dMCA-O).Irradiation with argon laser-activated 562nm dye laser after rose bengal (20mg/kg) iv was used to cause thrombotic dMCA-O. The ipsilateral common carotid artery was permanently occluded and contralateral carotid artery for one hour.In exp. 1, head temperature was controlled at 36°C.Three days after dMCA-O, brains were perfused and stained with H-E.Cortical infarct volume was 89±29 (mean±SD) mm3 in treated group, which was not different from 84±40 in control group.In exp. 2, head temperature was monitored but not controlled.Mean head temperature before ischemia was 34.4°C and decreased by 1-2°C after dMCA-O while rectal temperature was maintained at 37°C.Head temperature was not different between treated and untreated groups, thus excluding the confounding factor of temperature change.Further results of exp.2 will be presented.It is suggested from results of exp.1 that MK801 may be ineffective in head temperature controlled, thrombotic focal ischemia.

522.16

MK-801 PROTECTS IN A MODEL OF REPEATED EPISODES OF BRIEF NORMOTHERMIC FOREBRAIN ISCHEMIA IN RATS. B. Lin, W.D. Dietrich, S. Kraydieh, R. Busto, M.Y.-T. Globus and M.D. Ginsberg. Cerebral Vascular Disease Research Center, Dept. Neurology, Univ. of Miami School of Medicine, Miami, Florida, 33101.

We determined whether the non-competitive NMDA receptor antagonist, MK-801 (2mg/kg), would protect the brain if treatment was initiated after the first of three ischemic insults. Anesthetized rats were subjected to three 5-min periods of global forebrain ischemia by bilateral carotid occlusion plus hypotension (50 mmHg), separated by 1-hr periods. Ischemic brain temperature was maintained at 37°C. MK-801 (n=5) or water (n=6) was injected i.p. 45 min following the end of the first ischemic insult. Rats were perfusion-fixed at 7 days and regional ischemic cell change (ICC) assessed using a 3-point scale. Severe ICC was documented throughout the CA1 hippocampus, dorsolateral striatum, cerebral cortex and ventrolateral thalamus of nontreated rats. In treated rats, significant protection was documented in all brain regions ($p<0.01$, Kruskal-Wallis 1-way analysis by ranks). For example, in the ventrolateral thalamus, MK-801 treatment dramatically reduced damage from 2.6 ± 0.4 (mean ± SD) to 0.10 ± 0.1. These findings indicate that excitotoxic mechanisms are involved in ischemic damage produced by repeated ischemic insults and that significant protection can be demonstrated with MK-801 even when treatment is initiated after the first insult.

522.17

CGS-19755 IS NEUROPROTECTIVE DURING REPETITIVE ISCHEMIA. THIS EFFECT IS SIGNIFICANTLY ENHANCED WHEN COMBINED WITH HYPOTHERMIA. A. Shuaib*, S. Ijaz, W. Howlett, R. Mazagri. Dept. of Medicine, Div. of Neurology, Royal University Hospital, Saskatoon, Sask. S7N 0X0

In small animals the damaging effects of repetitive ischemia are cumulative and exceed that of a single similar duration insult. The more severe damage may be the result of prolonged release of glutamate. We looked at the protective effects of CGS-19755 (NMDA receptor blocker) or CGS-19755 in combination with mild hypothermia in a gerbil model of repetitive ischemia. We used 3 minutes of forebrain ischemia and repeated it 3 times at one hour intervals. Damage scores were assessed 2 days and 7 days after the insult. At 2 days, CGS-19755 treated animals showed significant protection in the cerebral cortex and hippocampus (CA1 and CA4), medial geniculate and the thalamus. At 7 days, milder protection was seen in the CA1 region of the hippocampus and cerebral cortex. The protective effects of CGS-19755 were better when the medication was started after the first ischemic insult as compared to animals where the medication was started prior to the initial insult. When CGS-19755 was combined with mild hypothermia, the effects of repetitive ischemia were completely abolished in all but one gerbil.

The use of NMDA receptor blockers may protect the brain in repetitive ischemia and this effect is significantly enhanced when combined with mild hypothermia.

522.18

THE NEUROBEHAVIORAL AND MORPHOLOGICAL PROTECTIVE EFFECTS OF CGS-19755 (AN NMDA RECEPTOR BLOCKER) IN AN ANIMAL MODEL OF ISCHEMIA. T.B. Wishart*, S. Ijaz, A. Shuaib, R. Mazagri, J. Kalra, W. Howlett. Depts. of Psychology, Medicine (Neurology) and Pathology, University of Saskatchewan, Saskatoon, Sask. Canada S7N 0W0.

CGS-19755 alone or in combination with mild hypothermia results in significant neuronal protection in an animal model of repetitive cerebral ischemia. This study was designed to examine sparing of behavioral function.

Groups consisted of sham-operated gerbils, gerbils with repetitive ischemia of 2 minutes with a reperfusion period of one hour, gerbils with ischemia + CGS, and gerbils with ischemia + CGS + hypothermia. Neural damage was assessed by examination of silver-stained sections.

Animals received 5 trials/day for 7 days in a Morris water maze. Gerbils subjected to repetitive ischemia had widespread neural damage and were significantly slower to learn the required response than were animals in all other groups. CGS or CGS + hypothermia had neuroprotective effects in thalamus, striatum, and substantia nigra. There were no behavioral differences between these groups and normal controls. CGS appears to be able to functionally protect animals from the behaviorally disruptive effects of cerebral ischemia.

522.19

ATTENUATION OF ISCHEMIC BRAIN INJURY BY THE NOVEL COMPETITIVE NMDA ANTAGONIST MDL 100,453.

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The excitatory amino acids glutamate and aspartate have been implicated in the delayed injury to brain tissue after cerebral ischemia through actions mediated by the N-methyl-D-aspartate (NMDA) receptor. The ability of the competitive NMDA antagonist MDL 100,453 to attenuate ischemic injury in a model of focal cerebral ischemia when administered either prior to, or after ischemia induction in spontaneously hypertensive rats was investigated. MDL 100,453 was administered via continuous intravenous infusion with Alza osmotic minipumps over a 24 hour time period starting either 30 minutes prior to (30 mg/kg/24 hr) or 30 minutes after (100 mg/kg/24 hr) tandem permanent occlusion of the right common carotid and middle cerebral arteries. MDL 100,453 was found to significantly reduce the volume of the resultant brain injury. The volume (mean ± sd) of infarcted brain was 174 ± 27 and 172 ± 15 mm^3, in saline treated control animals after pre- or post-occlusion administration, respectively, compared to 110 ± 21 and 124 ± 25 mm^3, respectively, in MDL 100,453 treated animals. The amount of protection seen was equivalent to that afforded by the prototype competitive NMDA antagonist CPP (91 ±11 and 119 ± 15 mm^3 after pre- and post-MCAO administration, respectively). These experiments therefore suggest that MDL 100,453 reduces ischemic damage and may be useful in the treatment of human strokes.

ISCHEMIA: GLUCOSE

523.1

HYPOGLYCEMIA OR INHIBITION OF GLUCOSE UPTAKE RESULTS IN A LACTATE PREVENTABLE INCREASE IN ADENOSINE AND DEPRESSION OF SYNAPTIC TRANSMISSION IN RAT HIPPOCAMPAL SLICES.

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The effect of glucose deprivation on adenosine levels and on synaptic transmission was investigated in rat hippocampal slices. Adenosine levels were measured, using absorbance HPLC, in aliquots taken from static chambers of 2 ml volume each containing 4 hippocampal slices. Slices were kept on a net at an interface between the physiologic medium and the humidified atmosphere at a temp of 33-34°C. Electrophysiological measurements were made from slices under identical conditions.

Incubation of hippocampal slices in either glucose-free medium or in the presence of the glucose transport inhibitor cytochalasin B (50 μM) increased bath adenosine levels and depressed the extracellularly recorded synaptic potential or population spike. The addition of lactate (10 mM), a precursor for mitochondrial ATP generation, prevented the elevation in adenosine and the depression of the population spike. These results indicate that the neuroinhibitory modulator adenosine is elevated during glucose deprivation and contributes to the hypoglycemic depression of synaptic transmission. The rise in adenosine during glucose deprivation can be prevented by providing substrate for mitochondrial ATP generation. It is hypothesized that glucose transport inhibition may be a relevant neuroprotective strategy during cerebral ischemia as it may reduce lactic acid accumulation and help maintain elevated levels of the endogenous neuroprotectant adenosine.

523.2

HYPERGLYCEMIC HYPOXIA AND INTRACELLULAR ACIDOSIS ENHANCE THE DEPOLARIZING AFTERPOTENTIAL OF RAT MYELINATED AXONS.

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The mechanism(s) underlying functional abnormalities of peripheral axons in diabetic neuropathy are not well understood. Here we report that the combination of hyperglycemia and hypoxia, two factors possibly involved in the pathogenesis of diabetic neuropathy, enhances the depolarizing afterpotential (DAP) of myelinated axons. Experiments were performed on isolated rat spinal roots maintained at 36°C in a HEPES-buffered solution containing 2.5 or 25 mM glucose. Electrical stimulation and d.c.-stable recordings of compound action potentials were performed in a three-chambered organ bath. Hypoxia was induced by replacing oxygen for 30 min by ultrapure argon (P_{O2} <2 mmHg).

Under normoxic conditions, a single action potential was followed by a DAP (duration about 10 ms). During hypoxia in a solution with 2.5 mM glucose, the DAP disappeared. In contrast, hypoxia in 25 mM glucose strongly enhanced the DAP and increased its duration by up to 40 ms. A similar enhancement in amplitude and duration of the DAP was observed after the axons had been acid-loaded by addition of propionate (20 mM) to the bathing solution (at constant interstitial pH). These results indicate that hyperglycemic hypoxia may change the DAP and, consequently, functional parameters of myelinated axons by an acidosis-induced decrease in internodal membrane conductance. *Supported by the DFG (SFB 220).*

523.3

MODERATE HYPERGLYCEMIA WORSENS ACUTE BLOOD-BRAIN BARRIER DAMAGE FOLLOWING BRAIN ISCHEMIA.

W.D. Dietrich*, O. Alonso, M. Halley, and R. Busto. Cerebral Vascular Disease Research Center, Dept. Neurology, Univ. of Miami School of Medicine, Miami, Florida, 33101.

We examined the effects of moderate hyperglycemia on the response of the blood-brain barrier (BBB) to normothermic (37°C) cerebral ischemia. Rats underwent 20 min of 4-vessel occlusion followed by 30 min of postischemic recirculation. Hyperglycemic rats received an i.p. injection of 50% dextrose 15 min prior to the ischemic insult. Horseradish peroxidase was used as an indicator of BBB dysfunction. Following normoglycemic brain ischemia (plasma glucose 115±4 mg/dl, n=5) extravasated protein was mainly restricted to the cerebral cortex. In contrast, hyperglycemic ischemia (plasma glucose 372±21, n=6) led to severe and widespread BBB disruption throughout the neuroaxis. Sites of protein leakage included the cerebral cortex, striatum, hippocampus, thalamus and cerebellum. Parenchymal damage, including dark shrunken neurons and swollen astrocytes, was detected at sites of BBB damage. Intraischemic hypothermia (30°C, n=4) significantly attenuated the vascular and neuronal consequences of hyperglycemic ischemia. Under normothermic ischemic conditions, hyperglycemia worsens the degree of BBB disruption compared to normoglycemia. Increased vascular permeability represents an important pathomechanism by which systemic hyperglycemia may aggravate ischemic outcome.

523.4

DURATION OF ISCHEMIA: EFFECTS ON OUTCOME IN NORMOGLYCEMIC (N) AND HYPERGLYCEMIC (H) RATS.

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Acute hyperglycemia, prior to global ischemia, often results in seizures and death. The duration of ischemia necessary to elicit such effects was determined in this experiment. Fasted male Sprague-Dawley rats were anesthetized and prepared for forebrain ischemia (FBI). Prior to FBI, rats received either saline (plasma glucose=112±18 mg/dL) or glucose (PG=343±50 mg/dL). After 4, 8, 12, or 15 mins of FBI (bilateral carotid occlusion; MAP=30±5 mmHg), rats (n=12) were recovered and observed for seizures, motor function, and survival. Rats surviving 7 days underwent perfusion fixation for neurohistologic analysis. Seizure and mortality rates were compared between N and H groups for each ischemic duration. Significant differences were observed for both variables at ≥8 mins of FBI. Seizure rates continued to increase with increased ischemic durations (p<.01) while mortality rates stabilized at 8 mins (p=.42). Motor scores amongst survivors (7d postischemia) were different with ≥12 mins of FBI. The glycemic state of the rat becomes relevant to neurobehavioral outcome between 4 and 8 mins of global ischemia.

Seizures	N, 4'	N, 8'	N, 12'	N, 15'	H, 4'	H, 8'	H, 12'	H, 15'
Yes	0	0	0	0	0	4	8	11
No	12	12	12	12	12	8	4	1

Survive	N, 4'	N, 8'	N, 12'	N, 15'	H, 4'	H, 8'	H, 12'	H, 15'
Yes	12	12	12	12	12	7	6	5
No	0	0	0	0	0	5	6	7

523.5

EXTRACELLULAR DOPAMINE IN HYPERGLYCEMIC ISCHEMIA.
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We tested the hypothesis that hyperglycemic (HG) exacerbation of ischemic striatal damage is associated with an increased elevation of extracellular dopamine (DA). **METHODS:** 16 fed male rats were used. Each rat underwent insertion of a striatal microdialysis (MD) probe and recovered overnight in soundproof isolation. On d2 vascular cannulae temp probe and EEG elec were inserted. GLC 40% or 5% 4 ml/kg/h was started in 8 rats/grp. After 1h of O_2/N_2O; 100% O_2, 0.5 mg Arfonad was given iv, blood removed to MAP 50mmHg, and both carotids occluded for 12m. Life support continued for 6h. MD pump speed was 2 ul/m. Dialysate samples were assayed for dopamine by HPLC. Path in the striatum was graded from 0-5 (0=norm). **RESULTS:** Five 40% glc and 2 5% glc rats had severe (grade 4 or 5) striatal damage ($p<.05$). Baseline (BL) DA was nondetectable. N_2O and ischemia both increased DA vs. BL ($p<.05$). DA was increased at 30m PI compared to BL and N_2O ($p<.05$). No between group DA differences occurred. **CONCLUSIONS:** 1) N_2O after minor surgery increases striatal DA. 2) HG exacerbation of ischemic striatal damage in rats is not due to increased extracellular DA.

523.6

PHYSIOLOGIC AND HISTOLOGIC EFFECTS OF PHENYTOIN IN A RAT GLOBAL ISCHEMIA MODEL. H. Qi, M. Maletic-Savatic, F. Hospod, G. Newman*. SUNY at Stony Brook, NY and VAMC at Northport, NY, 11794.

Phenytoin is frequently studied in rat models of ischemia and epilepsy. Despite this, the physiologic effects of phenytoin in the rat have not been studied in detail. Experiments testing phenytoin in a brain slice model of global ischemia revealed a profound concentration-dependent hyperglycemia up to 25 mM glucose as well as hypothermia to 34°C.

Male Sprague-Dawley rats (175 -275g) were injected with 50 to 200 mg/kg *i.p.* and sacrificed after 30 min in most experiments. Some rats were initially anesthetized with halothane - NO_2 for arterial catheterization and then given phenytoin *i.v.* 2 hours later with BP and arterial blood gas monitoring. Several Fischer-344 and Wistar Kyoto (WKY) strain males (175g) were also studied. Histology was assessed by counting the percentage of normal appearing CA1 neurons remaining in 500μ thick hippocampal slices after 5 hours *in vitro*.

Phenytoin, at serum concentrations from 6 to 60 μg/ml, causes a linear increase in serum glucose from 8.5 mM in saline controls to over 25 mM. The hyperglycemia is reversed by co-administration of insulin *i.m.*, is present to at least the same degree in Fischer-344 and WKY strains and is independent of animal temperature. Fos-phenytoin also induces hyperglycemia while propylene glycol vehicle does not. There is a concentration-dependent hypothermia produced by phenytoin. BP and blood gasses are not significantly affected by phenytoin. Phenytoin produces only inconsistent benefit in CA1 neuronal survival whether phenytoin is continued *in vitro* or not.

Phenytoin-induced hyperglycemia may affect results with rat models of cerebral injury. The present histologic results, along with our prior results in our thick slice model of ischemia, suggest that phenytoin will be more useful in treating focal than global ischemia.

ISCHEMIA: GENE OR PROTEIN INDUCTION

524.1

THE STRESS RESPONSE IS DEPENDENT ON HYPERTHERMIA AFTER BRIEF ISCHEMIA IN THE GERBIL. T. S. Nowak, Jr.* and S. Suga. Stroke Branch, NINDS, NIH, Bethesda, MD 20892.

Previous studies established 2 min ischemia as a threshold insult for producing several changes in gene expression in gerbil brain. Brief periods of ischemia, associated with accumulation of the heat shock / stress protein, hsp70, in vulnerable CA1 neurons, are also reported to induce tolerance to subsequent more severe insults. In our hands both hsp70 expression and induction of tolerance after brief ischemia have been variable. We now show that increases in hsp70 mRNA correlate with the presence of hyperthermia during early recirculation after 2 min insults. Gerbils were subjected to bilateral carotid artery occlusion under halothane/N_2O anesthesia. Hsp70 mRNA levels were evaluated by in situ hybridization and quantitative densitometry at 3 h or 24 h. Rectal temperature (T_R) was monitored during recirculation and release of anesthesia (Experiment 1) or anesthesia was continued and T_R was either maintained at 37 °C or elevated to 39-40 °C during 30-90 min recirculation (Experiment 2). In Experiment 1, pronounced hsp70 expression was observed only in gerbils with spontaneous $T_R \geq$ 39 °C. Hsp70 hybridization in all hippocampal fields was several-fold higher in hyperthermic animals at 3 h, and lasting hsp70 hybridization at 24 h was only detected in the hyperthermic group (Experiment 2). Earlier work identified temperature during early recirculation as an potential variable influencing the course of pathology in CA1 neurons after damaging insults. The present results implicate postischemic temperature as a factor affecting the reproducibility of transcriptional responses to threshold ischemia that may be of particular relevance to the expression of tolerance after brief challenges.

524.2

INDUCTION OF HEAT SHOCK PROTEINS IN ASTROCYTES AND NEURONS AFTER EXPOSURE TO PROSTAGLANDIN A1. R.N. Nishimura, B.E. Dwyer* and T. Yoshida. In Vitro Remyelination Laboratory and Molecular Neurobiology Laboratory, Sepulveda VAMC, Sepulveda, CA 91343.

The induction of heat shock proteins was investigated in cultured rat forebrain astrocytes and cortical neurons after exposure to prostaglandin A1 (PGA1) at doses of 0.05 to 4ug/ml of medium. Accumulation of the major inducible 68 kilodalton heat shock protein was undetectable by Western blotting at 3, 6, and 24 hours in neurons and minimally detectable in astrocytes. In contrast, the induction of a 32-34 kilodalton stress protein previously identified as heme oxygenase (Dwyer et al, Glia 5:300-305,1992) was detected in neurons after 6 hours of PGA1 exposure. Heme oxygenase induction in astrocytes was noted at 3 and 6 hours of exposure to PGA1 and was profoundly increased at 24 hours of PGA1 exposure. The results suggest that inflammatory cytokines are capable of inducing heat shock proteins in neurons and astrocyes and that they may be important in the induction of the acute phase protective response of CNS cells to injury.
Supported by the research service of the Department of Veterans Affairs.

524.3

DISTINCT PATTERNS OF HEAT SHOCK PROTEIN INDUCTION FOLLOWING FOREBRAIN ISCHEMIA IN RATS. H. Gaspary, S. Graham, S. Sagar, P. Weinstein*, and F.Sharp. Dept. of Neurology and Neurosurgery, UCSF Sch. of Med. SF, Ca. 94143.

During global ischemia, the synthesis of various heat shock proteins (HSP's), including HSP72, is induced in selectively vulnerable neurons, and occasionally in glial and endothelial cells, of the cortex, hippocampus, and striatum. The significance of HSP72 induction in these cells, however, is unclear.

The present study employs the model of simultaneous carotid occlusion and hypotension in rats for either 30 (n=6) or 15 (n=2) minutes. After 24 hours, immunohistochemistry was performed using a monoclonal antibody for HSP72. Sections were also stained with H&E or acid fuscin. In addition, sections from one brain were double-labeled for either HSP72 and glial fibrillary acidic protein (GFAP), or for HSP72 and isolectin B4, markers for astrocytes and microglia, respectively.

Mild ischemic injury was characterized by patchy HSP72 induction in neurons in the cortex and striatum, small columns of HSP72 positive neurons in Ca1 hippocampus, and no apparent abnormalities with H&E or acid fuscin. Moderate injury resulted in diffuse HSP72 induction in neurons throughout the entire forebrain, with occasional glial cell induction. On H&E stained sections, scattered necrotic neurons were evident in the cortex and hippocampus; Ca1-4 hippocampal neurons were acid fuscin-positive while the dentate gyrus appeared spared. HSP72 was not found in necrotic neurons when slides were counterstained with H&E. In severe injury, HSP72 induction was marked in glial and endothelial cells, with almost no neural expression, except in resistant regions (i.e. dentate gyrus). Areas with prominent glial induction corresponded to regions with widespread ischemic necrosis and pale staining on H&E, and acid fuscin positive cells throughout the hippocampus. Double-labeled sections revealed that both microglia and astrocytes (type I and II) express HSP72 in these severely injured brains.

These results confirm previous observations that HSP72 is expressed in injured neurons that survive ischemia. In addition, severe ischemic injury results in both microglial and astrocyte HSP72 expression in the absence of neuronal expression. Thus HSP72 is a valuable early marker to quantify ischemic injury.

524.4

FOCAL HSP-70 mRNA INDUCTION WITH LITTLE TO NO HISTOLOGIC INJURY IN RAT BRAIN. D.M.O'Rourke*, S.S. Glazier, F.A. Welsh, Div. of Neurosurg, Univ. of Penn., Phila., PA. 19104.

A variety of cell stresses including ischemia have been shown to induce heat shock proteins which may help defend cells against injury. The 70 k-Da protein, HSP-70, is one of the major proteins induced in ischemia. Although HSP-70 is a marker of regional metabolic compromise in brain ischemia, the relationship of HSP-70 production to neuronal cell injury or recovery is not well defined. We sought to determine whether HSP-70 could be induced by focal ischemia without producing neuronal injury in rat brain. Focal ischemia was induced in Wistar rats by transient, distal MCA occlusion for 20,30,or 60 minutes. After 24 h reperfusion, all animals undergoing 30 (n=5) or 60 (n=3) min of reversible ischemia exhibited micro-infarcts in the outer MCA neocortex with occasional extension to inner neocortex and limited selective neuronal necrosis in the peri-infarct zones. Brains undergoing 20 min (n=3) of ischemia exhibited either no histologic injury or occasional focal infarction nearly indistinguishable from that observed after occlusion for 30 or 60 min. Mean area of neocortical HSP-70 mRNA production (assessed by in situ hybridization after 2 h reperfusion) was larger than the core of infarction and was 3.4mm^2 at 20 min (n=3) and 9.4mm^2 at 30 min (n=3) at the level of the striatum predominantly in the outer layers of neocortex. Thus, distal, reversible MCA occlusion for 20-60 min results in focal HSP-70 mRNA production in neocortex with minimal histologic injury. Reversible ischemia for 20 min in this model may represent a window where HSP-70 mRNA is induced in cells which recover completely from metabolic stress.

524.5

NEURONAL PROTECTION FROM ISCHEMIC NECROSIS BY FOCAL INDUCTION OF HEAT-SHOCK PROTEINS. S.S.Glazier, D.M.O'Rourke, D.I.Graham, F.A.Welsh*. Div of Neurosurg, U of Penn, Phila, PA 19104.

Ischemic stresses inflict numerous changes on neural cellular elements. One such consequence is the production of several classes of stress-response proteins, or heat-shock proteins (HSPs). Both in-vitro and in-vivo models have correlated the induction of HSPs with a decrease in the expected injury from a subsequent stress. We have developed a model of cortical neuronal protection that allows direct comparison of a protected hemisphere with the contralateral, unprotected hemisphere. We used a distal middle cerebral artery occlusion in the rat as a priming insult to stimulate a stress response in the cerebral cortex without causing neuronal necrosis. Twenty-four hours later the animal was subjected to a ten minute period of bilateral common carotid artery occlusion with hypotension in a temperature and humidity controlled environment. This global ischemic insult has been characterized to result in symmetrical, selective cortical and subcortical neuronal necrosis in unprimed animals. In a preliminary set of three rats, we found a decrease in the number of necrotic neurons in the primed cortex compared to the unprimed side in two animals. In the third animal there was insufficient cortical injury on either side to detect protection. Subcortical structures were symmetrically affected in all three animals. This model suggests a correlation exists between the in-vivo induction of HSPs and resistance to ischemic cell death. The presence of a side-to-side control (opposing cortical regions), and a unilateral control (adjacent cortical and subcortical regions), makes this an attractive model for the further characterization and enhancement of neuronal protection during stress.

524.6

HEMEOXYGENASE mRNA AND PROTEIN LEVELS INCREASE IN NEURONAL CELLS IN RESPONSE TO HYPOXIA AND HYPOXIA-REOXYGENATION. C.M. Bitler, B.J. Murphy and L. Toll*, Department of Neuroscience, SRI International, Menlo Park, CA 94025

The presence of increased amounts of oxidized proteins in aged animals, particularly in the brains of such animals, have led to the discovery that compounds which react and stabilize free radicals generated during such oxidations can reverse degenerated behavioral parameters in older gerbils to levels comparable to those of young gerbils. In aerobic animals, a number of natural antioxidants exist which include superoxide dismutase, catalase, and the bile pigment, bilirubin. We have found that during a hypoxic episode in immortalized neuronal cells, hemeoxygenase (HO), the enzyme responsible for the generation of bilirubin, and the mRNA which encodes it, increase several-fold. Since free radicals are most prevalent in the brain following an ischemia-reperfusion episode, we mimicked this situation by reoxygenating the cells following hypoxia and found that the increased levels of HO mRNA are maintained. Interestingly, the regions of the brain most vulnerable to hypoxia-reperfusion are areas in which HO mRNA is most abundant. We propose that the generation of the antioxidants biliverdin and bilirubin protects the brain from the assault of free radicals generated during ischemia-reperfusion, and the extent to which hemeoxygenase is induced contributes to the resiliency or vulnerability of the brain (particularly, the hippocampus) to ischemia.

524.7

REGION-SPECIFIC CHANGES IN EXPRESSION OF SULFATED GLYCOPROTEIN-2 (SGP-2) FOLLOWING TRANSIENT GLOBAL ISCHEMIA AND INHIBITION BY LY231617. K.S.Fuson *, J.A.Clemens, J.A.Panetta, E.B.Smalstig, and P.C.May. Lilly Research Laboratories, CNS and Diabetes Divisions, Eli Lilly and Company, Indianapolis, IN 46285.

SGP-2 is emerging as an important marker for neurodegeneration. In this study, we examined SGP-2 RNA expression in rat hippocampus (HC), caudate nucleus (CN), and cortex (CX) 24 hours after transient global ischemia induced by four vessel occlusion (4VO). At 72 hours post-4VO, SGP-2 RNA is elevated in HC, CN, and CX (May et al, Mol. Brain Res., in press). However, at 24 hours post-4VO, SGP-2 RNA is increased only in CN and CX (1.5- and 1.7-fold, respectively); there is no change in SGP-2 RNA in HC. Induction of SGP-2 RNA at this early timepoint is limited to regions (CN and CX) where rapid neurodegeneration post-4VO takes place; this induction has not yet occurred in HC, where neurodegeneration is delayed. To further study the relationship between SGP-2 and neurodegeneration, we tested the effectiveness of LY231617, an antioxidant compound that is protective against ischemic damage in HC and CN, in preventing SGP-2 RNA expression following 4VO. LY231617 (50 mg/kg) or vehicle was administered orally before and after 30 minutes of 4VO, and the rats were sacrificed 72 hours later. Treatment with LY231617 prevented the induction of SGP-2 RNA in HC but not in CN, although 4VO-damage in both regions was markedly attenuated by the compound. These data suggest that ongoing neurodegeneration is not an absolute requirement for the induction of SGP-2 RNA in the brain.

524.8

CO-INDUCTION OF FOS AND JUN EXPRESSION FOLLOWING FOCAL CEREBRAL ISCHEMIA-REPERFUSION INJURY. G. An, T.N.Lin, J.J. Xue, Y.Y.He, C.Y.Hsu*. Division of Restorative Neurology, Baylor College of Medicine, Houston, TX 77030.

Alterations of immediate early gene expression have been observed during central nervous system ischemia. In the present study, the expression of the proto-oncogenes, c-fos, c-jun, jun B and jun D were investigated in a rat focal cerebral ischemia-reperfusion model. Northern blot analysis indicated that the expression of c-fos and jun B in control brains were very low and both were co-induced immediately following focal cerebral ischemia-reperfusion and peaked at 30 and 60 min after reperfusion respectively. c-jun was expressed constitutively in a significant level in all time intervals examined and was also enhanced in a similar pattern as c-fos and jun B but with a much lower magnitude. In contrast, no change in jun D expression was observed. The increases of c-fos, jun B and c-jun mRNAs were localized to the cortex and hippocampus, as shown by in situ hybridization. Nuclear run-on assays indicated that the increased mRNA levels of c-fos, jun B and c-jun are due to the increases of transcription rate in these genes. Mobility shift assays were used to determine the DNA binding activity of transcriptional factor AP1 (Fos-Jun heterodimer). Nuclear extract from control cortex showed a basal binding activity to the AP1 DNA sequence; however, 90 min ischemia followed by 4 hr reperfusion resulted in a 4 to 6-fold increase of AP1 binding activity, and the enhanced DNA binding activity persisted for at least 24 hr. These results suggest that, as general transcriptional factors, the changed expression of fos and jun may play a central role in post-ischemic alteration of genetic process.

524.9

LOCATION OF C-FOS AND C-JUN mRNA EXPRESSION AFTER NEONATAL HYPOXIA-ISCHEMIA. F. Munell, R.M. Gubits, R. E. Burke, A. Bandele, and D.C. DeVivo*. Dept. of Neurology and Dvsn. of Pediatric Neurology, Columbia Univ. Coll. of Phys. and Surg., NY, NY 10032.

The expression of c-fos and c-jun mRNAs was localized by in situ hybridization in a neonatal model of hypoxic-ischemic (H-I) brain injury (Rice et al.,1981). Rat pups (PND7) were exposed to unilateral common carotid ligation, followed by 3 h of hypoxia (8%O2/92%N2). Adjacent coronal brain sections were hybridized to 3'-end labeled oligomeric probes for c-fos and c-jun mRNAs. At 2 h post-hypoxia, c-fos and c-jun expression was observed in ipsilateral (I) striatum and hippocampus, as well as both vulnerable and spared regions of the cortex. A columnar pattern of expression was observed in the ipsilateral MCA cortex, which is similar to the pattern of permanent morphological damage observed in this area in neonatal, but not adult, models of unilateral H-I. Emulsion autoradiography of hybridized sections revealed cell-specific expression in these areas. In some animals, expression in the contralateral hemisphere occurred in sites that represented a partial mirror image of those on the I side. The ipsilateral striatum, MCA cortex, and hippocampus exhibit selective vulnerability to damage in the neonatal model. Since fos and jun mRNA expression occurs in both vulnerable and spared regions, AP1 transcriptional activity could mediate region-specific responses to H-I in the immature brain. Sup. by Colleen Giblin Fndtn. and NS26836.

524.10

IEG INDUCTION AFTER NEONATAL HYPOXIA-ISCHEMIA. R.M. Gubits*, R. E. Burke, H. Yu, G. Casey, A. Bandele,and F. Munell. Dept. of Neurology and Dvsn. of Pediatric Neurology, Columbia Univ. Coll. of Phys. and Surg., NY, NY 10032.

Immediate early gene (IEG) products, such as FOS and JUN, may partially mediate the longterm transcriptional response of CNS cells to specific changes in thier environment. To determine whether IEG products might play a role in the immature brain's response to hypoxia-ischemia (H-I), rat pups (PND7) were subjected to unilateral common carotid artery ligation followed by 3h of hypoxia (8%O2/92%N2) at 37°C, which results in pathological changes only in the ipsilateral hemisphere (Rice et al.,1981) . Following H-I, 3 animals were euthanized at each of 4 time points (1, 2, 18, and 24 hr). RNAs from 4 brain regions were analyzed by northern blot hybridization for their relative concentrations of 9 IEG mRNAs (c-fos, c-jun, junB, and TIS 1 (nur77), TIS7, TIS8 (zif268), TIS10, TIS11, and TIS21). Induction of all of the IEGs, except TIS7 and TIS10, was observed in ipsilateral forebrain, and less frequently in contralateral forebrain, at 1 and 2 hr post-hypoxia. In some animals, lower levels of expression were also detected at 18 and 24 hr. With minor exceptions, co-induction of all 7 IEGs was observed in a given RNA sample. In summary, exposure of neonatal rats to H-I produces a dramatic induction of 7 IEG mRNAs in their brains. Several convulsant drugs induce seizures, but not fos expression, before PND20 (Sakurai-Yamashita et al., (1991)). Thus, the extensive IEG response to H-I represents one of the few reported thus far in which IEG-encoded proteins could mediate longterm cellular responses in neonatal brain. Sup. by Colleen Giblin Fndtn. and NS26836.

524.11

ALTERATION OF NEUTROPHIN mRNA EXPRESSION AFTER FOCAL CEREBRAL ISCHEMIA-REPERFUSION. J.S.Liu, T.N.Lin*, J.J.Xue, Y.Y.He, G.An, C.Y.Hsu. Division of Restorative Neurology, Baylor College of Medicine, Houston, TX 77030.

Neurotrophins (NTs) have been shown to promote the differentiation and survival of certain types of neurons and may play a role in plasticity and regeneration after CNS injury. In this study, the influence of focal cerebral ischemia on NT family mRNA expression was examined using Northern blot analysis and in situ hybridization. Transient focal cerebral ischemia for 30 minutes with reperfusion of 30 minutes or 4 hours induced a transient increase in NGF and BDNF expression in ischemic cortex. A second peak was found two to four weeks after ischemic insult. The biphasic expression of NT mRNA was also observed to a lesser extent in the contralateral cortex which sustained only very mild ischemia. Similar biphasic NT mRNA expression was also noted if ischemia lasted for 90 minutes leading to a permanent infarct. Interestingly, in hippocampus, a monophasic peak happened between four hours to 1 week after reperfusion, which corresponded to a period of depressed NGF and BDNF expression in the ischemic cerebral cortex. There was no obvious increase in NT-3 expression. We also failed to detect CNTF mRNA signals in brain with or without ischemia. Results from this study suggest time-dependent expression of selected NTs.

524.12

THE EFFECT OF POST-OCCLUSION SURVIVAL TIME ON LESION VOLUME AND THE IMMUNOHISTOCHEMICAL LOCALIZATION OF MAP2 AND GFAP AFTER PERMANENT MIDDLE CEREBRAL ARTERY OCCLUSION IN THE MOUSE. C. Gonzales*, T.L. Sailer and J.A. Moyer. CNS Division, Wyeth-Ayerst Research, CN 8000, Princeton, N.J. 08543-8000.

Permanent middle cerebral artery occlusion in the mouse causes severe neuronal cell loss in the ipsilateral cortex presumably due to massive release of excitatory amino acids in the area of the lesion. Because this type of injury and the resulting cellular response is a dynamic process that occurs over days, the effect of time on infarct volume and the presence of neurons and glial cells in the area of an ischemic lesion were examined. Mice were anesthesized with 2% isofluorane and temperature was maintained at 37°C while the right middle cerebral artery was occluded by electrocautery. Animals were sacrificed 2, 4, and 6 days post surgery. Mean infarct volumes were determined at each timepoint using a Nissl stain and sections through the lesion were processed for immunohistochemistry using a polyclonal antibody against microtubule-associated proteins (MAP2) or a monoclonal antibody against glial fibrillary acidic protein (GFAP). The results showed that infarct volume decreased 50% from 2 days to 6 days post occlusion. GFAP staining was intense and localized throughout most of the ipsilateral hemisphere at 2 days post-occlusion and became more concentrated in the area of the lesion over time. In the perimeter of the lesion, there were surviving neurons that express MAP2, particularly at 4 and 6 days. These studies suggest that some neurons that appear abnormal 2 days after ischemia may show a recovery process over time.

524.13

INCREASES IN GAP 43 IMMUNOREACTIVITY AFTER CORTICAL INFARCTION. R.P. Stroemer *, T.A. Kent and C.E. Hulsebosch. Depts. of Anat. and Neurosci., Marine Biomed. Inst., Neurol., Univ. of Texas Med. Br., Galveston, TX 77550.

The denervation in the cortex following damage from ischemia should provide a stimulus for sprouting. Earlier work supported the existence of synaptogenesis after cortical infarction using antibodies to synaptophysin. The present study demonstrates the distribution of immunoreaction against GAP 43, a growth cone associated protein, as an indicator of neurite growth following cortical infarction. Hypertensive rats were subjected to permanent tandem occlusion of the right common carotid and right distal middle cerebral arteries. This procedure produces ischemia in the cortex with little damage to subcortical structures. After recovery times of three days to two months, the animals were perfused with paraformaldehyde and processed for conventional immunohistochemistry using monoclonal antibodies to GAP 43. These slices were evaluated on a Quantex image analyzer to determine the optical density of the reaction product. Preliminary optical density data is shown below (* P<.01 Student's T test):

Time Post-Op	O.D. ipsi. to Infarct	O.D. contra. to infarct
3 days *	17.0±5.5	-8.0±2.3
7 days *	47.0±6.1	30.1±6.6
14 days *	41.0±7	28.3±4.5
1 month	17.1±10.6	19.0±7.3
2 months	40.4±9.1	49.0±4.7

These results suggest that neurite sprouting occurs shortly after ischemia in the cortex, but is diminished by one month when synaptogenesis occurs.This supports the hypothesis of neurite growth and synaptogenesis in the cortex following ischemia. Supported by NS 11255, NS 01217, and Bristol Myers-Squibb.

524.14

TRANSIENT FOREBRAIN ISCHEMIA CAUSES AN IMMEDIATE INCREASE IN bFGF mRNA, AND A DELAYED DECREASE IN FGF RECEPTOR mRNA IN THE CA1 REGION OF THE HIPPOCAMPUS M. Endoh and J. A. Wagner*: Cornell University Medical College, 1300 York Ave., New York, N.Y., 10021

Using both in situ hybridization and RNAase protection assays we have studied the change in mRNA expression of both bFGF and FGF receptor in the rat hippocampus after a ten minute period of transient ischemia induced by the four vessel occlusion model. There was an increase in the expression of bFGF message at six hours and one day after ischemia with a subsequent decrease, possibly because of the presence of high concentrations of excitatory amino acids or increased metabolism in the injured tissue. At one week, bFGF message expression was again increased. This increase occurred after the period of neuron death and was associated with gliosis. Although no immediate change in message for the FGF receptor (flg) was observed, there was a decrease in this message that was temporally correlated with neuron death, suggesting either that the receptor was expressed on neurons or that its expression was dependent on the presence of viable neurons. These changes in the expression of FGF and FGF receptor are presumably a part of a coordinated response to ischemic injury that may be designed to minimize the severity of the effects of neuron cell loss.

524.15

EFFECTS OF INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) TREATMENT TWO HOURS AFTER HYPOXIC-ISCHEMIC INJURY. C E Williams, J Guan, M Dragunow*, and P Gluckman. Dept. Ped., Univ. Auckland, Private Bag, Auckland, New Zealand.

We have shown that following hypoxic-ischemic (HI) injury to the rat brain, there is marked induction of mRNA for IGF-1, IGF binding proteins BP2-4 and TGFβ in the areas of neuronal loss within 5 days. Induction of NGF-β, BDNF or neurotropin-3 was not detected. IGF-1 is known to be a potent non-selective neurotrophic agent. We examined the possibility that IGF-1 may be neuroprotective. Rats were subject to a unilateral HI injury by ligating one carotid artery then subjecting the animals to 10 min of 6% inspired O_2. This induces considerable neuronal loss and infarction in the middle cerebral artery territory of the ligated hemisphere. Growth factor or vehicle were administered as bolus injections into the lateral ventricles.

IGF-1 given 2 hours *after* the insult reduced the infarction rate. In controls the infarction rate was 86%: this reduced to 36% with 5μg IGF-1 (n=14) and to 27% with 50 μg IGF-1 ($p<0.05$, n=15). Similarly within each neuronal region IGF-1 caused dose dependent neuroprotection particularly in the striatum and cortex. IGF-1 treatment did not alter cortical temperature. Equimolar doses of insulin did not improve outcome. Indicating the protective effects of IGF-1 do not act via the insulin receptor or hypothermic mechanisms. This peptide may have therapeutic use in the rescue of the injured brain.

524.16

IDENTIFICATION OF CHANGES IN NUCLEOTIDE INTERACTING PROTEINS IN CONTROL VS ISCHEMIC RAT BRAINS Banumathi Sankaran, Boyd Haley* and James Clemens. Eli Lilly & Co., Indianapolis and College of Pharmacy, University of Kentucky, Lexington, KY, 40536.

The effects of ischemia on the phosphorylation and nucleotide binding properties of rat brain proteins were examined. Tissue homogenates were from the corpus striatum, dorsal hippocampus and paramedian cortex regions. Samples from both control rats and rats subjected to 30 minutes of 4VO followed by no reperfusion, 1hr or 24hrs reperfusion were phosphorylated or photolabeled. Either α or γ^{32}P labeled $8N_3ATP$ or $8N_3GTP$ were used. Major changes in phosphorylation and nucleotide binding of specific proteins in ischemic rat brains were observed. Most changes were observed in the striatum, a few in the hippocampus and almost none in the cortex. This agrees with the fact that after the ischemic insult, striatum degenerates in 24hrs, hippocampus dies after 3 days and cortex shows minimal degenerative changes. $8N_3ATP$ photoinsertion increases in ischemic brain proteins of 55-57 (CaM kinase), 42-43 (creatine kinase), 31 (unknown, but also a GTP binding protein that shows an increase in photoinsertion of $8N_3GTP$ in ischemic samples) and 14 (unidentified) kDa Mr values. Phosphorylation of a 110 kDa protein (CaATPase) is observed in controls but not in ischemic samples. Supported by Eli Lilly and Co. & NIH grant GM-35766-07.

524.17

GLOBAL CEREBRAL ISCHEMIA EFFECTS ON HIGH MOLECULAR WEIGHT PEPTIDES AND CA1 PYRAMIDAL NEURONS IN THE RAT HIPPOCAMPUS. T.M. Wengenack*[1], R. Slemmon[1], J.M. Ordy[2], P. Bialobok[2], W.P. Dunlap[3], and P.D. Coleman[1]. [1]Dept. of Neurobiol. and Anat., Univ. of Roch., and [2]Fisons Pharm., Rochester, NY and [3]Tulane Univ., New Orleans, LA.

The four-vessel occlusion (4-VO) rat model of global cerebral ischemia has been used widely to study selective hippocampal cell vulnerability, gliosis, and viability or plasticity of surviving cells. Intense interest exists on the molecular events of selective CA1 vulnerability and the plasticity of surviving neurons in response to injury. The specific aim of this study was to correlate the post-ischemic progression of CA1 cell death and the molecular events which may play a critical role in neuronal vulnerability and plasticity in response to injury.

Reverse phase HPLC was used to profile hippocampal peptide changes following 4-VO. Rats were sacrificed 1, 3, or 7 days after 30 min of 4-VO or sham surgery. Supernatants of denatured hippocampal homogenates were passed through G50 Sephadex columns and the high molecular weight fraction separated by HPLC. Peaks which exhibited a 25% or greater change after 4-VO were isolated for identification. Trypsinized peptides were sequenced and identified by homology match to GenBank. In two rats from each group, CA1 pyramidal neurons were counted in three anatomically-defined regions of CA1, in two sections of the dorsal hippocampus.

The number of CA1 pyramidal neurons was not decreased significantly on day 1 after 4-VO, but was on days 3 and 7. Peptides that exhibited a relative change of 25% or greater were: zinc binding protein (-26, -23, -24%; days 1, 3, 7), myelin basic protein (-7, -38, -28%), serum albumin (31, 58, 7%), calmodulin (8, -33, -19%), alpha hemoglobin (26, 50, 1%), and beta hemoglobin (62, -11, -21%). The correlation of post-ischemic CA1 cell death with specific peptide changes provided evidence on the critical role of specific molecular events in neuronal vulnerability and plasticity. Supported by NIH AG 00107 and LEAD Award AG 09016 to PDC.

ISCHEMIA: FREE RADICALS

525.1

DIFFERENTIAL VULNERABILITY OF HIPPOCAMPAL AND CORTICAL GLUTAMINE SYNTHETASE TO OXIDATIVE INACTIVATION. M.E. Harris,* T. Tatsuno, J.M. Carney, M.P. Mattson, and R.A. Floyd. Dept. of Pharmacology, Univ. of Kentucky Medical Center, Lexington, KY 40536.

Specific regions of the central nervous system are susceptible to age-related and degenerative changes. Oxygen free radicals may play a significant role. These oxygen radicals have been shown to oxidize membrane lipids and proteins such as glutamine synthetase (GS). GS plays a critical role in the metabolism of the excitotoxic neurotransmitter glutamate. Both hippocampal and cortical neuron/glia cultures were established from E18 rat fetuses. The cultures were exposed to the Fe^{2+} or Fe^{2+}/Ascorbate oxygen radical generating systems. Hippocampal GS exhibited a transient increase in activity at 2 uM Fe^{2+} (2 hr treatment) but was progressively inactivated at 10-100 uM Fe^{2+}. Cortical GS increased in activity at 2-100 uM Fe^{2+} and was inactivated only after longer exposure times to 100 uM Fe^{2+} (4-8 hrs). Hippocampal GS was also inactivated at 2-10 uM Fe^{2+} + 1mM Asc. whereas cortical GS was not inactivated until 200 uM Fe^{2+} + 1 mM Asc. or longer exposure times to 10 uM Fe^{2+} + 1mM Asc. This differential inactivation of GS was not seen in cell-free extracts of hippocampal and cortical cells. This data suggests that, in mammalian cells, there may be differences in cellular organization that render cells more or less vulnerable to oxidative damage. Supported in part by AG09690 and a gift from the Glenn Foundation.

525.2

OXIDATIVE INACTIVATION OF GLUTAMINE SYNTHETASE IN GERBIL BRAIN. T. Tatsuno*, J.M. Carney and R.A. Floyd. Dept. of Pharmacology, Univ. of Kentucky Medical Center, Lexington, KY 40536.

Glutamine synthetase (GS) plays an important role in the metabolism of the excitotoxic neurotransmitter glutamate. We have found the loss of forebrain GS activity associated with protein oxidation following transient ischemia and aging. We exposed the cytosolic fraction of gerbil brain to the ascorbate/Fe^{2+} oxygen radical generating system. Cytosolic GS activity was easily inactivated by ascorbate (3-30mM), Fe^{2+} (1-100μM) and ascorbate + Fe^{2+}. GS activity was protected by iron chelators, ATP and Mn^{2+} but not by GTP or spin-trap agents (PBN, POBN). Ascorbate-induced production of $\cdot$OH detected by the conversion of salicylate to dihydroxybenzoate was inhibited by deferoxamine, Mn^{2+} and partially by spin-trap agents but not by ATP, GTP or EDTA. We also detected Fe^{2+}-induced cytosolic protein oxidation (protein carbonyl formation), which was prevented by chelators. The loss of GS activity by ascorbate appeared to be due to a site selective oxidation. However, the ATP binding site on GS was unaffected as demonstrated by [^{32}P]8N_3-ATP photoinsertion. Oxidative inactivation of GS could be a potential marker for Fe-mediated damage within the tissue. Supported in part by NS23307 and AG09690.

525.3

THE EFFECT OF N-ACETYLCYSTEINE ON CHOROID PLEXUS TISSUE VIABILITY FOLLOWING TRANSIENT CEREBRAL ISCHEMIA. D. Palm*, P. Watson, N. Knuckey and C. Johanson. Dept. Clin. Neurosci., Program in Neurosurg., Brown Univ. & RI Hospital, Providence, RI 02902

Recent studies have revealed maximum ionic and ultrastructural changes in choroid plexus (CP) tissue 6 hr following 10 min of transient ischemia. The following studies evaluated CP tissue viability from rats pre-treated with, the free radical scavenger, n-acetylcysteine. Male, Sprague-Dawley rats (250-375g) were fasted overnight and anesthetized with a mixture of 2% halothane (HA), 70% NO_2, and 28% O_2. N-acetylcysteine (163 mg/kg i.p.) was administered 30 min and again 5 min prior to the ischemic insult. Ten min of ischemia was induced by bilateral common carotid artery occlusion with hypotension (50 mmHg). MABP, PCO_2, PO_2, and EEG were monitored and brain and body temperature were maintained at 37°C. CPs were dissected from lateral ventricles at 6 hr post ischemia and measured for tissue [Na] and [K]. The tissue [K]/[Na] ratio was used as an index of tissue viability. CP morphology was analyzed by electron microscopy (E.M.). The CP tissue [K]/[Na] ratio was reduced by 46% and 56% in halothane anesthetized rats (n=6) and animals pre-treated with n-acetylcysteine (n=6) respectively compared to control (n=6) values (2.01 ± 0.06). However, CP tissue [K]/[Na] ratio of HA animals (1.08 ± 0.13) did not differ from animals pretreated with n-acetylcysteine (0.88 ± 0.06). In reference to previous studies, the [K]/[Na] of HA rats was reduced by 30% compared to unventilated pentobarbital anesthetized (PA) rat (n=6) values (1.55 ± 0.16). Furthermore, E.M. of CP from HA animals demonstrated greater ultrastructural damage compared to PA animals. In conclusion, pentobarbital demonstrates a protective effect on CP tissue viability. However, no effect on CP tissue [K]/[Na], 6 hr post-ischemia, was observed with pretreatment of n-acetylcysteine. Further studies with pre- and post-treatment of n-acetylcysteine at various doses are indicated to characterize its possible protective effect on CP tissue viability.

(Supported by NIH grant NS 27601 and research funds from RI Hospital)

525.4

SUPEROXIDE DISMUTASE AMELIORATES NEURONAL DEATH FROM HYPOXIA IN CULTURE. D. M. Rosenbaum*, J. Kalberg, J. A. Kessler. Albert Einstein College of Medicine, Bronx, New York 10461.

Biochemical changes that accompany cerebral ischemia include formation of free radicals such as the superoxide anion O_2, and these highly reactive substances may be an important cause of neuronal damage. The purpose of this study was to test the hypotheses that free radical formation does play a role in the ischemic cascade, occurs intracellularly and that free radical scavengers, if taken up intracellularly, will protect from hypoxic damage. A tissue culture model of hypoxia was employed using both PNS (superior cervical ganglia) and CNS (hippocampal) cells. Pretreatment with free superoxide dismutase (SOD) had little effect on neuronal survival after hypoxia as measured by trypan blue exclusion. However, when SOD was taken up intracellularly under depolarizing conditions (55 mMK$^+$ in the medium) survival was increased significantly. During hypoxia, neurons reduced nitroblue tetrazolium to form the blue precipitate formazan, and the color change was blocked in neurons pretreated with SOD in depolarizing medium. Similar findings occurred in both SCG, as well as hippocampal neurons. Free radical formation appears to play a role in cell death caused by hypoxia and free radical scavengers, if taken up intracellularly, may ameliorate their effect.

525.5

PATTERN OF CELLULAR LOCALIZATION OF LIPID PEROXIDATION DURING RAT FOREBRAIN REPERFUSION FOLLOWING CARDIAC ARREST. A. Daya, B.C. White, and J.A. Rafols*, Departments of Anatomy and Cell Biology and Emergency Medicine, Wayne St. Univ. Sch. of Med., Detroit, MI 48201.

Following cardiac arrest and resuscitation an in situ reaction of thiobarbituric acid (TBA) and fluorescent microscopy were used to localize post-ischemic peroxidative damage in the rat forebrain. After reperfusion (90 or 240 mins.) the brains were fixed transcardially with 50% aldehyde-free methanol, 10% acetic acid, 2mM EDTA and 0.375% TBA. Formation of TBA-malondialdehyde (fluorescence peak of TBA-MDA adduct = 515 nm) was assessed in serial 40 µm frontal sections. Brains of normal and ischemic-only groups provided the controls. Intense granular fluorescence was found within the cell body of neurons in many of the selectively vulnerable zones (SVZ) after either reperfusion time. Fluorescent granules were disposed primarily in the perinuclear region coincident with the Golgi apparatus, and were absent in glial cells. The dorsolateral sector of the cerebral cortex at rostral levels contained the largest numbers of fluorescent neurons (FNs) which were distributed heterogeneously throughout the layers. Numerous FNs were also found in the pyramidal layer of Cornu Ammonis (CA_1, CA_3 and hilar region) but only a few FNs were detected in the dorsolateral striatum and septal nuclei. The present data indicates that neurons in the SVZ are targets of peroxidation during reperfusion. It further suggests that, within a zone, a time dependent vulnerability during reperfusion may exist among neurons of different morphological and chemical phenotypes. (Supported by NIH grants NS-24819 and GM08167).

525.6

Expression of glutathione reductase in ischemia sensitive brain regions.

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Damage as result of hypoxia and/or ischaemia affects the substantia nigra, the hippocampus, striatum and cortex more than other brain regions. The cause of this cell death is unknown but it is likely that free radical generation plays an important role. And although defense mechanisms against these radicals are present in the brain, these may be insufficient to counteract with the large radical generation during ischaemia or other radical generating circumstances as an MPTP injection.

In vitro studies of scavenger enzyme activities in homogenized samples of different brain regions are controversial.In vivo studies, describing expression of these enzymes in the rat brain are also not available. We studied the localization of glutathione reductase which reduces Oxidized glutathione (GSSG) to glutathione (GSH).

The hypoxia sensitive CA1 region in the hippocampus shows a high glutathione reductase appearance compared to the CA2/CA3. Furthermore cells in the zona compacta of the substantia nigra, probably dopaminergic, were labelled. In the SN pars reticulata a strong extracellular labeling was found with only few positive neurons. In the dorsolateral striatum many neurons expressed this enzyme whereas the medial part and the globus pallidus showed faint cellular staining.

This distribution might explain some features found after ischaemia.

ISCHEMIA: VASCULATURE

526.1

ATRIAL NATRIURETIC PEPTIDE BLOCKS PROTEOLYSIS OF THE BLOOD-BRAIN BARRIER BY BACTERIAL COLLAGENASE IN RAT.

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Atrial natriuretic peptide (ANP) is important in regulating brain fluid balance and in the control of brain edema. We have shown in collagenase-induced hemorrhages that brain edema is present by 4 hours and worsens over 24 to 48 hrs before recovery begins; the BBB is opened by 30 min. Therefore, we studied the effect of intravenous or intraperitoneal injection of ANP on edema formation and on the BBB. Adult rats (n=6) had lesions produced in the caudate/putamen by the stereotactic injection of 0.4U of bacterial collagenase in 2µl of saline. Other rats (n=7) had similar collagenase lesions, but had 5µg/kg/hr ANP infused for 24 hrs with an osmotic pump; 24 hrs after the lesions the brains were removed and water and electrolyte contents determined. Another group of rats (n=5) had ANP (50µg/kg/hr) infused intravenously for 90 min beginning 1 hr prior to the lesion; the permeability of the BBB was studied by the intravenous infusion of ^{14}C-sucrose given 10 min before the end of the experiment. Five control rats were similarly studied but without ANP. ANP significantly reduced the percent water at the injection site from 82.39±.24 to 80.55±.35 and the sodium from 402±17 to 309±15 mEq/mg dry wt (p<0.002). The percent uptake of ^{14}C-sucrose from blood into the brain was reduced from 17.3±2. in rats with lesions to 5.1±.4 in rats with ANP (p>0.0004). We conclude that intravenous ANP is effective in reducing brain edema in a hemorrhagic lesion by protecting the capillary from proteolytic damage by bacterial collagenase. ANP may be useful in treatment of brain edema.

526.2

FASTIGIAL STIMULATION ENHANCES EEG RECOVERY AND REDUCES TISSUE DAMAGE AFTER FOCAL ISCHEMIA. F. Zhang and C. Iadecola, Dept. of Neurology, Univ. of Minnesota, Minneapolis, MN 55455.

Stimulation of the cerebellar fastigial nucleus (FN) increases cerebral blood flow (CBF) but not metabolism and reduces the brain damage resulting from focal ischemia (JCBF&M 10:810, 1991). The FN may exert this effect by enhancing CBF to the ischemic tissue without increasing local metabolic demands. To test this hypothesis we studied whether the protective effect is restricted to the neocortex, a region in which the CBF increase is independent of metabolism, and whether stimulation of the dorsal medullary reticular formation (DMRF), a treatment that increases CBF and metabolism, also reduces the brain damage resulting from ischemia. In halothane anesthetized rats (1-3%) the middle cerebral artery (MCA) was occluded proximally or distally to the lenticulostriate branches. FN or DMRF were stimulated (50-100µA; 50Hz; 1 s on/1 s off) for 1 hr. Infarct volume was determined one day later. In unstimulated rats, proximal MCA occlusion (n=7) produced infarcts involving cortex (250±45mm^3) and striatum (57±8) (Mean±SD). Distal MCA occlusion (n=6) produced infarcts restricted to the neocortex (223±28). FN stimulation substantially reduced stroke size (p<0.001). The reduction was greater after distal (-69±8%) than proximal (-38±8%) MCA occlusions (p<0.001) and occurred in neocortex (-43±9; p<0.001) and not in striatum (-16±21; p>0.05). MCA occlusion reduced EEG amplitude in the ischemic cortex by 73±8%. One hr later the EEG recovered by 13% in unstimulated rat and by 48% with FN stimulation (p<0.003). DMRF stimulation (n=7) did not affect stroke size or EEG recovery (p>0.05). Thus stimulation of the FN, but not DMRF, attenuates the functional impairment and tissue damage resulting from cerebral ischemia. The finding that the protection is limited to the neocortex and that the amount of tissue salvaged is greater after distal MCA occlusion suggests that the FN may protect ischemic tissue from infarction by enhancing collateral flow without increasing local metabolic demands. Activation of selected neural networks protects the brain from the consequences of focal cerebral ischemia. (Supported by the American Heart Association of Minnesota).

526.3

SPINAL CORD BLOOD FLOW IN RESPONSE TO $PaCO_2$ WITH LASER DOPPLER FLOWMETRY. L. J. Mouw, P. W. Hitchon, R.K. Osenbach, J.C. Torner, and C.M. Loftus* Division of Neurosurgery, Univ. of IA Hosp. and Clinics, Iowa City, IA 52242.

Spinal cord blood flow (SCBF) is affected by numerous physiologic parameters including $PaCO_2$. The authors describe a new technique of measuring SCBF in a rat model using laser doppler flowmetry. Male Wistar rats underwent induction of anesthesia followed by tracheostomy and femoral vessel cannulation. A C7 bilateral laminectomy was performed to expose the dorsal spinal cord. Using a stereotactic frame with intraoperative magnification, a needle-tip fiberoptic probe was placed over the dorsal horn of the cord. Ventilation was adjusted to maintain the $PaCO_2$ ± 3 mmHg at one of three predetermined levels (30, 40, 50). SCBF was averaged over two minutes of continuous monitoring. Segmental SCBF at $PaCO_2$ levels of 30, 40, and 40 mmHg were 58.8, 96.2, and 106.4 cc/100gm/min respectively (P=0.0001). Linear regression revealed that the slope was 2.74 when the $PaCO_2$ changed from 30 mmHg to 50 Hg (P=0.0002). Laser doppler flowmetry is a useful tool in measuring relative changes as well as continuous on-line dynamic monitoring of segmental SCBF in the rat.

526.4

LEUKOCYTE BEHAVIOR DURING ACUTE REPERFUSION AFTER GLOBAL CEREBRAL ISCHEMIA IN THE RAT BRAIN CORTEX MICRO-CIRCULATION. G.Sixt, A.Villringer*, U.Dirnagl. Dept. of Neurology, University of Munich, 8000 Munich 70, F.R.G.

We tested the hypothesis that leukocytes (WBCs) are activated within the brain microcirculation by global ischemia, and that obstruction by WBC is responsible for postischemic hypoperfusion. Wistar rats were anesthetized with Inactin, tracheotomized, ventilated, and a closed cranial window was implanted (dura removed). Arterial blood pressure, endexspiratory pCO_2, body temperature, and cortical rCBF (Laser-Doppler flowmetry) were measured continuously. Global cerebral ischemia was induced for 10 min by reversible bilateral common carotid occlusion combined with hypotension. WBCs were labelled intravitally with Rhodamine 6G. WBCs within the microcirculation of the outer 100 µm of the cortex were imaged with confocal laser scanning microscopy. Over the 3 h observation period, in controls (n=5) there was no significant increase of stickers (WBCs adherent to the vessel wall) and rollers (WBCs rolling allong the vessel wall), and no extravasation of WBCs occured. Ischemia induced an increase of venular stickers, in capillaries only few occluding WBCs were seen. No changes were observed in arteries. Extravasation of WBCs was observed in all animals beginning 1.5 hours post ischemia. We conclude that WBCs are activated within the first hours after global cerebral ischemia, however, mechanical obstruction by WBCs may not cause postischemic hypoperfusion.

	Baseline n=5	Hyper n=5	Hypo (20min) n=5	Hypo (1h) n=5	Hypo (2h) n=3	Hypo (3h) n=2
Roller (vein)	1.8 ± 2.7	4.5 ± 4.7	3.0 ± 2.7	2.5 ± 1.0	2.0 ± 2.8	1.4 ± 2.0
Sticker (vein)	1.1 ± 0.8	2.3 ± 1.7	3.3 ± 3.0	4.7 ± 4.4	5.8 ± 6.5	5.9 ± 3.7
Sticker (cap.)	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 1.3	2.6 ± 3.3	2.7 ± 2.3	2.5 ± 3.5

Values are means ± SD per vascular segment/30 sec. n: number of animals, Hyper: Hyperperfusion, Hypo: Hypoperfusion, Cap: Capillary

526.5

ADHESION AND INFILTRATION OF MONOCYTE/MACROPHAGES IN BLOOD VESSELS OF HYPERTENSIVE RATS Y. Liu, A.-L. Sirén, F. Barone, G. Feuerstein, and J.M. Hallenbeck*. Dept. of Neurology, USUHS, Bethesda, MD 20814, Stroke Branch, NINDS, Bethesda, MD 20892, and Dept. of Pharmacology, SmithKline Beecham, King of Prussia, PA 19406.

The endothelial adhesion of monocytes and expression of the intercellular adhesion molecule-1 (ICAM-1) in the carotid arteries of rats with and without the stroke-risk factor hypertension were demonstrated using the immunohistofluorescence technique. Perfusion-fixed frozen sections (16 μ) of carotid arteries of spontaneously hypertensive rats (SHR), stroke-prone SHR (SHR-SP) and normotensive Wistar-Kyoto (WKY) rats were exposed to specific monoclonal antibodies against rat monocyte/macrophages (ED-1) or rat ICAM-1. All slides were double stained with anti-Factor VIII antibodies for the identification of endothelial cells which showed no ED-1 staining. In SHR and SHR-SP, single monocytes or clusters of monocyte/macrophages were found adhering to the endothelium. In SHR-SP, ED-1 positive cells were present also in a subendothelial location. The number of endothelium-attached monocyte/macrophages per millimeter of internal elastic lamina was significantly greater in SHR-SP than in SHR (5.1 ± 0.7 and 3.2 ± 0.4, $p<0.05$, $n=4$). No ED-1 staining was found around the endothelium in WKY rats. Patchy expression of ICAM-1 on endothelial cells was found in carotid arteries of SHR and SHR-SP, but not in WKY rats. The results suggest that the increased accumulation of monocyte/macrophages and activation of endothelial cells are associated with the stroke-risk factor hypertension increasing the tendency for the endothelium to convert from an anticoagulant to a procoagulant surface.

526.6

ATP-SENSITIVE POTASSIUM CHANNELS IN RAT BRAIN MICROVASCULAR ENDOTHELIAL CELLS

Damir Janigro, Ellen L. Gordon & H. Richard Winn*
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The endothelium plays an important role in the modulation of vascular tone and blood cell activation. Extensive work has demonstrated that the release of EDRF from the endothelium is evoked by a number of physical and chemical stimuli requiring Ca^{+2}. Since endothelial cells do not express voltage-dependent calcium channels, calcium influxes following receptor activation may be facilitated by cell hyperpolarizations mediated by the activation of potassium conductances. We have investigated the electrophysiological properties of an ATP-sensitive K^+ conductance in whole cell and membrane patches from rat aorta and brain microvascular endothelial cells. Whole cell as well as patch currents were increased by either intracellular dialysis of ATP or by application of glucose-free/NaCN (2 mM) solutions. Both currents were reversibly blocked by glibenclamide (1-100 μM). The $K_{(ATP)}$ channel opener pinacidil (30 μM) caused activation of an outward current in the presence of physiological $[ATP]_i$. In inside-out patches, ATP (10 μM-1 mM) invariably caused a dramatic decrease in channel activity. We conclude that both rat aorta and brain microvascular endothelial cells express $K_{(ATP)}$ channels. $K_{(ATP)}$ may play a role in the regulation of endothelial cell resting potential during impaired energy supply and therefore modulate EDRF release.

526.7

RESPONSE OF CEREBRAL CAPILLARY ENDOTHELIAL CELLS TO HYPOXIA AND RADIATION. G. T. Gobbel, T. Y. Y. Chan, J. R. Fike, P. H. Chan*. CNS Injury & Brain Edema Res. Center, Dept. Neurol., & Brain Tumor Res. Center, Dept. Neurosurg., Univ. of Calif., San Francisco, CA 94143.

Nitric oxide synthesis inhibitors, such as nitro-L-arginine, and polyamine inhibitors, such as alpha-difluoromethylornithine (DFMO), can inhibit ischemic and/or radiation-induced brain injury. The cellular basis of these effects may be related to inhibition of NMDA receptor activation. We have examined the effect of both radiation and hypoxia on cerebral capillary endothelial cells and modification of that effect by nitro-L-arginine (0.5 mM) and DFMO (1 mM). Cells were isolated from 14 day old Sprague-Dawley rats. At 4-7 days after plating, cells were treated with 24, 48, or 62 hrs of complete hypoxia or a 16, 32, or 64 Gy radiation dose. There was an initial increase in cell injury, as detected by an increase in lactate dehydrogenase (LDH) within the media, at 48 hrs of hypoxia, and the release of lactate dehydrogenase (LDH) intensified with increasing time of hypoxia or increasing radiation dose. The hypoxia-induced LDH release correlated with an increase in the ratio of dead to viable cells (ethidium bromide and fluorescein diacetate staining). While DFMO did not reduce release of LDH due to radiation, it did appear to reduce radiation-induced shedding of endothelial cells from the substrate. Neither DFMO nor nitro-L-arginine seemed to alter hypoxic injury under our experimental conditions. DFMO inhibited a hypoxia-induced increase in total LDH measured within cells attached to the substrate, suggesting that hypoxia either induces an increase in intracellular LDH or stimulates cellular proliferation. These results suggest that activation of NMDA receptors are not involved in hypoxia-induced injury to cultured cerebral endothelial cells. In addition, the modification of radiation-injury by DFMO may be related to a reduced rate of release of damaged endothelial cells and thus maintenance of the endothelial cell layer. Supported by NIH Grants AG 08938, NS-14543, NS-25372, and National Brain Tumor Foundation.

526.8

Hypoxia-induced changes in endothelial layer permeability with cultured cells from the middle cerebral artery. K. Takenaka, N.F. Kassell, J.A. Jane*, K.S. Lee Dept. of Neurological Surgery, Univ. of Virginia, Charlottesville, VA 22908.

The effect of hypoxia on endothelial layer permeability was examined in cells cultured from the middle cerebral artery. Permeability was assessed by measuring the passage of [^{14}C] labeled sucrose or albumin across a cellular monolayer seeded onto semipermeable polycarbonate filters. Cultures were placed in an hypoxic chamber for 1 to 18 h. During this time, no gross morphological changes were observed using phase-contrast microscopy. In addition, no increases in lactate dehydrogenase activity were observed in the medium. Permeability of sucrose increased gradually in a time-dependent manner with hypoxia; the rate of sucrose passage after 18 h of hypoxia was 120% of control levels ($p<0.05$). Re-oxygenation after hypoxia enhanced this effect to 200% of control levels. No significant change in the passage of albumin across the cellular monolayer was observed during 18 h of hypoxia. However, after re-oxygenation, albumin permeability was increased to 136% of the control level ($p<0.05$). In contrast, endothelial cells derived from the carotid artery were resistant to such permeability changes after hypoxia. These results suggest that re-oxygenation is an important factor in changing permeability of the intracranial, endothelial layer after hypoxia.

526.9

EFFECTS OF THE CYTOPROTECTIVE AGENT TIRILAZAD MESYLATE (U-74006F) ON THE TIME COURSE OF NEUTROPHIL INFILTRATION IN CEREBRAL ISCHEMIA. J.A. Oostveen* and L.R. Williams, CNS Diseases Res., The Upjohn Co., Kalamazoo, MI.

Using the gerbil unilateral carotid occlusion (UCO) model of transient cerebral ischemia, initial experiments identified a substantial accumulation of cytochrome oxidase (CO) - positive neutrophils (NLs) in the ischemic hemisphere after 24 hrs of reperfusion. The NL accumulation correlated with a severe neuronal death in the CA1 region of the hippocampus (H). Treatment with tirilazad at 10 mg/kg i.p. both immediately before and after the UCO resulted in a significant 64% reduction in NL accumulation, and a significant 25% reduction in the incidence of neuronal death. In the present experiments, animals were collected after 0 to 24 hrs of reperfusion, and frozen coronal sections were stained with CO and cresyl violet histochemistry. The incidence of neuronal death was scored semiquantitatively with a Viability Index (VI) of 0 (no damage) to 4 (complete neuronal loss). After 3 hrs UCO and 2 hrs reperfusion, beginning neuronal death was apparent ($VI = 0.36 \pm 0.10$, $n=11$), but NLs were not observed. After 4 hrs reperfusion, neuronal death was the same ($VI = 0.28 \pm 0.12$, $n=14$), and NLs were observed adherent to the endothelium. At 6 hrs reperfusion, migration of NLs into the parenchyma was extensive, and neuronal death was more advanced ($VI = 0.78 \pm 0.20$, $n=9$). After 6 hrs of reperfusion, tirilazad treatment resulted in an 80% reduction in NL accumulation, and an 85% improvement in the VI ($n=9$). Thus, tirilazad may act directly on the endothelial cell layer to limit peroxidative membrane damage and to limit chemotactic signaling, and may have a direct action on NLs to limit their activation, adhesion, diapedesis, or migration, thus limiting consequent NL-mediated oxidative neuronal destruction.

527.1

EARLY ISCHEMIC INJURY IN THE BASAL GANGLIA: AN IMAGING AND NEUROPATHOLOGICAL STUDY.
R.N. Bryan[1]*, C.A. Pardo[2], L. Monsein[1], V. Mathews[1] and P. Barker[1]. Neuroradiology Div.[1] and Neuropathology Lab[2]. The Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

Early magnetic resonance imaging (MRI) and neuropathological changes were evaluated in a model of regional ischemia in baboons. Models included complete ischemia (CI) and incomplete ischemia with reperfusion (IIR). The earliest changes in basal ganglia (BG) appeared in IIR as detected by MRI (three hours postischemia). Immunocytochemical studies included the analysis of neurofilaments, microtubule-associated protein-2 (MAP2), and calcium-binding proteins (calbindin and parvalbumin). The external portion of the striatum appeared to be the initial focus of necrosis. Loss of patch and matrix organization of striatum was one of the earliest changes, as demonstrated by a disappearance of compartmental distribution of MAP2. The magnitude of ischemic injury in IIR appeared to be more severe than CI. These findings suggest that disruption of neurocytoskeletal organization is an early change during ischemic injury and that reperfusion enhances ischemic damage.

527.2

DIFFUSION/PERFUSION MAGNETIC RESONANCE IMAGING OF CEREBRAL EMBOLISM IN RATS.
M. Tsuura, A.J. de Crespigny and J. Kucharczyk* Department of Radiology, UCSF, San Francisco, CA 94143.

To determine whether an intracerebral distribution of emboli can induce perfusion deficits and ischemic brain injury, diffusion/perfusion magnetic resonance(MR) imaging techniques were employed using a General Electric 4.7 Tesla imager before and after cerebral embolism in rats. Clot emboli made from venous blood which had been drawn 48hours earlier were injected into the right internal carotid arteries of male Sprague-Dawley rats(n=5). Diffusion-weighted spin echo imaging was used to detect early ischemic injuries. Sequential echo planar imaging(EPI)or gradient echo imaging(GRE) was carried out following bolus i.v. injection of the magnetic susceptibility contrast agent, DyDTPA-BMA(0.25mmol/kg), or after one minute apnea during which deoxyhemoglobin provided endogenous contrast. Images were also acquired during clot embolization to localize the intravascular distribution of the emboli.

Images acquired during blood clot injection showed signal loss in the right cerebral hemisphere which corresponded to the distribution of the emboli due to the magnetic susceptibility effect of deoxyhemoglobin. The spatial distribution of signal loss corresponded to perfusion deficits and hyperintense areas on diffusion-weighted images. On perfusion images, images acquired during one minute apnea demonstrated no signal changes in ischemic tissue. In normally perfused tissue there was less signal decrease during apnea in white matter than gray matter. Signal overshoot following rebreathing is also greater in gray matter as apnea-induced hypercapnea produces a greater increase of blood flow. Perfusion deficits in cerebral white matter were more visible on DyDTPA-BMA enhanced EPI or GRE.

MR imaging thus provided excellent in vivo mapping of the distribution of emboli in relation to cerebral perfusion deficits and acute ischemic injury.

527.3

EARLY DETECTION OF EMBOLIC STROKE BY CONTRAST ENHANCED MAGNETIC RESONANCE IMAGING.
K.H.Taber*, S.R. Northrup, J. Kirkpatrick and L.A. Hayman. Departments of Radiology and Pathology, Baylor College of Medicine, Houston, TX 77030.

Sequential magnetic resonance (MR) images were acquired at 2.35 Tesla in rabbits 30 minutes to 6 hours after induction of embolic stroke. The normal evolution on T2 weighted images was a gradual appearance of bright signal, with the area(s) of stroke moderately well delineated by 2-4 hours after onset. Contrast-enhancement with Gd-DTPA resulted in development of extremely bright signal on both T1 and T2 weighted images by 30-60 minutes after administration. This delayed enhancement indicates that the contrast agent is gradually pooling in the stroked tissue. Comparison of the bright areas on the MR images with the areas of stroke as determined by TTC staining indicated that the stroke was accurately portrayed. Thus, contrast administration can be used in this model of stroke to allow earlier identification of the affected area than is possible on non-enhanced MR images.

527.4

DIFFUSION MR IMAGING: CORRELATION WITH HISTOPATHOLOGICAL FINDINGS IN EVOLVING STROKE.
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Neuroimaging Branch,(NINDS, NIH) Bethesda MD 20892

Conventional T2 weighted magnetic resonance imaging (T2WI) is able to detect the vasogenic edema developing during cerebral ischemia but is inadequate for the evaluation of the size, severity and location of the lesion within the edematous area. In this study we investigated the ability of diffusion-weighted MRI (DWI), a technique sensitive to the Brownian motion of water molecules, to offer more specific information about the histopathological changes encountered in the evolving and complete stroke.

Focal cortical infarction was induced in rats by transcranial photoactivation of I.V. injected Rose Bengal . Axial T2-weighted and diffusion-weighted spin-echo images were acquired at various times for five days. The images obtained were matched with histological sections stained with Cresyl violet, H&E and triphenyltetrazolium chloride.

DWI was able to identify changes in the injured area as soon as 20 minutes after the induction of the ischemia. Moreover a clear discrimination among the necrotic core, a surrounding rim in which the tissue architecture was still preserved and the peripheral edematous gray matter was observed at 24 hours.

Our data suggest that DWI may be suitable for improving diagnostic capabilities as well as for testing "in vivo" the effectiveness of stroke therapies.

528

SYMPOSIUM. MOLECULAR BIOLOGY OF OLFACTION.
G.M. Shepherd, Yale (Chairperson); R.R. Reed, Johns Hopkins; L. Buck, Harvard; R. Axel, Columbia; D. Lancet, Weizmann Institute.

The molecular mechanism of odor discrimination has long been a central unsolved problem in sensory neurobiology. The last decade has seen enormous progress, from audacious hypotheses to a profound molecular understanding. These developments have culminated recently in the molecular cloning of candidate olfactory receptor genes. The purpose of this symposium is to convey the excitement generated by this breakthrough, summarize current understanding of the molecular basis of olfaction, and delineate the areas where this new knowledge impacts on the neurosciences.

G.M. Shepherd will provide an overview, correlating the molecular physiology and pharmacology of olfactory transduction with the molecular components of the transduction pathways, and delineating implications of the new studies for neural mechanisms of odor processing. **R.R. Reed** will summarize his studies in cloning and sequencing the G protein, adenylate cyclase, and membrane conductance channel in the sensory transduction pathway in the olfactory sensory neuron. He will describe his current work in identifying new members of the receptor gene family. **L. Buck** will describe the innovative strategy and methodology used in cloning and sequencing the large gene family for the candidate receptor proteins belonging to the 7 transmembrane domain receptor superfamily. She will discuss the complexities of the olfactory receptor repertoire in mammals. **R. Axel** will summarize the extension of his collaboration with Buck to a comprehensive characterization of the molecular and cellular organization of a fully tractable olfactory system in fish. He will discuss the implications of this model for the organization of the peripheral olfactory pathway. **D. Lancet** will delineate mechanisms for olfactory signal modulation: desensitization, possibly by receptor phosphorylation, and termination, by binding proteins, cytochrome P-450s and UDP glucuronosyl transferase. He will also report on genome mapping of human olfactory receptors, with implications for diversity and polymorphisms in human odor recognition.

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SYMPOSIUM. REGULATION OF NEURONAL PEPTIDE GENE EXPRESSION: LESSONS FROM THE NEUROHYPOPHYSEAL SYSTEM.
C. D. Sladek, Chicago Med. Sch. (Chairperson); E. Mohr, Univ. Hamburg; F. Bloom, Scripps Clin. Res. Found.; F. W. Van Leeuwen, Netherlands Instit. Brain Res.; D.M. Dorsa, Univ. Washington.

The goal of this symposium is to present and discuss several recent and exciting developments in our understanding of the mechanisms involved in neurosecretion. For decades, the neurohypophyseal system has served as a model system for elucidating cellular mechanisms of neurosecretion which have proved, in general, to be applicable to other neuronal systems. **Evita Mohr** will present evidence for transport of endogenous vasopressin (VP) and oxytocin (OT) mRNA from the hypothalamus to the posterior pituitary and discuss the potential functional significance of these mRNAs in the neural lobe. **Floyd Bloom** will discuss the finding that intrahypothalamic microinjection of VP mRNA leads to selective uptake, retrograde transport and expression of VP exclusively in the magnocellular neurons of the Brattleboro rat leading to a temporary reversal of diabetes insipidus for up to 5 days. **Fred Van Leeuwen** will present evidence for VP gene repair in Brattleboro rats, and discuss its impact on the expression of co-localized peptides. **Celia Sladek** will discuss in vitro approaches to studying the regulation of gene expression in the neurohypophyseal system including the role of second messenger systems, and **Daniel Dorsa** will present new information on the regulation of VP gene expression by hormone activated transcription factors. These new findings in the neurohypophyseal system promise to alter our view of the regulation of peptide secretion by neurons in general.

532.1

VALIDATION OF DUAL-DETECTOR PROBE SYSTEM FOR MONITORING HUMAN DOPAMINE D-2 RECEPTOR OCCUPANCY BY HALOPERIDOL IN VIVO K.J. Jeffries*, C.A. Tamminga, D.E. Dubois, R.R. Conley, D.F. Wong, H.L. Loats, E.K. Shaya, L.T. Young, C.J. Gounaris, B.B. Chin, B.C.K. Yung, R.F. Dannals, T.R. Guilarte, and H.N. Wagner University of Maryland, MPRC, Balto., MD 21228, Johns Hopkins Medical Institutes, Balto., MD 21205-2179

A dual detector probe (Bice, A.N., J Nucl Med 27:184-191, 1986) permits positron emission studies of the brain at much reduced radiation dose and cost in comparison to conventional PET. Coincidence counts obtained from the device can be used either directly or in an MRI-registered computer model if measurement of activity within specific regions of the brain is desired. Studies of the correlation between probe measurements and PET region-of-interest (ROI) data in humans have been carried out. In these studies, subjects were scanned with the probe immediately following a PET scan. Multiple studies were performed on each subject in either a haloperidol dose response or withdrawal paradigm. Haloperidol occupancy of dopamine D-2 receptors in the striatum was measured by its blockade of 3-N-[C-11]methylspiperone binding. The ratio of counts from a position directed at the striatum over a position directed at the cerebellum was used for the probe measure of receptor occupancy. The ratio of the patient's PET ROI sample for striatum over cerebellum was extrapolated to the time of the probe scan. Comparison of the probe measurements with the PET measurements in 17 studies demonstrated a linear correlation (r=.93). These data suggest that a simple dual-detector probe system can provide estimates of dopamine receptor occupancy that are useful for drug kinetic studies and reflect ratios obtained by positron emission tomography.

532.2

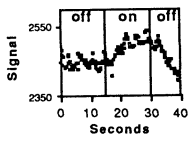
BLOOD VOLUME MEASURE OF BRAIN ACTIVITY DURING VISUAL STIMULATION USING MRI. F.A. Barrios[a], J.R. Zigun[a], J.A. Frank[b,c], K. Vladar*[,a], D.W. Jones[a], G. Sobering[d], C.T. Moonen[d], D.Z. Press[a], D.R. Weinberger[a]. Clinical Brain Disorders Branch, NIMH, Neuroscience Center at St. Elizabeths[a], Washington, DC. 20032. Diagnostic Radiology Research Program, OIR[b]. Diagnostic Radiology Department, CC[c]. Biomedical Engineering Instrumentation Program, NCRR[d] NIH, Bethesda MD 20892.

Advances in ultra fast image acquisition and in the application of contrast susceptibility effects have made it possible to perform dynamic physiological scanning with magnetic resonance imaging (MRI). We studied fourteen subjects during visual activation and during a dark control state. Scans were obtained every 2.05 seconds for two minutes using an unmodified GE SIGNA 1.5T scanner and a Turbo GRASS pulse sequence. Signal potentiation from a surface coil (N=7) and a standard quadrature head coil (N=7) was used. A bolus of Gd DTPA was injected during each condition. Cines produced from the series of rapid scans clearly depict an arterial, parenchymal, and venous phases. A pixel by pixel analysis of serial concentration maps derived from the rapid scans revealed expected cerebral blood volume (CBV) differences between gray and white matter as well as significantly increased CBV in calcarine cortex during visual activation versus control state (average increase of 15.59%, t=2.674, p<0.05 for the surface coil and 20.29%, t=3.495, p<0.05 for head coil).These results confirm earlier findings and demonstrate that dynamic CBV can be assessed with MRI during physiological activation.

532.3

REAL-TIME MAGNETIC RESONANCE IMAGING (MRI) OF BRAIN ACTIVITY IN HUMANS KK Kwong, JW Belliveau, CE Stern, JR Baker, DA Chesler, IE Goldberg, BP Poncelet, DN Kennedy, RM Weisskoff, MS Cohen, R Turner, H-M Cheng, TJ Brady, BR Rosen* MGH-NMR Center, 13th St. Building 149, Charlestown MA 02129 and Harvard Medical School. *RT at NIH NHLBI*

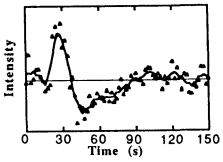
High-speed, noninvasive MRI methods sensitive to changes in cerebral blood flow (CBF) and oxygenation have been used to generate real-time functional MRI maps of human visual and motor cortex activation. Two different techniques, which do not require the injection of contrast agents, were used to study perfusion changes associated with neuronal activity. The first approach is sensitive to changes in blood flow. As regional CBF increases during brain activation, the number of magnetic spins flowing out of an activated volume leads to a shortening of tissue T1, and thus, higher signal in T1 weighted images. In the second approach, signal intensity is inversely proportional to the concentration of deoxyhemoglobin. As CBF exceeds local oxygen consumption, this leads to an increase in venous blood oxygenation and thus higher signal in T2* weighted images. Over 15 subjects underwent dynamic MR imaging using an echo planar imaging (EPI) technique. Changes in blood oxygenation were detected using a gradient echo imaging sequence sensitive to variations in T2* (TR=250-3000 ms, TE=50 ms). Changes related to CBF were evaluated using a spin echo inversion recovery, T1-sensitive pulse sequence (TI=1100 ms, TR=3500 ms, TE=42 ms). Typically, a series of 80-160 images were acquired in under 5 minutes. The figure shows signal intensity changes as a function of time for a region-of-interest within primary visual cortex, during darkness and during 8 Hz visual stimulation. Our non-contrast agent dependent, non-invasive MRI methods can be repeated safely in human subjects, thereby expanding the spatial-temporal window of *in vivo* brain investigation.



532.4

CEREBRAL BLOOD OXYGENATION AND BLOOD FLOW IN HUMAN SUBJECTS: MRI EVIDENCE FOR DECOUPLING DURING FOCAL BRAIN ACTIVITY. CE Stern*, KK Kwong, JR Baker, JW Belliveau, TJ Brady, BR Rosen Harvard Medical School and MGH-NMR Center, Charlestown, MA 02129.

High speed echo planar imaging techniques were used to track MR signal changes which reflect cerebral blood flow (CBF) and cerebral blood oxygenation levels. Gradient echo (GE) imaging sequences were used to track changes in blood oxygenation, while changes reflecting CBF were evaluated using an inversion recovery (IR) technique (for methods see abstract by Kwong *et al.*). Neuronal activity was altered in primary visual cortex using 8 Hz pattern-flash visual stimulation goggles. Activity within primary motor and somatosensory cortex was induced by active finger movements and passive tactile stimulation. In eight normal volunteers, the duration of the visual stimulus was altered repeatedly between 15, 30, 60, and 90 seconds to examine the effect of prolonged neuronal activation on the recovery time course of the MR measurements. During the stimulation period, MR signal intensity rose significantly above baseline on both the CBF and oxygenation sensitive sequences. Following the cessation of stimulation, the GE signal fell below the pre-stimulus baseline (see Figure); whereas, the flow sensitive IR signal did not drop below baseline. The recovery time of the GE signal undershoot ranges between 30 s and 50 s, and does not appear to be sensitive to variations in stimulus duration. The increased GE signal intensity during activation is strong evidence for increased postcapillary blood oxygenation, as the GE signal intensity is sensitive to changes in paramagnetic deoxyhemoglobin. In agreement with previous PET results, the results suggest that during normal neuronal activity, blood flow and oxygenation levels become uncoupled, with CBF far exceeding increases in O_2 utilization. The relatively long recovery time course of the undershoot may reflect increased O_2 extraction as a result of activity induced changes in pH.



532.5

QUANTITATIVE REGISTRATION OF FILM AUTORADIOGRAPHY AND ITS APPLICATION TO 3D ANALYSIS OF CEREBRAL BLOOD FLOW. W. Zhao*, J.Y. Chang, D.W. Smith and M.D. Ginsberg. CVDRC D4-5, Neurology, Univ. of Miami Sch. of Med., P.O. Box 016960, Miami, FL 33101.

Rat models of MCA occlusion are highly useful approximations of ischemia hemispheral infarction in humans. Three-dimensional (3D) representation and calculation of ischemia infarction has long been attracting people's interests. Quantitative registration of serial autoradiographic images can provide detailed 3D information of the entire neural system. We analyzed and compared several existing registration algorithms and applied them to 3D data set of rat brains with MCA occlusion. The principal axes scheme characterized by its efficiency is based on the calculated moments which indicate the centers of mass and the orientations of serial sections. With the cross-correlation method, one image is held fixed, while the second is repositioned by translation to overlay the first in every possible position. The point of maximum cross-correlation provides the necessary information for correction of translational misregister. Since the algorithm repeatedly uses the pixel gray values, it does not require well-defined serial section shapes and is less sensitive to noise. A novel image registration scheme using disparity analysis developed at this center is based on a linear affine model to analyze point-to-point disparities in two images. It is a direct method that estimates scaling, translation and rotation parameters simultaneously with mapping accuracy up to 100 microns. Quantitative comparisons and registered serial rat brain sections using these algorithms are presented. We also demonstrate the 3D shape of ischemia infarction at MCA occlusion studies.

532.6

SPECT STUDIES OF COGNITIVE ACTIVATION: INITIAL STUDIES OF A NOVEL DUAL-ISOTOPE TECHNIQUE. D.S. O'Leary*, M.T. Madsen, R. Hurtig, P.T. Kirchner, K. Rezai, M. Rogers, and N.C. Andreasen. Depts. of Psychiatry, Radiology, and Speech Pathology and Audiology, University of Iowa Hospitals and Clinics, Iowa City, IA, 52242.

We report an initial series of human studies using a novel SPECT technique to assess changes in regional cerebral blood flow (RCBF) due to cognitive activation. I^{123}-IMP and Tc^{99m}-HMPAO are administered to the same individual during different tasks. Images of the two tracers can subsequently be separated using a high-energy resolution scanner because the photopeaks of the two radionuclides differ (140 KeV for Tc99m versus 159 KeV for I123). A number of initial control studies were performed with the two tracers administered simultaneously, or at different times, but during performance of the same cognitive task. These studies indicated that the two tracers distribute similarly in the brain under similar physiological conditions. The variability in distribution *in vivo* was similar to that found in phantom studies. In two subjects we then administered HMPAO during an eyes-closed baseline task and IMP during visual checkerboard stimlation. Ratio images comparing baseline to activation condition showed a localized increase in RCBF in the occipital lobe. In two additional subjects we administered HMPAO during an easy baseline task and IMP during a difficult language task involving dichotic listening with directed attention instructions. The ratio images showed hemispheric asymmetry, with increased blood flow to the left temporal lobes while subjects listened for the semantic target. The dual-isotope technique permits analysis of RCBF during two different tasks with absolute anatomical registration of the resulting images. This strategy appears promising for study of localization of cognitive processing in normal subjects and in groups suffering from psychopathological conditions such as schizophrenia.

532.7

DECREASED GLUCOSE METABOLISM IN NORMAL AGING. W.J. Jagust, J.L. Eberling, B.C. Richardson*, T.E. Nordahl, N. Kusubov, B.R. Reed. Center for Functional Imaging, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720, and University of California, Davis, 95616.

Decreases in both regional cerebral blood flow (rCBF) and regional cerebral metabolic rates for glucose (rCMRglc) reportedly occur in normal aging, although this remains a controversial area. Here we present the results of a PET study of rCMRglc in 6 young (mean age = 27, SD = 4.6) and 8 old (mean age = 66, SD = 5.0) normal control subjects using a high-resolution (2.6 mm in-plane) PET tomograph and ^{18}F-fluorodeoxyglucose (FDG). We scanned three contiguous tomographic levels through the temporal lobes. Previously determined rate constants and an operational equation were used to determine rCMRglc. The rCMRglc values for temporal lobe structures were averaged over the three tomographic levels.

The results of a repeated measures analysis of variance revealed that, while the main effect was not significant ($p>0.05$), there was a significant ($p = 0.0001$) group x region interaction indicating that the pattern of metabolism differed for the two groups. A series of Bonferroni corrected t-tests revealed that the older controls had significantly lower rCMRglc only in the right and left anterior temporal cortex. Decreases in rCBF have also been reported for the temporal cortex in aged controls, and more severe decreases in both rCBF and rCMRglc occur in age-related diseases such as Alzheimer's disease. Thus, the current findings may reflect either a regionally specific rCMRglc decrease that occurs with normal aging, or early indications of cognitive dysfunction that is associated with age-related disorders.

532.8

REGIONAL CEREBRAL BLOOD FLOW (rCBF) AND GLUCOSE METABOLISM (rCMRglc) AT REST AND DURING ACTIVATION IN HEALTHY HUMAN AGING ASSESSED BY POSITRON EMISSION TOMOGRAPHY (PET). P. Pietrini *, B. Horwitz, C.L. Grady, J. Maisog, A.Gonzales-Aviles, E. De Micheli, S.I. Rapoport, M.B. Schapiro. Lab. of Neurosciences, Natl. Inst. on Aging, Bethesda MD 20892.

To understand the effects of age on rCBF and rCMRglc, we studied two groups of healthy men (6 young: mean age 24 ± 2 yr, range 22-26; 5 older: 65 ± 5 yr, range 60-74) using PET with [15-O]-water and [18F]FDG. In the same scanning session, subjects were studied under two experimental conditions (EC), at rest (ears/eyes covered; minimal room noise) and during sensory activation (SA) (watching a documentary film), using a Scanditronix PC-2048-15B (FWHM 6mm) PET scanner, and a double FDG injection procedure (Brooks et al., J Nucl Med 28: 53, 1987; Chang et al., J Nucl Med 28: 852, 1987), which allows two sequential FDG injections. rCBF and rCMRglc values were measured in absolute units of ml/100g/min and mg/100g/min, respectively. To reduce inter-subject variability, data were "normalized" to mean sensorimotor rCBF and rCMRglc values, respectively. Effects of age, EC, and age*EC were analyzed by ANOVA. Significant increases of absolute and normalized rCBF and rCMRglc values were found during SA bilaterally in the occipital regions in both age groups. Significant age and age*task effects were shown by frontal regions: at rest, rCBF and rCMRglc were higher in the young than in the old; SA induced increases in the young and decreases in the old, so that difference between the two groups became greater during SA. Coupling between rCBF and rCMRglc was present in both age groups and was stronger during SA. Although the meaning of the differential effects induced by SA on rCBF and rCMRglc in the frontal areas in young and old subjects is not clear and needs to be further investigated in a larger sample, these results suggest that rCBF and rCMRglc undergo more age-related changes in frontal than in other brain regions, and support brain activation as an ideal condition to enhance differences in brain metabolism between young and old subjects.

532.9

IN VIVO METABOLIC ABNORMALITIES OF THE FRONTAL LOBE IN AUTISM

N.J. Minshew, G. Goldstein, K. Panchalingam, S.M. Dombrowski, and J.W. Pettegrew*. Western Psychiatric Institute and Clinic, Univ. of Pgh. Med. Ctr., Pittsburgh, PA 15213.

Recent neurophysiologic and neuropsychologic studies in autism have implicated the cerebral cortex including the frontal lobes in the pathophysiology of this disorder. In this pilot study, the high-energy phosphate and membrane phospholipid metabolism of the brain were examined in the dorsal prefrontal cortex of 11 high functioning autistic adolescent and young adult males and 11 age, sex, IQ, race, and SES matched normal controls using *in vivo* ^{31}P NMR spectroscopy. When compared to the normal control group, the autistic group had decreased levels of phosphocreatine ($p<0.04$) and α-ATP ($p<0.02$). The levels of the high energy phosphate and membrane phospholipid metabolites were then compared within each subject group with IQ, and selected scores from tests of reasoning ability, secondary memory, and pragmatic-semantic language comprehension. Across clinical indices, a common pattern of clinical-metabolic correlations emerged in the autistic group. As test performance declined, the levels of the labile high energy compounds and of the membrane building blocks decreased, and the levels of the membrane breakdown products increased. Significant correlations were not observed in the control subjects. These data provide preliminary evidence of alterations in brain high energy phosphate and membrane phospholipid metabolism in the frontal lobes in autism which correlate with the clinical deficits. The findings suggest a hypermetabolic energy state and hypometabolism of brain membranes. The increase in phosphocreatine utilization is consistent with PET findings and may reflect less efficient pathways for processing information. The alterations in membrane phospholipid metabolism may be related to the truncation in dendritic tree development which has been observed in histoanatomic studies of autism.

ISCHEMIA I

533.1

DIFFERENT VULNERABILITY OF HIPPOCAMPAL GABA, PARVALBUMIN AND CALBINDIN IMMUNOREACTIVE NEURONS TO NEONATAL ANOXIA IN RATS. M.E.Dell'Anna, M.Molinari, M.Magarelli, M.C.Geloso, M.L.Rodrigo-Angulo*. Experimental Neurology Lab., Inst. of Neurology, Catholic University, Rome (Italy).

In adulthood, anoxic lesions of the hippocampus primarily affect GABAergic neurons while calcium binding proteins (CBPs) containing neurons are relatively spared. It has been recently demonstrated that neonatal anoxia induces a permanent cell reduction in the CA1 sector of the hippocampus (Dell'Anna et al.,Behav. Brain Res, 1991,45,125-134). The neurochemical characterization of this neuronal loss is still largely unknown. The present study was aimed at investigating the effects of neonatal anoxia on the development of the immunoreactivity (IR) for GABA or for the CBPs parvalbumin (PV) and 28 kd-calbindin (CB) in the hippocampus. Anoxia was induced by exposing rats at 30 hours after birth to nitrogen 100% for 25 minutes. Adjacent sagittal 40 um sections, obtained from animals sacrified at P7, P21, P30, P45 and P60, were processed for GABA, PV or CB according to the ABC-peroxidase immunohistochemistry. Animals exposed to neonatal anoxia, when compared with age-matched controls, showed a highly significant reduction of the number of GABA-IR neurons in CA1, CA2, CA3 sectors and in GD of the dorsal hippocampus at all the stages of life analyzed. Significant effects of neonatal anoxia on PV-IR neurons were evident only at P7 and P21 in CA1 and CA3, while no differences between the two experimental groups were observed at the P30 and P60 analyses. CB-IR neurons were significantly reduced in anoxic rats, with respect to the controls, at P7, P21 and P60 in CA1, CA2 and CA3 sectors, while no statistically significant differences between the two groups were evident at P30 and P45. In spite of the effects of neonatal anoxia on the number of neurons IR for GABA, PV or CB, the postnatal pattern of development of these antigens was similar in the two experimental groups. During postnatal development of both anoxic and control rats GABA-IR did not show significant modifications; PV-IR presented a progressive increment from P7 to P60, while CB-IR had a more variegated pattern with a decrement from P7 to P21 and a subsequentnt increment from P30 to P60. The present data demonstrate that, contrarily to that described after adult anoxia, neonatal anoxia induces permanent reduction of the number of GABA and CB-IR hippocampal neurons while it affects only temporarily the PV-IR neuron density.

533.2

IMMUNOHISTOCHEMICAL OBSERVATIONS ON GABAERGIC NEURONS FOLLOWING CARDIAC ARREST CEREBRAL ISHEMIA. K. Kawai, C. Ruetzler, L. Nitecka, J. Lohr, and I. Klatzo*. Stroke Branch, NINDS, NIH, Bethesda, MD 20892

Description of characteristic, early changes affecting predominantly GABAergic neurons (J. CBF&Metab. 12: 238-249, 1992), prompted the present study concerned with evaluation of changes in parvalbumin (PV) and glutamate decarboxylase (GAD), both proteins related to the GABA neurotransmitter. Observations with application of specific antibodies to these compounds revealed a marked reduction of both PV and GAD immunoreactivity in the neurons of the n. reticularis thalami (NRT), at 1 hr-interval following ischemia,Staining with GAD at that time also showed an intense immunoreactivity of enlarged and seemingly degenerating terminals and boutons in the ventral thalamic nuclei, medial geniculate, inferior colliculus and other structures. During the first week after ischemic insult, the GABAergic terminals and boutons in these areas appeared inconspicuous or absent. After 2 weeks, there was an indication of the regeneration and sprouting of new terminals, especially conspicuous in the ventral thalamic nuclei. Our study confirms early reactivity of GABAergic system following global ischemia, which may secondarily affect non-GABAergic neurons in various locations connected by GABAergic circuitry. Also, our study demonstrates a postischemic attempt at regeneration as shown by sprouting of presumably GABAergic terminals.

533.3

GLUTAMATE RELEASE INDUCED BY OXYGEN/GLUCOSE DEPRIVATION IN CORTICAL CELL CULTURES. D. Lobner* and D.W. Choi. Dept. of Neurology, Washington Univ. School of Med., St. Louis, MO 63110.

Murine cortical cell cultures, containing both neurons and glia, exposed to combined oxygen-glucose deprivation for 45-75 min developed acute neuronal swelling, and widespread neuronal degeneration by the next day. At termination of oxygen-glucose deprivation, an increase in bathing medium glutamate could be detected by HPLC using phenylisothiocyanate derivatization and UV detection: typically the glutamate concentration in the extracellular medium increased from about 200 nM to 2-3 μM. Assuming the glutamate originated from neurons (about 180,000 neurons per 16 mm well containing 375 μL medium), this would correspond to the release of 2.3 X 10^9 molecules of glutamate per neuron. Distributed in available neuronal cell body volume (mean neuronal cell body radius 7.8 microns), a concentration of 2 mM would result. Most likely, much of this glutamate originates from presynaptic terminals where original glutamate concentrations may be much higher. Both the increase in extracellular glutamate and neuronal death were attenuated by addition of 10 μM N6-cyclohexyladenosine, an A1-adenosine receptor agonist, or 100 μM Di-(o-tolyl)guanidine, to the cultures during the oxygen-glucose deprivation, consistent with the idea that the increase in extracellular glutamate is related to observed neuronal injury. While the measured increase may be too small to cause excitotoxic injury itself, higher glutamate concentrations may build up local to synaptic release sites.

533.4

EFFLUX OF (3H)-D-ASPARTATE FROM RAT HIPPOCAMPAL SLICES DURING IN VITRO ISCHEMIA IS NOT BLOCKED BY THE NA-COUPLED CARRIER INHIBITOR, DIHYDROKAINATE. V. Roettger* and P. Lipton. Dept. of Physiol., Univ. of Wisconsin, Madison, WI 53706.

Excess release of glutamate (GLU) very probably contributes to ischemic brain damage. The mechanism of ischemia-induced release is unknown but one widely suggested possibility is reversal of the Na-coupled high-affinity glutamate transporter. We examined this hypothesis using the transporter substrate (3H)-D-aspartate (ASP) and the transporter inhibitor dihydrokainate (DHK). 500 μM DHK completely blocked Na-coupled uptake of ASP.

Slices were loaded with ASP, then exposed to in vitro ischemia (IVI, 0-glucose/N_2 equilibrated buffer/37°C) for 3 successive 5 minute periods. ATP fell to <5% within 5 minutes. ASP efflux increased x1, x2.5, and x3.6 in the three periods. These increases were unaffected by 20 min. pre-incubation in 0-Ca buffer. 500 μM DHK did not affect ASP efflux during control period or during IVI.

50 μM veratridine increased ASP efflux by x1, x1.7, and x2.3 during 3 successive 5 minute periods. DHK completely blocked this increase, showing DHK very probably blocks ASP release when it occurs via reversal of the coupled transporter. Thus, Ca-independent GLU release during ischemia does not appear to occur via reversal of the Na-coupled transporter. Release of 3H-leucine from slices is not increased by IVI, indicating release does not result from membrane "leakiness". Other mechanisms are being investigated.

533.5

SODIUM REGULATES ANOXIA-INDUCED INTRACELLULAR CALCIUM CHANGES IN CA1 HIPPOCAMPAL NEURONS. J.E. Friedman* and G.G. Haddad, Dept. Pediatrics, Section of Respiratory Medicine, Yale Univ. Sch. of Med., New Haven, CT 06510

Anoxia is believed to induce neuronal damage by causing a sustained increase in Ca_i^{2+}, possibly via glutamate excitotoxicity. Using freshly dissociated adult rat hippocampal CA1 neurons, fluo-3 and confocal microscopy, we have previously shown that anoxia causes a rapid (within 2 min) increase in Ca_i^{2+} accompanied by swelling, but that this increase is not glutamate mediated. In addition, neuronal injury does not have to be accompanied by an increase in Ca_i^{2+}. To further understand the mechanisms underlying swelling and injury, we focused on the role of Na_o^+. Upon replacing Na_o^+ with N-Methyl-D-Glucamine (NMDG), Ca_i^{2+} increased, but anoxia caused a sharp decrease to below baseline level. Neurons remained morphologically intact, without swelling, for up to 90 minutes after anoxia. Adding Na^+ at any time after reoxygenation resulted in immediate swelling and injury. Addition of Bepridil (10μM), a Na^+/Ca^{2+} exchange blocker, caused this same sequence of events to occur as with NMDG, although the neurons did eventually swell following anoxia. EIPA (10μM), a Na^+/H^+ exchange blocker, had no effect. The Na^+-channel blocker TTX (9μM) did not affect baseline fluorescence, but caused a rapid decline in Ca_i^{2+} after an anoxia-induced transient rise. We conclude that 1) the increase in Ca_i^{2+} that occurs with anoxia is Na^+-dependent; 2) anoxia-induced neuronal swelling is caused by Na^+ entry and removal of Na_o^+ can protect against anoxia-induced injury; 3) to a great extent Na^+ does not enter through voltage-sensitive Na^+ channels and 4) possible reoxygenation injury requires the presence of Na_o^+.

533.6

REGIONAL DISTRIBUTION AND PHARMACOLOGICAL MODULATION OF BDNF mRNA EXPRESSION IN THE RAT BRAIN FOLLOWING THE PHOTOCHEMICAL STROKE. M.C. Comelli, M.S. Seren*, R. Canella, D. Guidolin and H. Manev. FIDIA Research Laboratories, 35031 Abano Terme, Italy

In order to understand the molecular mechanisms involved in degeneration and survival of neurons following an ischemic insult, we studied the expression of the brain-derived neurotrophic factor (BDNF) mRNA in the photochemical model of unilateral cortical stroke in rat (Ann. Neurol. 17: 497, 1985). We followed: 1) the time course (2,4,18 hrs after stroke) of expression of BDNF and NGF mRNAs by quantitative in situ hybridization; 2) the involvement of different glutamate receptors in BDNF mRNA regulation by pretreating the rats with MK 801, a non-competitive N-methyl-D-aspartate (NMDA)-sensitive glutamate receptor antagonist, or with 1S,3R-ACPD, a metabotropic glutamate receptor agonist (i.c.v.); 3) the effect of the neuroprotective drug GM1 (ganglioside which antagonizes glutamate excitotoxicity) on the expression of BDNF. The BDNF but not the NGF mRNA content increased and peaked in the ipsilateral cortex at 4 hours after the stroke (no changes were observed contralaterally at any time studied). MK 801 diminished the BDNF mRNA upregulation, thus indicating that most of the BDNF mRNA expression is related to the NMDA sensitive glutamate receptor activation. Both the 1S,3R-ACPD and GM1 pretreatment potentiated the BDNF mRNA upregulation consequent to the stroke.

533.7

RAPID RECOVERY OF INTRACELLULAR pH DURING RESUSCITATION FROM CARDIAC ARREST IN RATS. J.C. LaManna*, J.K. Griffith, B.R. Cordisco, C.-W. Lin, H.N. Ferimer, H.M. Kabert, K.L. Kutina and W.D. Lust. Dept. Neurology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

Brain intracellular pH (pHi) falls dramatically during the total cerebral ischemia that occurs during cardiac arrest. The level of acidosis depends upon the pre-arrest plasma glucose level and the amount of lactate accumulated during the arrest. Because hyperglycemia at the time of ischemia has been associated with poor recovery, tissue acidosis has been proposed as being responsible for tissue damage due to ischemia. The purpose of this study was to determine the time course of pHi and tissue lactate recovery and to find out if they are correlated in the postischemic recovery period.

Reversible cardiac arrest was produced in male Wistar rats (200-300g) by KCl administration, followed after 5-7 min by resuscitation by chest compression and mechanical ventilation. The pHi was determined in frozen rat brains by histophotometry of neutral red before cardiac arrest, at the end of the 7-10 min ischemic duration, and at 5, 15 and 60 minutes after succesful resuscitation. Control brain cortical pHi was 7.13 ± 0.06 (SE, n=3). During ischemia, pHi fell below 6.5 and then returned to control values within 5 min of resuscitation (7.15 ± 0.03, n= 3) and remained constant thereafter. This also occurred in rats made hyperglycemic before arrest. In both groups brain lactate levels after 5 min of resuscitation remained at ischemic levels. Reversal of acidosis was delayed by treatment with inhibitors of Na+/H+ exchange transport.

In conclusion, reversal of brain intracellular acidosis after resuscitation from cardiac arrest is rapid, and thus postischemic acidosis can play no continued role in tissue damage.

533.8

EXCITATORY AMINO ACIDS STIMULATE NEURONAL LACTATE PRODUCTION. S.H. Graham* and R.A. Swanson. Neurology Dept., U. California and VAMC, San Francisco, CA 94121.

It is well known that lactic acidosis occurs during stroke and may injure glia. Excitatory amino acids do not kill glia, yet N-methly-D-apartate (NMDA) antagonists prevent the death of glia in stroke. The effect of EAAs upon lactic acid production was studied in primary rat cortical cultures. Experiments were performed in pure neuronal cultures at day 11-13 after they developed NMDA sensitivity and at day 24-31 in astrocyte cultures. Medium lactic acid was measured by an enzyme-linked flurometric assay. Glutamate increased lactate production in pure neuronal cultures in a dose dependent manner from 57±11 in controls to 449±40 nmole/mg protein/hr at 1mM. NMDA had similar effects, but 100mM K+ increased lactate formation to only 160±11 nmole/mg protein/hr. Glutamate induced lactate formation was partially inhibited by MK801 and calcium free medium. Glutamate had no effect in astrocyte culture, but 100mM K+ doubled lactate production. These results suggest that EAAs could increase lactic acid formation in brain by two mechanisms: 1) by direct effects on neurons, and 2) by depolarizing neurons resulting in K+ release and increased glial lactate production. Since lactic acidosis may selectively injure glia, inhibition of EAA induced lactic acid production could explain why NMDA antagonists spare glia as well as neurons in stroke.

533.9

GLUCOCORTICOID PREVENTION OF HYPOXIC-ISCHEMIC DAMAGE: ROLE OF HYPERGYLCEMIA AND OXIDATIVE ENZYMES. U.I. Tuor*C.S. Simone, R. Arellano, K. Tanswell and M. Post. Neonatology, Hosp. for Sick Children and University of Toronto, M5G 1X8, Toronto, Canada.

Pre-treatment of neonatal rats with dexamethasone prevents brain damage associated with hypoxia-ischemia (unilateral carotid occlusion + 3hr hypoxia) (Ped Res 29:558-63, 1991). We presently investigate whether hyperglycemia or an induction of endogenous free radical scavengers explains dexamethasone's neuroprotective effect.

Pathological damage was examined in rats maintained hyperglycemic during hypoxia-ischemia by the administration of 10% dextrose (.1ml,ip) at 0, 1, 2 and 3 hr of hypoxia (n=14) and compared to that in control (n=15) or dexamethasone (.1 mg/kg ip, n=15) treated animals. Despite similar elevations in blood glucose at the end of hypoxia, dextrose treated animals had greater damage than dexamethasone treated animals and both of these groups had less damage than controls (volumes of damage of approx. 32.3±9.5, 3.1±2.2, 59±7.2 % ipsilaterally, respectively; $p<0.0001$).

Antioxidant enzymes were measured within brains of animals treated with dexamethasone or vehicle (n=44). Cerebral concentrations of catalase, glutathione peroxidase and CuZn or Mn superoxide dismutase were similar in both treatment groups, with or without exposure to hypoxia-ischemia.

Thus, the relative hyperglycemia associated with dexamethasone treatment may partially contribute to the protective effect of dexamethasone. However, an additional glucocorticoid mediated effect other than hyperglycemia or a change in antioxidant enzyme activity must also be involved in dexamethasone's prevention of hypoxic-ischemic damage. (Supported by the Heart and Stroke Fndn of Ont (T2065) and MRC (PG-42).

533.10

DEXAMETHASONE POTENTIATES HEAT SHOCK PROTEIN 72 INDUCTION IN PRIMARY ASTROCYTE CULTURES.
**S.C. Zhang, R.A. Swanson, F.R. Sharp and S.M. Sagar*
Dept. of Neurology, Univ. of California and VAMC, San Francisco, CA 94121.**

Hsp 72, one of the 70 kDa family of heat shock proteins, was induced in primary rat astrocyte cultures subjected to heating to 44°C for 40 minutes. Pretreatment with dexamethasone, 10^{-9} to 10^{-5}M increased hsp72 expression in the heated astrocytes with its peak effect at 10^{-7}M. Other steroids (cholesterol, estradiol, progesterone and aldosterone) had little effect. RU486, a glucocorticoid receptor antagonist, blocked the effect of dexamethasone. Heat shock decreased protein synthesis, as measured by incorporation of [^{35}S]-methionine into perchloric acid precipitable material; and this effect was antagonized by dexamethasone but not by the other steroids. These results suggest that dexamethasone potentiates the heat shock response in astrocytes by acting through the glucocorticoid receptor. The functional significance of this effect is being investigated.

533.11

PHOTOCHEMICALLY INDUCED CORTICAL LESIONS CHANGE THE IONOTROPIC GLUTAMATE RECEPTOR EXPRESSION PATTERN. D.M. Armstrong*, A.N. Kharlamov, D. Grayson, L. Marlier, R.J. Wenthold, E. Costa, A. Guidotti. FGIN, Georgetown Univ. Med. School, Washington, D.C. 20007 & (RJW) NIH, Bethesda, MD 20892.

The neuronal damage surrounding the primary foci of brain ischemia is in part mediated by the persistent stimulation of ionotropic glutamate receptors. It is therefore conceivable that the expression of these receptors may be modified in the process. In the photochemical rodent model (Watson B.D. et al., Ann. Neurol. 17:497-504, 1985) we studied whether there was a change in ionotropic glutamate receptor expression in the focal and perifocal areas. The ionotropic glutamate receptor subunits were visualized immunocytochemically using antibodies directed against GluR1, GluR2/3 or GluR4 or by in situ hybridization using cRNA probes that detect mRNA's for GluR1 and GluR2 and their alternatively spliced forms. In control brains the various antibodies and the two RNA probes yielded very unique cytological and topographical profiles. Following focal ischemia and in an area immediately surrounding the foci of the lesion (i.e., perifocal area) GluR1 and GluR2/3 immunolabeled cells were dramatically reduced in number and in staining intensity as early as 6 hours post-lesion. A similar rapid decrease was also observed for GluR1 and GluR2 mRNA. Importantly, in this perifocal area the relatively early decrease in the expression of glutamate receptor subunit proteins and message expression probably precedes any neuronal loss. In fact, these neurons still maintain the ability to respond to a glutamate induced event as demonstrated by the increased expression of c-fos mRNA and FOS. In contrast, GluR4 immunoreactivity was not reduced but rather in several instances displayed elevated levels which correspond both temporally and topographically with the rise in vimentin-positive and glial fibrillary acid protein-labeled elements both within the perifocal area and throughout the ipsilateral cortex. Importantly, the perifocal area is a region in which profound cellular reorganization occurs and is a key region to target various neuroprotective drugs.

533.12

ALTERATIONS IN THE DISTRIBUTION OF CYTOSKELETAL PROTEINS FOLLOWING TRANSIENT CEREBRAL ISCHEMIA. L.C. Pettigrew*, C. Schwab, S. Craddock, and J.W. Geddes. Stroke Program, Sanders-Brown Center on Aging and Dept. Anatomy & Neurobiology, Univ. Kentucky, Lexington, KY 40536.

The rat 4-vessel occlusion model of transient cerebral ischemia results in the loss of hilar neurons within a few hours and a delayed loss of CA1 pyramidal neurons. The purpose of this study was to determine whether alterations in the localization of cytoskeletal proteins precede neuronal death. We examined the distribution and levels of several cytoskeletal proteins (tau, MAP1, MAP2, MAP5, ß-tubulin and neurofilaments) at various reperfusion times (30 min, 2h, 6h, 24h and 72h) following 20 min of ischemia. Thirty min following reperfusion, a loss of tau immunoreactivity was evident in the inner 1/3 of the dentate gyrus molecular layer (DGML) and tau staining was increased in DG granule cells and in non-neuronal cells. By 6h the staining pattern in the granule and non-neuronal cells was similar to controls. At 24h, there was an accumulation of tau in the soma and proximal dendrites of CA1 and subicular neurons. By 72h, tau staining was normal except for the inner DGML and the necrotic CA1 neurons. In contrast to tau, the axonal distribution of phosphorylated neurofilaments was not altered following ischemia and reperfusion. With dendritic markers such as MAP2, a loss of staining was evident in CA3c 30 min following reperfusion. At 2h and 6h, the staining was similar to controls but at 24h there was a loss of MAP2 in the dendrites of CA1 and subicular neurons. At 72h, MAP2 had recovered except in necrotic CA1 neurons. The results demonstrate that alterations in the distribution of microtubule-associated proteins precede neuronal death in vulnerable neurons but that similar alterations occur, and are reversible, in other neuronal populations.

CARDIOVASCULAR REGULATION II

534.1

THE 10-HZ RHYTHM IN SYMPATHETIC NERVE DISCHARGE IS GENERATED BY A SYSTEM OF COUPLED NONLINEAR OSCILLATORS. S. Zhong*, Z.-S. Huang, G.L. Gebber, and S.M. Barman. Dept. Pharmacol. & Toxicol., Michigan State Univ., East Lansing, MI 48824.

We previously reported that the interval between the coherent discharges of different postganglionic sympathetic nerves can be frequency dependent in the 8- to 12-Hz band. In the current study we consider the possibility that the frequency-dependent interval is a property of a system of weakly coupled nonlinear oscillators. This model was tested by forcing the central system responsible for the 10-Hz rhythm in sympathetic nerve discharge (SND) with single or repetitive electrical stimuli applied to sympathoexcitatory sites in the medullary lateral tegmental field (LTF) or sympathoinhibitory sites in the medullary raphe. Recordings were made simultaneously from the postganglionic inferior cardiac, vertebral, and renal nerves of baroreceptor-denervated, decerebrate cats. Single shocks applied to the LTF or raphe reset the 10-Hz rhythm in SND whereas periodic stimulation at frequencies between 8 and 12 Hz entrained the rhythm (phase locking ratio of one). Lower frequencies (<6 Hz) of medullary stimulation entrained the 10-Hz rhythm in SND to a harmonic of the stimulus frequency. Most importantly, the preferred phase locking ratio of 10-Hz bursts of SND to each stimulus for one postganglionic nerve could be different than that for a second nerve when the stimulus frequency was <6 Hz. On the basis of these observations, we propose that the circuits responsible for the 10-Hz rhythms in SND may be modelled as a system of coupled nonlinear oscillators, each of which either influences one postganglionic nerve or nonuniformly affects different postganglionic nerves. (Supported by NIH grants HL-13187 and HL-33266.)

534.2

ARE THE DISCHARGES OF INDIVIDUAL ROSTRAL VENTROLATERAL MEDULLARY (RVLM) AND CAUDAL MEDULLARY RAPHE NEURONS CORRELATED TO BOTH THE 10-HZ AND 2-to 6-HZ RHYTHMS IN SYMPATHETIC NERVE DISCHARGE? S.M. Barman* and G.L. Gebber. Dept. Pharmacol. & Toxicol., Michigan State Univ., East Lansing, MI 48824.

We have used spike-triggered averaging of sympathetic nerve discharge (SND) to identify RVLM and caudal medullary raphe neurons with activity correlated to the 10-Hz rhythm in SND of decerebrate cats and to the 2- to 6-Hz rhythm in SND of anesthetized cats, but no one has yet determined if the discharges of individual medullary neurons are correlated to both rhythms. In many baroreceptor-denervated, decerebrate cats SND contains a mixture of 10-Hz and 2- to 6-Hz rhythms. In 13 such experiments we constructed spike-triggered averages of inferior cardiac SND that was selectively filtered with a low-pass setting of 6 Hz or a bandpass of 8 to 14 Hz. This allowed us to test for a relationship between medullary unit activity and each rhythm independently. We identified six raphe and two RVLM neurons with activity correlated to both rhythms in SND. Spike-triggered averaging revealed that the discharges of 17 other neurons (seven RVLM and 10 raphe) were correlated to the 10-Hz but not to the 2- to 6-Hz rhythm in SND. We also recorded from four RVLM neurons with activity correlated to the 2- to 6-Hz but not to the 10-Hz rhythm in SND. Since the discharges of ~38% of the raphe and 15% of the RVLM neurons were correlated to both the 10-Hz and 2- to 6-Hz rhythms in SND, to some extent the networks responsible for these rhythms used a common pool of medullary neurons. There was more convergence from the sources of the two rhythms onto raphe neurons than onto RVLM neurons. The significance of this finding remains to be determined. It is also not known whether RVLM and raphe neurons with activity correlated to only one of the rhythms in SND would have developed a discharge pattern correlated to the other rhythm under different experimental conditions. (Supported by NIH grants HL-33266 and HL-13187.)

534.3

THE 2- TO 6-HZ RHYTHM IN SYMPATHETIC NERVE DISCHARGE (SND) IS AN EMERGENT PROPERTY OF A BRAINSTEM NETWORK COMPOSED OF NONRHYTHMICALLY ACTIVE NEURONS. G.L. Gebber*, Z.-S. Huang, S. Zhong, and S.M. Barman. Dept. Pharmacol. & Toxicol., Michigan State Univ., East Lansing, MI 48824.

Individual medullary neurons with activity correlated to the 2- to 6-Hz rhythm in SND of baroreceptor-denervated cats have firing rates that generally are lower than the frequency of the rhythm recorded from postganglionic nerves. These neurons miss firing in a variable number of cycles of SND. Thus the rhythm in SND is not strictly represented in the spike trains of single medullary neurons. This suggests that each cycle of SND reflects synchronization of the discharges of a small and continuously changing subset of the neurons comprising the brainstem network responsible for this rhythm. We tested this hypothesis by determining whether the 2- to 6- Hz rhythm appears in population recordings (i.e., field potentials) made from the rostral ventrolateral medulla (n=6), medullary raphe (n=5), and lateral tegmental field (n=1). Autospectral analysis revealed peaks in the 2- to 6-Hz band of activity recorded from these brainstem sites and the inferior cardiac and renal sympathetic nerves. The shapes of the autospectra of brainstem activity and SND were indistinguishable. Coherence analysis demonstrated that the rhythms in brainstem activity and SND were significantly correlated. Peak coherence values were as high as 0.56. Cervical spinal cord transection reduced inferior cardiac and renal SND to 12±4 and 11±4% of control, respectively, and eliminated the coherence of brainstem activity to SND. In contrast, spinal cord transection only modestly reduced the power in brainstem population activity to 85±2% of control. Most importantly, the shape of the autospectrum of brainstem activity was unchanged. We conclude that the 2- to 6-Hz rhythm in SND arises from a network of brainstem neurons whose discharges are probabilistically related to the phases of the population rhythm. (Supported by NIH grants HL-13187 and HL-33266.)

534.4

ROLE OF THE SPINAL CORD (SC) IN GENERATING THE 2-6 Hz PEAK IN THE POWER SPECTRUM OF RAT SYMPATHETIC DISCHARGE (SND). A.M. Allen*, J.M. Adams and P.G. Guyenet. Depts. of Pharmacology and Biomedical Engineering, Univ. of Virginia, Charlottesville, VA 22908.

The existence of a 2-6 Hz "rhythm" in SND, and the fact that the discharge of various types of medullary neurons is statistically correlated with this rhythm, have fueled arguments against the pacemaker theory of SND generation. The latter states that the basal SND largely arises from the intrinsic pacemaker discharges of excitatory premotor neurons located in the rostral ventrolateral medulla (RVL PMNs). The main objection to the theory is that the discharge rate of these cells (5-35 Hz) is much faster than the peak frequency of the SND "rhythm" in rats (3-4 Hz). This objection rests on the assumption that the discharge rate and/or pattern of RVL PMNs is critical to the generation of the 2-6 Hz "rhythm". The following data (in debuffered, urethan-anesthetized rats) suggests this assumption may be invalid. SND synchronization was determined by measuring the fractional power contained in the 2-8 Hz band of 0-50 Hz power spectra derived from bipolar recordings of lumbar SND (0.3-1000 Hz recordings). Unilateral microinjection of muscimol ($GABA_A$ agonist), or bilateral injection of a mixture of kynurenic acid (KYN, EAA receptor antagonist) and bicuculline ($GABA_A$ receptor antagonist), into RVL, produced little effect on the 2-6 Hz rhythm. Intrathecal injections of KYN, at mid-thoracic levels slightly reduced the 2-6 Hz peak but SC transection at C_2 had no effect, after SND had been restored close to control level by intrathecal injections of kainic acid (EAA receptor agonist). We conclude that i) the firing rate and pattern of the RVL input to the SC is not critical for production of a 2-6 Hz peak in SND power spectra, ii) in the rat this synchronization may be essentially of SC origin and iii) the existence of a 2-6 Hz rhythm of SND does not constitute an objection to the pacemaker theory of SND generation. Support: HL 28785 from NIH and Medical Research Council of Australia.

534.5

DIRECT PROJECTIONS FROM A VASODEPRESSOR AREA IN THE GIGANTOCELLULAR RETICULAR NUCLEUS ONTO NEURONS IN THE THORACIC SPINAL CORD. S.A. Aicher*, D.J. Reis and T.A. Milner. Dept.of Neurol. & Neurosci., Cornell Univ. Med. College, NY, NY 10021.

We have recently identified a vasodepressor area within the ventral and caudal region of the medullary reticular nucleus gigantocellularis, the gigantocellular depressor area (**GIDA**), that is topographically distinct from other vasoactive sites in the medullary reticular formation (Aicher & Reis, Neurosci. Abstracts, 1991). We sought to determine if efferents from the GiDA project to the intermediolateral cell column (IML) and lamina VII of the thoracic spinal cord and to characterize their synaptic relations with neurons in these areas. The anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L) was iontophoresed into the GiDA of rats. In some cases, the GiDA was identified as a vasodepressor site by microinjection of glutamate into the site prior to the PHA-L iontophoresis. Ten days later, rats were anesthetized and perfused with acrolein and paraformaldehyde. Sections of the thoracic spinal cord (T2) were processed for immunocytochemical localization of PHA-L. The injection sites were located and verified as being confined to the GiDA. PHA-L labelled punctate fibers were seen in the medial aspect of the IML and throughout lamina VII. Electron microscopic analysis of the IML and lamina VII in these cases showed that PHA-L immunoreactivity was seen in myelinated and unmyelinated axons, as well as terminals (0.3 - 0.8 μm diameter). PHA-L labelled terminals contained small, clear vesicles and formed symmetric synaptic contacts primarily on large and small dendrites of neurons in the IML and lamina VII. These data demonstrate that neurons of the GiDA project directly to autonomic laminae of the thoracic spinal cord. Moreover, the present results indicate that this may be a direct and novel medullospinal sympathoinhibitory pathway. (Support: NIH #HL08251 & #HL18974).

534.6

BAROSENSORY NEURONS IN THE CAUDAL VENTROLATERAL MEDULLA MONOSYNAPTICALLY PROJECT ONTO BULBOSPINAL NEURONS IN THE ROSTRAL VENTROLATERAL MEDULLA. I. Jeske*, D.J. Reis and T.A. Milner. Div. of Neurobiology, Dept. of Neurology & Neuroscience, Cornell Univ. Med. Coll., New York, NY 10021.

Neurons of caudal ventrolateral medulla (CVL) may function as interneurons in the baroreceptor reflex arc by inhibiting sympathoexcitatory bulbospinal neurons in rostral ventrolateral medulla (RVL). While some CVL neurons are excited orthodromically by baroreceptors and antidromically from RVL (Jeske et al., *Am. J. Physiol.*, 1992, in press), evidence is lacking that these neurons monosynaptically innervate bulbospinal neurons of RVL. We sought to establish a direct CVL-RVL bulbospinal synapse. The CVL was explored in 10 anesthetized rats with microelectrodes containing biocytin (BCT). Six neurons excited by elevating arterial pressure and with cardiac-related activity met criteria of barosensory interneurons. BCT was iontophoresed into the CVL site, while gold-conjugated wheat-germ agglutinated apo-horseradish peroxidase (WAHG) was injected into the spinal (T3) intermediolateral cell column. After 18-24 h, sections of RVL were processed for both markers. By light microscopy, numerous BCT-labeled varicose processes overlapped RVL neurons containing WAHG. Ultrastructurally, the BCT-labeled terminals were large (0.6-1.2 μm in diameter) and contained small clear vesicles. These formed symmetric (inhibitory) synapses on somata and large dendrites within the RVL, many containing WAHG (associated with lysosomes). The results indicate: (a) barosensory sympathoinhibitory neurons in the CVL terminate on bulbospinal neurons in the RVL; (b) the synaptic profile is consistent with an inhibitory interaction, possibly GABAergic. The findings demonstrate a direct CVL-RVL projection supporting the hypothesis that the CVL is an intermediate relay in the baroreceptor reflex arc. (Supported by HL 18974 & HL 08326)

534.7

ACTIVATION OF Ca^{2+} CURRENTS IN RETICULOSPINAL PACEMAKER NEURONS OF RAT ROSTRAL VENTROLATERAL MEDULLA BY CYANIDE AND HYPOXIA: ARE THEY CENTRAL OXYGEN SENSORS? M.-K. Sun* and D.J. Reis. Div. of Neurobiol., Cornell Univ. Med. Coll., New York, NY 10021.

Reticulospinal sympathoexcitatory (pacemaker) neurons of rostral ventrolateral medulla (RVL) are rapidly excited (<2 sec) *in vivo* by systemic hypoxia or by cyanide (CN) applied by microinjection and/or ionophoresis (Sun et al., *Am. J. Physiol.* 262:R182, 1992). It is not known whether hypoxia/CN directly excite RVL neurons and if so by what cellular mechanism. Electrophysiological studies were conducted in slices of rat medulla maintained in an interface-type chamber at 31°C. Pacemaker neurons were spontaneously active. Hypoxia (75% N_2, 5% CO_2, 40s) or CN (30-300 μM, 40s application) rapidly and substantially increased their activity. The activity of adjacent neurons was unaffected or reduced. The excitatory response to CN was dose-dependent, reversible and reproducible at 10 min intervals. It was characterized by membrane depolarization (21.4±4.2 mV with 300 μM CN) and a decrease in membrane resistance (38.2±4.1 M ohm at 300 μM CN). The response persisted in the presence of tetrodotoxin (TTX, 10 μM), which eliminated evoked and spontaneous action potentials indicating that hypoxic excitation was not a consequence of synaptic excitation. Under single electrode voltage clamp, CN evoked an inward current (peak: 0.58±0.08 nA) which was voltage-dependent and transient at membrane potentials between -70 and -40 mV (when stepped from -100 mV). The current was abolished by 2 mM $CoCl_2$ or 100 μM $NiCl_2$, but not 50 μM $CdCl_2$. The results are consistent with a view that RVL pacemaker neurons are excited by hypoxia by increasing low voltage-activated calcium channel conductance. RVL neurons may, like glomus cells of carotid body, act as oxygen sensors. They may be responsible for initiating the sympathetic excitation associated with cerebral ischemia.

534.8

ELECTROPHYSIOLOGICAL IDENTIFICATION OF DEPRESSOR NEURONS IN THE CAUDAL VENTROLATERAL MEDULLA WITH PROJECTIONS TO ROSTRAL VENTROLATERAL MEDULLA IN THE RABBIT. W.W. Blessing* and Z.I. Gieroba. Centre for Neuroscience, Flinders University, Bedford Park, SA 5042, Australia.

The caudal ventrolateral medulla oblongata (CVLM) contains depressor neurons whose activity tonically inhibits sympathetic vasomotor tone, probably by inhibiting sympathoexcitatory neurons in the rostral ventrolateral medulla (RVLM). In urethane-anesthetized rabbits (1.5 g/kg, i.v.) we recorded extracellularly from CVLM neurons identified by antidromic activation from RVLM and examined their responses to changes in blood pressure and electrical stimulation of the aortic depressor nerve. Peristimulus time histograms were constructed using an ITC16 interface and a Macintosh IIfx computer programmed with IGOR. We identified 134 spontaneously active neurons in the CVLM with projection to or through the RVLM. Twenty nine of 107 neurons tested (27%) were activated by increase in blood pressure, 60% were inhibited and 13% were unaffected. Injection of noradrenaline increased discharge rate of 29 neurons from 1.7 ± 0.3 spikes/s to 6.5 ± 1 spikes/s. Electrical stimulation of the ipsilateral aortic depressor nerve (3 pulses, 200 Hz, 0.5 ms) activated these neurons (latency 55 ± 4 ms, conduction velocity 0.6 m/s). Neurons excited by an increase in blood pressure were located in the previously defined caudal vasodepressor region and also as far rostrally as 0.5 mm rostral to the obex. These neurons may form part of the central inhibitory link in the baroreceptor-vasomotor pathway. Other neurons may be inhibitory vasomotor cells with functions independent of baroreceptor inputs or they may be A1 catecholamine neurons with axons passing through the RVLM.

534.9

CONNECTIONS BETWEEN PONTINE RETICULAR FORMATION AND ROSTRAL VENTROLATERAL MEDULLA IN CARDIOVASCULAR CONTROL. A.V. Krassioukov* and L.C. Weaver. The John P. Robarts Research Institute and Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada N6A 5K8

A previous study in our laboratory established that the pontine reticular formation (PRF) is involved in the neural control of circulation (Hayes and Weaver. Neurosci. Abst.17: 706,1991). The connections between PRF and the well-known rostral ventrolateral medulla (RVLM) are undefined. To determine these connections we investigated responses of renal sympathetic nerve activity (RSNA), arterial pressure (AP) and heart rate (HR) to microinjection of the inhibitory amino acid glycine (1.0M, 60nl) into the PRF before and after unilateral or bilateral injection of the synaptic blocking agent cobalt (4.0mM, 200nl) into the RVLM in propofol-anesthetized rats (n=19). Glycine injection into the PRF caused decreases in AP (-20±2 mmHg), HR (-7±1 bpm) and RSNA (-38±4%). Following microinjection of Co^{++} into the ipsilateral RVLM, responses to PRF blockade were significantly decreased: AP (-9±3 mmHg), HR (-4±0 bpm), RSNA (-6±2%). In contrast, injections of Co^{++} into the RVLM contralateral to the site of PRF blockade did not affect responses to this PRF blockade. Responses to PRF blockade after bilateral injection of Co^{++} into RVLM were similar to responses after unilateral injection of Co^{++}. Somato-sympathetic reflexes elicited in the renal nerve by electrical stimulation of the sciatic nerve were significantly inhibited after unilateral injection of Co^{++} into the RVLM and, after bilateral injection, they were abolished. The Co^{++} injections had no effect on basal firing of the sympathetic nerve or on arterial pressure or heart rate. These results suggest that the PRF influences on RSNA are relayed ipsilaterally from pons to RVLM to influence spinal sympathetic neurons. Supported by the Ontario Heart and Stroke Foundation.

534.10

ACTIVITY OF NEURONS IN THE PONTINE RETICULAR FORMATION PROVIDES TONIC EXCITATORY DRIVE TO NEURONS IN THE ROSTRAL VENTROLATERAL MEDULLA IN RATS. K. Hayes* and L.C. Weaver. The John P. Robarts Research Institute and Department of Physiology, University of Western Ontario, London, Ontario, Canada.

Recent studies have shown that neurons located in the pontine reticular formation (PRF) make an important contribution to ongoing activity of sympathetic nerves. To determine if the PRF is a source of tonic activity for sympathoexcitatory neurons in the rostral ventrolateral medulla (RVLM), discharge of PRF neurons was inhibited by unilateral microinjections of glycine (1.0M;50nl) while recording spontaneous discharge of neurons in the RVLM in 14 Saffan-anesthetized rats. Each RVLM unit was characterized by means of three tests. Units that displayed spontaneous activity inhibited by baroreceptor activation and synchronized to the cardiac cycle were regarded as "cardiovascular" neurons. RVLM neuronal spike-triggered averages of renal sympathetic nerve discharge were also constructed to determine temporal correlation of unit activity to sympathetic activity. Spontaneous activity was recorded from 15 cardiovascular neurons in the RVLM. Glycine injection into the ipsilateral PRF silenced the spontaneous activity of 10 cardiovascular units. Activity of these units returned to control discharge rate after 53±6s. Glycine injection had no effect on the discharge of 5 cardiovascular units. Spontaneous activity of 6 control units was not changed by baroreceptor activation and was not synchronized to the cardiac cycle. PRF blockade had no effect on the spontaneous activity of these control units. Glycine injection into the PRF caused decreases in arterial pressure (-28±5mmHg), heart rate (-23±3bpm) and renal nerve activity (-43±9%) that also returned to control in 50±6s. These results indicate that PRF neurons provide significant tonic excitatory drive to some cardiovascular neurons located in the RVLM. (Support: Ontario Heart & Stroke Foundation).

534.11

EFFECTS OF KAINIC ACID MICROINJECTIONS IN THE LATERAL TEGMENTAL FIELD ON THE SYMPATHOLYTIC ACTION OF 8-OH-DPAT M.E. Clement* and R.B. McCall, Cardiovascular Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

Sympathetically related neurons have recently been identified in the lateral tegmental field (LTF). A sub-population of these, the sympathoexcitatory (LTF-SE) neurons, have been shown to project to and provide an excitatory input to the rostral ventrolateral medulla. We have demonstrated that these LTF-SE neurons are inhibited by i.v. 8-OH-DPAT and iontophoretic 8-OH-DPAT and 5-HT. On the other hand, i.v. 8-OH-DPAT increased the firing rate of sympathoinhibitory neurons in the LTF (LTF-SI). LTF-SI units were insensitive to iontophoretic 8-OH-DPAT but were excited by iontophoretic 5-HT. The present experiments were designed to investigate the effects of kainic acid microinjections into the LTF on the sympatholytic effects of 8-OH-DPAT. Kainic acid has been reported to destroy cell bodies while leaving fibers of passage intact. Bilateral microinjections of kainic acid resulted in slight (6% ± 5.5 %) increases in MAP, negligible alterations in HR, and a 20% ± 16% increase in CNA. The maximum dose of 8-OH-DPAT caused a 19% decrease in MAP, an 8% decrease in HR, and a 26% increase in CNA (n = 14). Cardiac nerve activity could subsequently be inhibited by administration of clonidine. These values differed significantly from control animals in which 8-OH-DPAT reduced CNA and MAP 95% and 40% respectively. Histological examination of microinjection sites revealed the extent of diffusion of kainic acid to range from 300 to 500 microns from the point of injection, with diffusion being slightly greater along the dorso-ventral axis. These results indicate that the sympathetically related neurons in the LTF play an important role in the sympatholytic action of 8-OH-DPAT, and suggest disparate central sites for the action of 8-OH-DPAT and clonidine.

534.12

CARDIOVASCULAR PROJECTIONS FROM THE AMYGDALA (AMG) TO THE BED NUCLEUS OF THE STRIA TERMINALIS (BST). S. Roder* and J. Ciriello, Dept. of Physiology, Univ. of Western Ontario, Canada, N6A 5C1.

Two series of experiments were done to investigate the possibility that the depressor responses elicited by electrical stimulation of AMG were mediated via projections to BST. In the first series, to determine whether AMG neurons innervate the cardiovascular region of BST, projections to BST from different subnuclei within AMG were investigated in the rat using the retrograde tracer Fluorogold (FG; 2%) and the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L; 2.5%). FG (50-100nl) injections overlapping the cardiovascular region of BST resulted in retrogradely labelled neurons throughout the AMG. Iontophoretic PHA-L injections in the central nucleus of the AMG (ACe) and basolateral nucleus of the AMG (ABL) resulted in dense fiber and presumptive terminal labelling within the cardiovascular region of BST. In the second series, the effect of blocking synaptic transmission in BST with $CoCl_2$ or chemical lesions of BST with ibotenic acid (5ug/ul) on the depressor response elicited by stimulating AMG was investigated in the chloralose anesthetized, artificially ventilated and paralyzed rat. Five mM microinjections (200nl) of $CoCl_2$ into BST significantly reduced (41.2±7.3%) the depressor response during stimulation of ACe. On the other hand, the depressor response to stimulation of ABL was significantly potentiated (57.0±16%) after $CoCl_2$ injections into BST. Microinjections of ibotenic acid (200nl) into BST resulted in similar affects on the depressor responses to AMG stimulation. Taken together, these results suggest that AMG neurons innervate neurons in cardiovascular regions of BST and that these BST neurons are a component of the neuronal circuit that mediate cardiovascular responses elicited during stimulation of AMG. (Supported by MRC and HSFO).

CALCIUM CHANNEL MODULATION

535.1

TWO VOLTAGE INDEPENDENT CALCIUM CHANNELS ARE ACTIVATED BY PERTUSSIS TOXIN IN PC12 CELLS. S.A. Scott* and J.A. Strong, Dept. of Biol. Sci., Purdue University., W. Lafayette, IN 47907.

Voltage independent calcium channels have been described in a variety of cell types. This broad class of Ca^{++} channels includes channels that are regulated by either extracellular or intracellular ligands. We have observed two novel voltage-independent Ca^{++} channels with different slope conductances (3pS and 16pS using 20mM Ca^{++} as the charge carrier) in undifferentiated PC12 cells. We found that these channels open rarely during cell-attached recordings. The 3pS channel was activated by patch excision into a defined K-aspartate EGTA solution (n=13). Channel activity could be observed even at very negative potentials (eg. -110 mV). However, if the cells were pretreated with pertussis toxin (8-10 hours), both the small conductance Ca^{++} channel and the larger conductance Ca^{++} channel were active even in the cell-attached configuration (n=10). Like the 3pS channel, the 16pS channel also was seen over a wide range of potentials. Activity of both channels was observed at the cells' resting membrane potential. The effect of pertussis toxin suggests that these channels are normally under the control of a GTP-binding protein. The 3pS channel maybe similar to the pertussis toxin activated Ca^{++} channel reported by Ceña *et al* [1] in bovine chromaffin cells. Preliminary results suggest that bradykinin (10uM) also activates the smaller conductance channel (n=3) via an intracellular pathway. This suggests that this channel maybe responsible for the sustained Ca^{++} infux seen in cells treated with bradykinin. A physiological activator of the 16pS channel has yet to be found .

[1] V. Ceña *et al* ., *J. Membrane Biol.* **122**:23-31.

535.2

SUBSTANCE P INHIBITS Ca^{2+} CURRENTS IN RAT SYMPATHETIC NEURONS VIA A PERTUSSIS TOXIN-INSENSITIVE G-PROTEIN. M. S. Shapiro and B. Hille*. Dept. Physiology and Biophysics, Univ. Washington, Seattle, WA 98195.

We studied inhibition of N-type Ca^{2+} channels in adult rat superior cervical ganglion (SCG) neurons by substance P (SP) using the patch clamp. In whole-cell configuration with external 5 mM Ca^{2+}, 70 of 82 acutely dissociated SCG cells showed inhibition by 500 nM SP. Peak currents elicited by 12 ms voltage steps to 0 mV were reduced by 37 ±2% (mean±SE). Treatment of overnight SCG cultures with 500 ng/ml pertussis toxin (PTX) had no effect on SP inhibition compared to treatment with 500 ng/ml heat-inactivated PTX (59±7%, n=8 *vs.* 60±8%, n=7). Dialysis with 2 mM GDP-β-S for >7 min. reduced SP inhibition (to 16±3%, n=12 *vs.* 33±5%, n=12), implicating G-proteins. Inhibition measured by tail currents to -60 mV following voltage steps to 0 mV (38±6%) and 120 mV (38±5%) was not voltage dependent, and current activation was not slowed or biphasic, indicating a mechanism other than a "reluctant-willing" type. Exposure of cells to 1 μM neurokinin A (n=10) or 1 μM neurokinin B (13 of 14 cells) was without effect, suggesting NK_1 but not NK_2 or NK_3 subtypes of tachykinin receptors. Intracellular Ca^{2+} levels seem unimportant since SP inhibition with 0.1 mM BAPTA in the pipette (48±8%, n=9) was only slightly greater than with 20 mM BAPTA (33±5%, n=9). Cell-attached patches with SP applied to the bath were used to test for a diffusible messenger. With 120 mM $BaCl_2$ in the pipette and 150 KCH_3SO_4, 0 Ca in the bath, only 1 of 12 cells showed inhibition of currents in the patch upon addition of 500 nM SP. We conclude that the SP signaling pathway is BAPTA insensitive, membrane delimited and may operate by direct action of a PTX-insensitive G-protein on Ca^{2+} channels in SCG neurons.

535.3

MU-OPIOID-RECEPTOR-MEDIATED REDUCTION OF N-TYPE NEURONAL CALCIUM CURRENT OCCURS VIA A Go-TYPE GTP-BINDING PROTEIN. H.C. Moises* and R.L. Macdonald#, Dept. of Physiology and #Neurology, Univ. of Michigan, Ann Arbor, MI 48109.

Mu-opioid receptor activation can decrease calcium currents in sensory neurons of the dorsal root ganglion (DRG) in rat (Schroeder et al.1991) and in the SH-SY5Y human neuroblastoma cell line (Seward et al., 1991), and in the latter case a pertussis-toxin (PTX) sensitive G-protein was shown to be involved. In this study, we sought to determine whether a PTX sensitive G-protein also mediates the inhibitory coupling between mu-opioid receptors and calcium channels in rat sensory neurons, and if so to identify the specific subtype (Gi or Go) involved. We recorded whole cell calcium currents from acutely dissociated DRG neurons from 19-25 d rats, using Ca^{++} (5mM) as charge carrier. The effects of puffer application of the mu-selective agoinst PLO17 (300-3000 nM in the pipet) were examined on low-threshold transient (T-type) and high-threshold inactivating (N-type) and sustained (L-type) current components, isolated on the basis of their voltage dependency of inactivation and sensitivity to ω-conotoxin (ω-CTX, 1μM). PLO17 selectively reduced high threshold ω-CTX sensitive inactivating currents, whereas T-type and L-type currents were largely unaffected. The effects of PLO17 (1 μM) were blocked by naloxone (10-300 nM) and by ß-FNA (10 μM), but not by nor-BNI (10-50 μM), a kappa specific antagonist. The inhibition by PLO17 was irreversible in cells dialysed with GTPγS and was blocked by pretreatment with PTX. Moreover, intracellular application of antibodies specific for Go_α markedly attenuated the response to PLO17, whereas cells injected with antibodies for $Gi1_\alpha$/$Gi2_\alpha$ responded normally. These data indicate that Go is involved in coupling mu-opioid receptors to calcium channels in DRG neurons. (DA03365 and DA04122)

535.4

DYNORPHIN A-MEDIATED REDUCTION OF CALCIUM CURRENTS IN RAT DORSAL ROOT GANGLION NEURONS IS ANTAGONIZED BY ANTI-$G_{O\alpha}$ ANTISERUM. J.W. Wiley*, R.A. Gross#, H. Moises, & R.L. Macdonald, #University of Minnesota, Minneapolis, MN. & University of Michigan, Ann Arbor, MI. 48109

Kappa opioid receptors are important regulators of neural function. Previous studies suggested that dynorphin A(Dyn)-mediated activation of the kappa opioid receptors was associated with a reduction in calcium current via a pertussis toxin-sensitive (G_i/G_O-type) G-protein. The goal of this study was to examine the selectivity of the kappa receptor- G-protein coupling mechanism. Calcium currents were recorded in media that blocked Na^+ and K^+ currents in acutely dissociated postnatal rat dorsal root ganglion (DRG) neurons using the whole cell version of the patch clamp technique. Selected sera and antisera were included in the internal medium allowing diffusion into the cell after patch rupture. Intracellular delivery of sera was confirmed using a fluorescein-tagged IgG.

DRG neurons demonstrated at least three voltage-gated calcium currents, transient low-threshold (T-type), transient high-threshold (N-type) and slowly inactivating high-threshold (L-type) components. Dyn (3 μM) reduced the peak and slowed activation of the N-type current. Dyn's inhibitory action was antagonized by a selective anti-$G_{O\alpha}$ antiserum in a concentration-dependent manner but was unaffected by either nonimmune serum or a selective anti-Gi_α antiserum. We conclude that kappa receptor-mediated reduction of a transient high-threshold calcium current in rat DRG neurons involves selective coupling via the G_O subtype of G-proteins.

535.5

ETHANOL SUPRESSES NEURONAL CALCIUM CURRENTS BY G-PROTEIN ACTIVATION. A. HERMANN*, E. LAHNSTEINER and H. KERSCHBAUM. University of Salzburg, Dept. of Physiology, 34, A-5020 Salzburg, Austria.

Recent research indicates that voltage- and ligand gated ion channels are involved in the action of ethanol. In mollusc neurons calcium currents are suppressed by ethanol (CAMACHO-NASI and TREISTMAN, 1987), its mechanism of action is not understood, however. To study the action of ethanol in more detail we have used neurons in the central nervous system of the snail, Helix pomatia. Voltage clamp experiments show that ethanol (0.5-1%) suppresses calcium currents in a time- and voltage dependent manner. Intracellular buffering of calcium using BAPTA abolished the ethanol effects. Employing confocal laser microscopy imaging techniques revealed that ethanol at low doses does not alter the intracellular calcium concentration. GTP-γ-S injection also suppressed the calcium current whereas GDP-ß-S was ineffective. Staurosporin completely abolished the ethanol effects on calcium currents. Phorbol ester (OAG) also decreased the calcium current, addition of ethanol after a maximum effect was obtained was ineffective. A block of ethanol effects by staurosporin and phorbol ester (PdBu) have recently also been found in hippocampal slice preparations (LAHNSTEINER et al., 1992). Addition of ethanol, after application of dopamin which also reduces calcium currents, had no further suppressing effect. Employment of cAMP activators (8-Br-cAMP, bt-cAMP) decreased the peak calcium current as well as its time course of inactivation; application of ethanol further decreased calcium currents. The results taken together suggest that the suppression of calcium currents by ethanol is caused by activating a G-protein - protein kinase C transduction pathway.

CAMACHO-NASI & TREISTMAN, Cell. Mol. Neurobiol., 7, 191, 1986.
LAHNSTEINER, E., H. KERSCHBAUM & A. HERMANN, 1992, Neurosci. Lett. Abstr., (in press).

535.6

Facilitation of Ca Current in Bovine Adrenal Chromaffin Cells May be Due to Voltage-Dependent Phosphorylation

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Calcium currents in chromaffin cells exhibit facilitation; currents during test depolarizations are greatly potentiated by large pre-depolarizations or by repetitive depolarizations in the physiological range. Facilitation of the Ca current was also recruited by the activation of protein kinase A (PKA), in response to D_1 dopamine receptor agonists or to cAMP analogues. Thus, phosphorylation of the facilitation channel itself or a regulatory protein can play a role in facilitation. We have now investigated the role of phosphorylation in the recruitment of Ca currents by pre-pulses or repetitive depolarizations. Recruitment of facilitation by pre-pulses was a rapid ($\tau \sim 30$ ms) first order process. Activation of G-proteins was not required for recruitment of facilitation by pre-pulses. Broad spectrum protein kinase inhibitors such as H-7 or K252a inhibited the recruitment of facilitation by pre-pulses or repetitive depolarizations. PKA was not involved in the recruitment of facilitation by voltage. After recruitment by a pre-pulse to +120 mV, facilitation was normally transient, lasting ~60 s; in the presence of the phosphatase inhibitor okadaic acid, however, facilitation became irreversible. Substitution of ATPγS for ATP in the pipette solution also greatly prolonged facilitation. Further, recruitment of facilitation was slowed with ATPγS present in the pipette. Intracellular injections of the catalytic subunit of phosphatase 2A inhibited the recruitment of facilitation by pre-pulses. Taken together, these results indicate that recruitment of facilitation Ca currents in bovine chromaffin cells by pre-pulses or repetitive depolarizations involves the voltage-dependent phosphorylation of the facilitation Ca channel or a closely associated regulatory protein; voltage-dependent phosphorylation may represent a novel mechanism for modulating ion channel activity.

535.7

ENHANCEMENT OF NEURONAL N- AND L-TYPE Ca^{2+} CHANNEL ACTIVITY BY PROTEIN KINASE C. J. Yang & R. W. Tsien*. Dept. of Mol. & Cell. Physiol., Stanford Univ., Stanford, CA 94305

We studied effects of phorbol esters on Ca^{2+} channel activity in frog sympathetic neurons. High-voltage activated Ba^{2+} currents in whole-cell recordings were consistently augmented (27 ± 3%, mean ± s.e.m., n=12) by 1 μM phorbol dibutyrate (PDBu), a stimulator of PKC. The inactive congener 4-α-phorbol produced no such increase (-3 ± 3%, n=10). The PDBu-induced enhancement was blocked by the inclusion of the PKC-blocking peptide PKC(19-31) in the pipette (internal) solution (5 ± 4%, n=4), or staurosporin in both the external and internal solutions (-12 ± 2%, n=6). These observations indicate that Ca^{2+} channel activity can be increased via stimulation of PKC.

Enhancement by PDBu was not caused by removal of tonic G-protein inhibition since it was observed in cells dialyzed with 2 mM GDP-ß-S (35%, n=2). N-type current (defined as resistant to 10 μM nimodipine but susceptible to inhibition by ω-CgTx or norepinephrine) was increased by 31 ± 5% (n=7); L-type current (resistant to 3 μM ω-CgTx but susceptible to block by nimodipine) was also enhanced (31 ± 14%, n=5). Moreover, PDBu augmented L-type tail currents made prominent by Bay K 8644 in the presence of ω-CgTx.

Dramatic increases in unitary N- and L-type channel activity were seen in cell-attached patches following application of PDBu to the bulk of the cell. In most recordings, the control activity was of the low open probability (p_o) mode. PDBu did not affect the unitary current size or mean open time but increased p_o several-fold by sharply decreasing closed time intervals between adjacent openings. Enhancement of Ca^{2+} channel activity by PKC may be important for various neuronal functions such as synaptic plasticity and development.

535.8

PHORBOL ESTERS ENHANCE N-TYPE CA CHANNEL CURRENT AND REDUCE ITS SUPPRESSION BY THE METABOTROPIC GLUTAMATE RECEPTOR

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We studied the actions of phorbol esters on Ca channels in rat CA3 pyramidal and superior sympathetic neurons using whole-cell patch clamp recordings. Phorbol-12-myristate-13-acetate (PMA; 500 nM) had two actions on CA3 neurons. First, high-voltage activated Ca channel current was enhanced (29 ± 3 % at -10 mV; 12 cells). Enhancement typically began within 10 seconds of PMA application and reached steady-state within ~ 1-3 min.. In most cells the enhancement was larger for small depolarizations; in one CA3 neuron enhancement was 49 % at -20 mV, 31 % at 0 mV, 20 % at +20 mV and 12 % at +40 mV. Second, the ability of metabotropic glutamate receptor agonists to modulate Ca channel current is greatly reduced after application of PMA; 1S,3R-ACPD suppressed Ca channel current by 22 ± 4 % prior to, and 2 ± 0.4 % following, PMA application. Both of these actions were blocked by including PKC 19-36 in the pipette (10 cells). Neither of these effects were seen after similar application of 4-α-PMA (6 cells). In 10 control cells 10 μM ω-CgTx-(GVIA) blocked 17.4 ± 1.3 pA/pF of current while in 9 cells where PMA was applied 10 μM ω-CgTx-(GVIA) blocked 27.7 ± 1.9 pA/pF of current. In CA3 neurons 10 μM ω-CgTx-(MVIIC) reversibly blocked 10 μM ω-CgTx-(GVIA) sensitive current; block by 10 μM ω-CgTx-(GVIA) occluded further block by 10 μM ω-CgTx-(MVIIC). In 4 cells 10 μM ω-CgTx-(MVIIC) blocked 17.2 ± 2.4 pA/pF of current prior to, and 32.1 ± 2.4 pA/pF of current following, PMA application. In contrast 10 μM nimodipine blocked 12.6 ± 2.5 pA/pF of current prior to PMA and 10.2 ± 2.1 pA/pF of current following PMA. PMA also enhanced Ca channel current in rat sympathetic neurons (25 ± 2.7 %; n=9). Experiments with blockers in sympathetic neurons confirm the notion that PMA stimulates N-type Ca channels.

535.9

Metabotropic glutamate receptor stimulation increases calcium currents in rat cerebellar granule cells. J.L. Bossu, L. Fagni, J.M. Nooney, J. Bockaert and A. Feltz*, Neurobiologie Cellulaire, 5 rue Blaise Pascal, F-67087 Strasbourg, Pharmacologie, rue de la Cardonille, F-34094 Montpellier.

Stimulation of metabotropic glutamate (Qp) receptors on cerebellar granule cells in culture increases IP3 and, subsequently, internal [Ca^{2+}] with a rise of Ca-dependent K^+ channel activity (Fagni *et al.* (1991) Eur. J. Neurosci.,3, 778-789). The latter effect is sensitive to external [Ca^{2+}] which suggests that Qp receptor stimulation may promote Ca influx. The effect of t-ACPD, a selective Qp agonist, on Ca whole-cell and channel activity were studied on granule cells maintained in elevated K^+ culture media for 5-14 days.

10^{-4} M t-ACPD increases both macroscopic and unitary Ba currents in a percentage of cells. Both activities are sensitive to dihydropyridines. Application of t-ACPD to the cell body during cell-attached recordings indicate that a second messenger pathway is involved. t-ACPD increases single channel activity following pre-treatment of the cells with the membrane permeable Ca chelator BAPTA-AM (50 μM for 30 min). Moreover 100 μM thapsigargin does not mimic the action of t-ACPD on Ca channels. Both observations suggest that intracellular Ca^{2+} is not the second messenger.

535.10

ENKEPHALIN INDUCES TWO DISTINCT MODULATORY EFFECTS ON CALCIUM CURRENTS IN ADULT RAT SENSORY NEURONS

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Aim of this study was to investigate the electrophysiological caracteristics of Ca channel modulation.Neurons were acutely isolated and recorded with whole-cell patch-clamp technique. D-ala-D-leu-enkephalin (DADL) (400 to 1000 nM), a potent analgesic, reduces high voltage activated (HVA) Ca current amplitude (-60% ±2.7 SEM n=40) in 90% of the cells tested. In 27 out of 40 cells sensitive to DADL, the reduction in Ca current amplitude was associated with a prolongation of current activation. The activation time course was single exponential in control, with time constant τ=2.3 msec and double exponential with DADL, with τ_1 =2.4 and τ_2 =11 msec (22°C). A conditioning depolarizing prepulse [Formenti A. & Sansone V. (1991) Neurosci.Lett. 131:267-272] speeded up the activation time course completely eliminating the slow, voltage sensitive component, but it was only partially effective in restoring the current amplitude to control values. After the conditioning pulse, the voltage-sensitive component returned and its amplitude increase with a monoexponential time-course (τ=230msec). The voltage range at which the conditioning pulse was effective was that in which HVA channels were activated. The current amplitude could be restored close to control values only washing out DADL. In the remaining 13 cells, DADL decreased Ca channel current amplitude without prolongation of Ca channel activation. In these cases the conditioning pulse was not effective in relieving the inhibition of DADL, therefore this type of inhibition was voltage independent. The voltage-dependent inhibition occurred slowly after DADL superfusion (30-60sec), whereas the voltage-independent one developed rapidly (at least one order of magnitude less in time). These two types of modulation could play different roles in controlling nociceptive sensory information.

535.11

SELECTIVE ADENOSINE RECEPTOR ACTIVATION BLOCKS N-TYPE BUT POTENTIATES P-TYPE HIPPOCAMPAL Ca CURRENT

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Adenosine, released in response to increased neuronal electrical activity or metabolic demand, has been shown to both decrease synaptic transmission (Ginsborg & Hirst, *J. Physiol.* 1972; Dunwiddie & Hoffer, *Br. J. Pharmac.* 1980; Silinsky, *J. Physiol.* 1984) and to produce an excitatory response (Sakurai & Okada, *J. Physiol.* 1991) in hippocampal synapses. Previous reports have shown that adenosine or its analogues reduce Ca current in dorsal root ganglion and hippocampal neurons (Dolphin et al., *J. Physiol.* 1986; Madison et al., *Biophys. J.* 1987; Gross et al., *J. Physiol.* 1989). The effect of selectively activating adenosine receptors was examined on Ca channels from acutely isolated pyramidal neurons from the CA3 region of guinea-pig hippocampus using the whole-cell voltage-clamp technique. Activation of A_1 receptors inhibited primarily ω-Conotoxin-sensitive N-type Ca current although a fraction of Ca current inhibition by A_1 activation was not ω-Conotoxin sensitive. In contrast, activation of A_{2b} receptors resulted in significant potentiation of ω-Agatoxin-IVA-sensitive P-type but not N-type Ca current. This potentiation occurred via a cAMP-dependent pathway and was blocked by inclusion in the patch pipette of WIPTIDE (10 μM), a specific protein kinase A inhibitor. Conditions which augment Ca current have been implicated as an important component of synaptic transmission, excitotoxicity, and long-term potentiation. Because of the ubiquity of adenosine, the differential effects of receptor subtype activation on Ca channels may have significant implications for neuronal excitability.

535.12

THE ω-3 FATTY ACID DOCOSAHEXANOEIC ACID INHIBITS L-TYPE CALCIUM CURRENT.

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Docosahexaenoic acid (DHA), a C22:6 ω-3 fatty acid found in high concentrations in fish oil, is thought to protect both the brain and cardiovascular tissues from cytotoxicity during periods of acute ischemia, though the exact mechanism by which this is accomplished is unclear. Recently Hallaq et al. (PNAS, 1992, 89: 1760) reported that concentrations of DHA which had no effects of its own blocked the agonist and antagonist effects of dihydropyridines (DHPs) on cardiac muscle contraction. It also noncompetitively inhibited the binding of the DHP antagonist ^{3}H-nitrendipine to membranes of cardiac myocytes. Here, we have examined whether DHA acts as a modulator of L-type Ca current in a manner similar to that of DHPs in undifferentiated PC12 cells, a pheochromocytoma cell line that contains mainly L-type Ca channels. Bath application of DHA at a concentration that had no effect of its own (1 μM), did not block the effects of the DHP agonist (+)-202-791 or antagonist (-)-202-791 on the whole cell L-type Ba current. 5 μM and 10 μM DHA decreased whole cell Ba currents 36 ±7% (N=10) and 78 ± 4% (N=3) respectively. Arachidonic acid (5 μM; C20:4 ω-6) also decreased the peak current 64 ± 6% (N=7) while oleic acid (10 μM; C18:1 ω-9) had no significant effect (N=3).

The effects of DHA at the single channel level were consistent with its effects at the whole cell level. 10 μM DHA puffed onto cells decreased the mean average current (67% ± 7%, N=6) and the open probability of multichannel patches recorded in the cell-attached patch configuration in the presence of 500 nM (+)-202-791. Mode 1 and 2 activity of single L-type currents decreased in the presence of DHA, but were still apparent. These results indicate that DHA and DHPs have overlapping but distinct effects on L-type Ca channels.

AXON GUIDANCE MECHANISMS AND PATHWAYS V

536.1

FASCICLIN IV: STRUCTURE, EXPRESSION, AND FUNCTION DURING GROWTH CONE GUIDANCE IN GRASSHOPPER.

A.L. Kolodkin*, D.J Matthes, T.P. O'Connor, D. Bentley, and C.S. Goodman, HHMI, Dept. of Molecular and Cell Biology, U. of California, Berkeley, CA 94720

Three different surface glycoproteins were previously identified (fasciclin I, II, and III) that are expressed on subsets of axon pathways during embryonic development. To identify additional pathway recognition molecules, we used the 6F8 MAb to study the structure, expression, and function of fasciclin IV in the grasshopper embryo. Fasciclin IV is initially expressed in the embryonic CNS on the U longitudinal pathway, on the three medial axon tracts and commissural bifurcations from the MP4, 5, and 6 progeny, on a set of anterior axon tracts in the segmental nerve root, and on axons in the intersegmental nerve root. Fasciclin IV is also expressed in circumferential stripes on the surface of epithelial cells in the developing limb bud. The boundaries of these stripes correspond to the limb segment boundaries and, in particular, to the precise location where the growth cones of the limb bud pioneer neurons (Ti neurons) make a characteristic ventral turn during their extension to the CNS. Embryos cultured in the presence of 6F8 MAb and FAb during Ti axon migration exhibit aberrant formation of this pioneer pathway specifically at the trocanter/coxa segment boundary, where normally both fasciclin IV is expressed and the Ti axons turn ventrally. Based on protein microsequence data, oligonucleotides were used for both PCR and library screens to isolate cDNA clones encoding fasciclin IV. Conceptual translation of these cDNAs suggests that fasciclin IV is an integral membrane protein with a signal sequence, a long extracellular domain, a transmembrane domain, and a short cytoplasmic domain. The extracellular domain has three clusters of cysteine residues (16 in total). No homologies have been found between fasciclin IV and any other protein in the available data base. We are looking for the fasciclin IV homologue in Drosophila.

536.2

AXONAL PROJECTION PATTERNS ARE ALTERED IN A NEW *DROSOPHILA* MUTANT, *midline uncoordinated (muc).*

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Our objective was to uncover new mutations that alter the neural circuitry of the thoracic nervous system of flies. New mutants were isolated in an F2 screen of lines mutagenized by the controlled mobilization of a P element (P[lacW]; Bier et al. 1989). Mutant flies were identified in a screen for abnormal grooming behavior; mutants did not remove dust from their bodies as efficiently as wildtype. In one of these mutants (*muc*) the legs are brought into the proper position at the midline, rubbing movements occur, but the legs do not touch at the midline and dust particles are not removed.

This defect in coordination of movements along the midline led us to examine sensory neurons whose axons normally cross the midline in the CNS and arborize bilaterally. The axonal projections of the sensory neurons innervating large tactile hairs called ASC and PSC in mutant flies lacked the axonal branch contralateral to the cell body. This branch is present in 100% of the wildtype flies but was reduced or missing in more than 50% of the mutant flies. When the P element was excised from the second chromosome, both the behavioral and the axonal phenotypes reverted to wildtype, confirming that the defect was caused by the insert. Analysis of the lacZ reporter gene expression patterns in the peripheral nervous system demonstrated that the cells of the tactile receptors were stained. A variety of cells in the central nervous system are also stained and analysis of these lacZ expression patterns is continuing. The results suggest that the mutation is causing a defect in the assembly of the midline. Supported by NIH grant NS15571 to R.K.M.

536.3

MUTATIONS WHICH PERTURB THE FORMATION OF LONGITUDINAL AXON PATHWAYS IN DROSOPHILA. G. Tear, M. Seeger*, D. Ferres-Marco, and C.S. Goodman, HHMI, Dept. of MCB, U. C., Berkeley, CA 94720

In order to elucidate the molecular mechanisms that guide the growth cones that pioneer the longitudinal and commissural axon pathways in the developing CNS of the Drosophila embryo, we performed a large-scale F2 genetic screen for mutations that disrupt the formation of these pathways. Approx. 13,000 independent lines, with ~3 lethal hits per chromosome, were screened for mutations that produce specific defects in the major CNS pathways as recognized by MAb BP102. Last year we reported on mutations in *commissureless* which perturb the commissural pathways (Seeger et al., 1991). Here we report of mutations which perturb the longitudinal pathways.

We saved ~250 mutant lines, and separated them into several discrete phenotypic classes; some appear to have normal pattern formation and cell fate decisions, but show specific guidance defects. Mutations in one gene, *longitudinals lacking* (*lola*), lack most longitudinal tracts while having nearly normal commissures, peripheral nerves, muscles, and body organization. The neurons that pioneer the MP1 pathway project their growth cones at the normal time and initial orientation; however, they often stall and fail to form the pathway. The longitudinal glia are born, initially migrate, and divide as normal; the earliest defect is seen about the time that the pioneering growth cones contact these glia and fail to extend along them. A mutation in a second gene, *roundabout* (*robo*), leads to a dramatic misrouting of the MP1 pathway. Defects are seen in a number of pioneering growth cones, including the MP1 growth cone, which extends posteriorly, but at the anterior commissure of the next posterior segment, makes a 90° turn towards the midline where it often contacts its contralateral homologue. The MP1 and other axons then form large circles of axons around the midline of two adjacent segments. We believe that these genes and others under investigation may encode proteins which function in the guidance of pioneer growth cones. The cloning of several of these genes is currently underway.

536.4

GENETIC ANALYSIS OF MOTONEURON PATHFINDING AND NEUROMUSCULAR CONNECTIVITY. D. Van Vactor and C.S. Goodman*, HHMI, Dept. of Molecular and Cell Biology, U. C., Berkeley, CA 94720

The ability of motoneuron growth cones to find and recognize their correct muscles has been a model system for studies on the mechanisms of target recognition; in both vertebrates and invertebrates, motoneuron growth cones extend toward and innervate the appropriate target muscles in a highly stereotyped fashion. Neuromuscular specificity in Drosophila is particularly well suited for studies on the molecular mechanisms of pathway and target recognition. The body wall musculature of Drosophila embryos and larvae consists of 31 individually identified muscle fibers in each abdominal hemisegment. Most if not all of these muscle fibers are innervated by one or only a few motoneurons. Extensive analysis from several labs of normal and manipulated embryos in both grasshopper and Drosophila argue for a high degree of specificity in the ability of motoneuron growth cones to recognize particular muscle fibers. Monoclonal antibody 1D4 directed against the cytoplasmic domain of fasciclin II specifically labels a large subset of motoneuron growth cones and axons (but not sensory neurons) in the periphery. We have used this and other probes to characterize the specific pathfinding of the aCC, three U's, and RP2 growth cones as they extend from the ventral nerve cord towards the most dorsal muscle fibers. In order to gain insights into the molecular mechanisms underlying pathway and target specificity, we have embarked upon a large-scale F2 mutant screen using MAb 1D4 to evaluate the pattern of neuromuscular connectivity directly in whole-mount embryos. Our goal is to nearly saturate the Drosophila genome for mutations that produce specific defects in pathfinding by identified motoneurons. Even though the screen is still in its infancy, we have already isolated a number of new mutants that perturb the projection of motoneuron growth cones.

536.5

AXONAL RECEPTOR-LINKED PROTEIN TYROSINE PHOSPHATASES IN THE EMBRYONIC DROSOPHILA CENTRAL NERVOUS SYSTEM. Shin-Shay Tian, Bruce Hamilton, Sarah Fashena, Chand Desai, and Kai Zinn*. Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

Receptor-linked protein tyrosine phosphatases (PTPases) often contain adhesion molecule-like extracellular domains, and may thus couple cell recognition to signal transduction *via* control of tyrosine phosphorylation. We previously showed that three adhesion molecule-like PTPases are selectively expressed on CNS axons during the period of axon outgrowth. We have now found that another *Drosophila* PTPase, $DPTP_{69D}$, is also expressed primarily on CNS axons. $DPTP_{69D}$, which was previously sequenced, contains two immunoglobulin-like domains and two fibronectin type III (FN) domains. To define the roles these PTPases play in neural development, we are making mutations in the genes encoding them. We have developed a 'local transposition' method for isolating insertions of P-element transposons at specific locations, and have been able to use this method to make a mutation in the $DPTP_{99A}$ gene. We have shown that $DPTP_{99A}$ and $DPTP_{10D}$ do not act as homophilic adhesion molecules in transfected tissue culture cells, suggesting that if they are involved in cell recognition they must recognize other cell surface ligands. In preliminary experiments, we have obtained evidence that a ligand for the distal 4 FN domains of $DPTP_{10D}$ is also localized to CNS axons, and we are attempting to clone cDNAs encoding this ligand. Support: NS28182, MOD 5-816, Pew Foundation, McKnight Foundation, Sloan Foundation, American Cancer Society.

536.6

GUIDANCE OF MOTONEURON OUTGROWTH BY A SERIES OF PERIPHERAL CUES DURING DEVELOPMENT OF DROSOPHILA. **L.S. Wang*and H. Keshishian, Dept. of Biology, Yale University, New Haven, CT 06511.**

During neural development of Drosophila, axons of motoneurons follow stereotypic pathways and innervate specific body wall muscle fibers. Muscle fibers 5 and 8 are innervated by a group of up to 4 motoneurons whose axons (here termed group 1 axons) form part of the efferent component of segmental nerve A (SNa). Other motor axons (group 2 axons) in the SNa make connections with transverse muscle fibers. It was shown previously that group 1 axons are capable of reaching the normal innervation region in the absence of their target muscle fibers 5 and 8 (Cash et al., J. Neurosci. 1992, in press). Our study of the temporal and spatial development of SNa shows that efferent axons first extend in the anterior half of the segment along the preexisting afferent axons of the ventral campaniform sensilla 4 and 5 (vc4 and vc5). The two groups of motor axons contact the cell bodies of vc5 and branch at the lateral edge of muscle fiber 12 where group 1 axons turn posteriorly and ventrally to make contact with muscle fibers 5 and 8. Laser ablation of muscle fiber 12 results in the early posterior turning of both group of axons. Ectopic endings on muscle fiber 4 by group 2 axons are also observed. In the absence of both muscle fibers 12 and 5, delayed branching and turning of the group 1 axons are seen. We propose that the outgrowth of the motor axons of the SNa is guided by a series of peripheral cues provided by afferent axons, sensory cell bodies, and both intermediate and target muscle fibers.

536.7

DOUBLE MUTATION OF FASCICLIN I AND FASCICLIN III CAUSES ERRONEOUS GROWTH CONE BEHAVIOR OF *DROSOPHILA* EMBRYONIC MOTONEURONS. A. Chiba*, T. N. Chang, & H. Keshishian, Dept. of Biology, Yale Univ., New Haven, CT 06511.

The membrane proteins fasciclin I (fas I) and fasciclin III (fas III) are expressed by overlapping populations of *Drosophila* embryonic neurons in spatio-temporally specific manners. The two molecules do not interact directly *in vitro* (Elkins et al., Cell 53,577). To assess their possible involvement during axon pathfinding and synaptic targeting, we have examined null mutants of fas I and/or fas III using immunocytochemistry and intracellular dye-injections. Although the CNS of these viable mutants appears normal at a gross level, upon closer examination some identified motoneurons demonstrated consistent errors when both fas I and fas III were missing. Within the embryonic CNS the axons of motoneurons RP1, RP3, and RP4, revealed by antibody 22C10, repeatedly failed to maintain tight fasciculation within the normal pathways. The motoneuron axons in the periphery, labeled by neuron-specific (anti-HRP) antibody, showed aberrant filopodial spread during synaptogenesis. These observations were further confirmed by dye-injections into the RP motoneurons. The motor endings in the mature (third instar) larvae sometimes exhibited anomalous branch extensions. In contrast to the double mutant, the mutants lacking either protein alone had no detectable differences from wild type. The results suggest that a subset of *Drosophila* embryonic growth cones may respond to at least two functionally redundant signals during their pathfinding and synaptic targeting.

536.8

HIERARCHICAL GUIDANCE CUES AND THE FORMATION OF MOLECULARLY DISTINCT AXONAL TRACTS DURING LEECH DEVELOPMENT. K.K. Briggs*, K.M. Johansen and J. Johansen, Department of Zoology and Genetics, Iowa State University, Ames, Iowa 50011.

In the CNS of leech, the central projections of peripheral sensory neurons during early development segregate into three distinct axonal tracts, which are labeled by the monoclonal antibody lan 3-2 (Johansen et al., Neuron 8:559, 1992). We have also shown that a subset of these neurons, recognized by the mab lan 4-2, projects its axons selectively into only one of these fascicles. Here we report on a molecular and developmental characterization of another fascicle specific antigen recognized by the monoclonal antibody lan 3-6. We have found that as is the case with the lan 4-2 epitope the lan 3-6 epitope is only expressed by a subset of the peripheral neurons. The axons of these neurons also show selective fasciculation in the CNS, but only to a single one of the three lan 3-2 positive tracts, which, very interestingly, is a different one from the lan 4-2 positive tract. Thus, these observations provide further evidence for the existence of a hierarchy of guidance cues mediating specific tract formation in this system.

We have screened an expression vector library with the lan 3-6 antibody and pulled out several potential lan 3-6 positive clones. Partial sequencing of one of these clones have revealed a protein with homology to proteins with EF-hand Ca-binding domains. However, apart from this general domain homology the sequence obtained appears to be unique. Immunoprecipitations followed by SDS-PAGE and silver-staining suggests that the antigen has a molecular weight of approximately 200 kd. Experiments with Northern and *in situ* hybridizations with the isolated clone are in progress in order to verify that the identified clones are indeed corresponding to the lan 3-6 antigen. Supported by NIH grant NS 28857.

536.9

IN VITRO-STUDIES ON THE TOPOGRAPHIC PROJECTION OF NASAL RETINAL FIBERS ONTO THE CHICK POSTERIOR TECTUM. Ysander v. Boxberg, Silvia Deiss, and Uli Schwarz*.
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The stripe assay has been introduced as a model system to study the projection of retinal fibers from embryonic chicken onto their topographically correct target regions on the tectum (Walter et al., 1987, Dev. 101, 909-913). By combining it with a novel membrane protein fractionation method we have been able to demonstrate that not only the temporal but also the nasal fibers can recognize guiding cues present on cell membranes derived from their proper target area. In addition, we could show that the survival of nasal fibers *in vitro* can be substantially prolonged by supplementing the culture system with membrane preparations from posterior tectum. We therefore suggest that tropic as well as trophic interactions may be involved in the homing process of nasal axons within the posterior tectum.

536.10

RETINAL GANGLION CELL AXONS FAIL TO FORM AN OPTIC CHIASM FOLLOWING EMBRYONIC ABLATION OF VENTRAL DIENCEPHALON NEURONS. D.Sretavan*, M.Siegel & L.Reichardt. Howard Hughes Med. Inst., Univ. Calif. San Fran. & Genentech, South San Francisco, CA.

Developing retinal ganglion cell axons arriving at the ventral diencephalon establish an "X" shaped intersection of axonal projections from the two eyes known as the optic chiasm in which axons from specific retinal regions sort into the correct optic tract. We previously found that the region of the future chiasm, prior to arrival of retinal axons, contains a population of neurons arranged in an inverted "V" formation straddling the midline; and suggested that they may play a role in axonal guidance and formation of the optic chiasm.

We have now identified a monoclonal antibody (Mab) recognizing a ~90kd cell surface molecule selectively expressed on these early chiasm neurons which is capable of labeling these neurons in live embryos. Injection of this Mab together with complement into the CNS ventricles of mouse embryos at E11-E11.5, prior to arrival of retinal axons, ablates early chiasm neurons within 24 hours. Examination of injected embryos subsequently at E16 shows the ventral diencephalon at light and EM levels to be histologically normal, but the "X" shaped optic chiasm which is normally present by this age is not observed. Axons in injected embryos have grown from both retinas into the optic nerves, but have failed to enter the diencephalon to reach the midline and form optic tracts. The formation of the optic chiasm was not prevented in animals treated with either complement or Mab alone or with a control Mab combined with complement. These results indicate that early ventral diencephalon neurons play a crucial role in the guidance of retinal axons and chiasm formation in the embryonic diencephalon.

536.11

CUTANEOUS AND MUSCLE AFFERENTS: INTERACTIONS WITH POTENTIAL TARGET TISSUES IN VITRO. S.A.Scott*. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

During embryonic development, cutaneous and muscle afferents appear to use different cues as they grow toward their respective targets. To test whether cutaneous and muscle afferents also differ in their response to the targets themselves, explants of the trigeminal mesencephalic nucleus (TMN), which contains only muscle afferents, or the dorsomedial pole of the trigeminal ganglion (DM-TG), which is largely cutaneous, from E10 chick embryos were co-cultured with explants of epidermis or dermis from E7 embryos; interactions of individual growth cones with these potential targets were followed with time lapse videomicroscopy. *In vivo* few cutaneous axons penetrate the epidermis and most terminate in the dermis. Interactions of growth cones with myotubes, the target of muscle afferents *in vivo*, are also being investigated.

Neither DM-TG nor TMN growth cones advanced onto epidermal explants, but over half of the TMN neurites remained in contact with the epidermis for >1 hr. In contrast, many (10/19) DM-TG growth cones collapsed and retracted within 15 min of touching epidermis, and very few (1/19) maintained contact for >1 hr. The behavior of both types of neurons was different upon encountering dermis. The majority (9/14) of DM-TG growth cones grew readily across dermis at rates comparable to their growth on the polyornithine-laminin substrate. In contrast, growth cones of TMN neurons did not advance well on dermis, and most (5/6) subsequently retreated to the substrate. Thus, there appear to be differences in the behavior of growth cones of E10 trigeminal cutaneous and muscle afferents. These differences could play a role in the selection of targets or could instead be a consequence of target innervation. Additional studies involving younger neurons that have not yet innervated targets will investigate these possibilities. (Supported by NIH grant NS16067)

536.12

THE LAMINATION OF HIPPOCAMPAL AFFERENTS IS NOT CAUSED BY THEIR SEQUENTIAL ARRIVAL DURING ONTOGENETIC DEVELOPMENT. B. Heimrich and M. Frotscher*, Inst. Anat., Univ. Freiburg, D-7800 Freiburg, FRG.

Afferents to the hippocampal formation are known to terminate in a laminated fashion. Bayer and Altman (1987) have provided evidence that the lamination of hippocampal afferents correlates with the neurogenetic gradients between cells providing input to the hippocampus. Thus, afferents from progressively later originating cells terminate progressively closer to the cell bodies of pyramidal neurons and granule cells. Early arriving entorhinal fibers meet the yet short dendrites of the hippocampal target neurons. With further dendritic growth the entorhinal fibers eventually terminate on the most peripheral dendritic segments, whereas later arriving hippocampal (associational and commissural) fibers impinge on proximal dendritic portions. We have tested this temporal hypothesis of hippocampal lamination in an in vitro system. Two slices of hippocampus were co-cultured. Seven days later a slice of the entorhinal cortex was added to the two hippocampal slices. Fiber connections between the cultures were then labeled by using biocytin as an anterograde tracer. In contrast to the normal situation in vivo, the entorhinal fibers arrived later than the hippocampal fibers in this experimental paradigm. However, like in vivo, the entorhinal fibers terminated on the most peripheral dendritic segments of the co-cultured hippocampal target cells. We conclude that the sequential arrival of hippocampal afferents does not determine their termination in distinct layers.

Bayer, S., and J. Altman (1987) Progr. Neurobiol. 29:57-106.

(Supported by the DFG: SFB 325)

VISUAL CORTEX: BEHAVIOR AND BEHAVIORAL CORRELATES

537.1

CATS WITH STRIATE CORTEX LESIONS CAN DETECT FINE GRATINGS BUT CANNOT IDENTIFY THEIR ORIENTATION T. Pasternak*and †C. R. Olson, Dept. Neurobiology and Anatomy, Univ. of Rochester, Rochester, NY 14642; †Univ. Maryland; Baltimore, MD.

In the cat, areas 17 and 18 receive largely separate and distinct inputs from the lateral geniculate nucleus, the X-cells and the Y-cells respectively. Similarly to X-cells, neurons in area 17 respond linearly to spatial frequencies that are about three times higher than neurons in area 18. In this study, we examined the contribution of area 17 to visual function in two cats with fixation controlled by means of scleral search coils. Ibotenic acid lesions were made within the physiologically identified representation of the lower left visual field of area 17. Since neurons in this area respond to higher spatial frequencies than those in area 18, we expected to find deficits in the ability to detect higher spatial frequencies.

Initially, contrast sensitivity and visual acuity for the intact and the lesioned hemifields were measured in a detection task in which the cats indicated the presence or the absence of a vertical grating. With this task, sensitivity loss increased with spatial frequencies and then decreased again at higher spatial frequencies, with no loss in spatial resolution. The preserved sensitivity to high frequencies appeared inconsistent with loss of area 17 neurons. To examine the possibility that the preserved detection of fine gratings involved non-veridical perception, we repeated the measurements with a task in which the cats discriminated between vertical and horizontal gratings. With this task, sensitivity loss was minimal at low spatial frequencies and increased drastically at higher spatial frequencies. Visual acuity was reduced by about an octave. These results suggest that, in the cat, area 17 is critical to the veridical perception of stimuli containing high spatial frequencies. The apparent preservation of sensitivity to fine gratings suggested by the detection performance may be due to signals from Y-cells which respond non-linearly to frequencies that are as high as those carried by linear X-cells to area 17.

(Supported by EY06175, EY01319, NS 27287)

537.2

EFFECTS OF V1 AND V2 LESIONS ON THE VISUAL PERFORMANCE OF MACAQUES. William H. Merigan*, Tara A. Nealey, and John H. R. Maunsell. Depts. Ophthalmology and Physiology and Center for Visual Science, University of Rochester, Rochester, N.Y. 14642

This study measured the detection and discrimination performance of macaques, tested with controlled fixation, after localized cortical lesions produced by multiple injections of ibotenic acid. Primary visual cortex, area V1, is the target of most cortical afferents from the geniculate, and there is substantial evidence that under most conditions, lesions of this area result in blindness. A V1 lesion of about 6 by 6 mm caused a 1.5 deg diameter scotoma at 4 deg eccentricity for all functions tested, which persisted throughout the 10 month post lesion test period.

Cortical area V2 receives a substantial portion of the output from V1, although there are also pathways through V3, MT and other areas. Visual acuity and chromatic and luminance contrast sensitivity were measured by testing discrimination between gratings of horizontal and vertical orientation and no deficits were found. Measurements were also made of more complex visual discriminations. In the first task, the monkey discriminated vertical from horizontal orientation of two lines of irregularly spaced dots. The number of background dots that brought this discrimination to threshold was measured as an index of the ability to extract collinear groups of dots from a display of irregularly placed dots. V2 lesions substantially reduced the tolerable number of background dots. A second task required that the animal discriminate the orientation of a group of three distinctive neighboring stimuli embedded in a six by six array of otherwise identical stimuli. The three stimuli could be discriminated from the others on the basis of orientation, color, or luminance. We found that this task could not be done in the region corresponding to a V2 lesion when the three stimuli differed from the others in orientation, but could be done when they differed in color or luminance. These results are consistent with the hypothesis that utilization of second-order spatial relationships requires the activity of cortical area V2.

(Supported by AFOSR 890041, and EY08898)

537.3

SELECTIVITY FOR FIGURE-GROUND DIRECTION AT OCCLUDING CONTOURS IN MONKEY AREA V2. E. Peterhans* and R. von der Heydt, Department of Neurology, University Hospital Zurich, CH-8091 Zurich, Switzerland.

Visual processing in situations of spatial occlusion involves the detection of occluding contours and the distinction between foreground and background. We studied the neural mechanisms of such processing in the alert monkey during behaviorally induced fixation. In area V2 we recorded the responses of single neurons to light and dark edges and to stimuli that mimicked situations of spatial occlusion, for example a light rectangle overlaying a grating of dark lines, or the same figure in reversed contrast. In neurons responding to the borders of such rectangles we studied the effects of figure-ground direction and contrast polarity.

About half of the neurons studied (19/40) were selective for figure-ground direction. In 9 of these cells this selectivity was independent of contrast polarity, 10 cells showed interaction. These cells preferred stimuli with a certain figure-ground direction and a contrast polarity consistent with their edge preference. Even with the non-preferred contrast polarity most of them preferred the same figure-ground direction. These findings can be interpreted in terms of a previously proposed model assuming that the figure-ground direction is inferred by asymmetric end-stopped cells (see TINS 14:112-119, 1991).

We conclude that mechanisms for the segregation of figure and ground are present at the level of area V2.

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537.4

NEURAL RESPONSES TO TEXTURE BORDERS IN MACAQUE AREA V1. H. C. Nothdurft, J. L. Gallant*, D. C. Van Essen, Div. Biol., CalTech, Pasadena, CA 91125, and Max Planck Inst. Biophys. Chem., Goettingen, FRG.

We have studied the responses of neurons in area V1 of anesthetized macaque monkeys to oriented texture elements that were part of a large texture field containing a texture border across which the element orientations differed by 90 deg.. The texture border was presented at different positions and different orientations relative to the cell's classical receptive field. The single texture element within the classical receptive field was either matched or was orthogonal to the preferred orientation. Out of 161 cells tested, about one third showed a significantly greater response when the texture border was adjacent to the receptive field than when the texture border was more distant. In most instances, the border enhancement was evident for only a subset of the border orientations tested. However, ~10% of the total population showed a significant border enhancement for all orientations. We also tested for an orientation contrast ('popout') effect: the responses to an oriented texture element were determined for an isolated element, an element embedded within a uniform texture field, and a single element within a field of elements of the orthogonal orientation. There was a clear, but imperfect correlation between the two tests; many cells showing a border enhancement effect also showed a differential effect in the single-element popout test.

Altogether, these data support the hypothesis (derived from psychophysical studies) that texture segmentation is based on local feature contrast rather than on feature analysis extending over large texture regions (Nothdurft, Vis. Res., 1991).

537.5

DO CELLS IN AREA V2 RESPOND TO THE ORIENTATION OF KINETIC BOUNDARIES ? V.L. Marcar*, S.E. Raiguel, D. Xiao, H. Maes & G.A. Orban, Lab. Neuro- en Psychofysiologie, Medical School, KULeuven, B-3000-Leuven, Belgium.

Human observers and monkeys can perceive the orientation of a boundary defined by differences in the direction of motion of two random dot fields. We reported (Marcar et al, Soc. Neurosci. Abstr. 1991, 17: 525) that MT cells of macaca fascicularis were not selective for the orientation of such kinetic boundaries. We have now extended this investigation into area V2.

We tested V2 cells with contrast luminance gratings and edges as well as kinetic boundaries generated using two random dot fields moving coherently in opposite directions. Their receptive fields were mapped, to enabled us to align the center of the display with the centre of the receptive field and select the optimal stimulus size. In one type of kinetic boundary the motion was parallel, while the other type the motion was orthogonal to the orientation of the boundary. Only cells tuned to the same orientation for two types of boundaries qualify as selective.

Five out of 44 V2 cells were tuned to the same orientation for both types of kinetic boundary and were considered selective. Their orientation preference for the kinetic boundary was also found to be within 23° of their orientation preference for a luminance contrast boundary. Four of these kinetic boundary cells have been placed in layer V. These results together with that of others (e.g. Peterhans & von der Heydt, J. Neurosci. 1989, 9: 1749-1763) suggests that area V2 plays an essential role in the perception of boundaries.

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537.6

FACILITATION ALONG THE LONG AXIS OF VISUAL CORTICAL RECEPTIVE FIELDS: A POSSIBLE EXPLANATION FOR THE BARBER-POLE PHENOMENON ON THE CELLULAR LEVEL ? F. Wörgötter* and U.T. Eysel Inst. Physiol. Univ. Bochum, FRG.

Usually, the strongest response of a visual cortical cell is obtained when the receptive field (r.f.) is *crossed* with an oriented moving stimulus (orientational component). Recently, we have shown that small dots moving *along* the long axis of the r.f. elicit equally strong reactions in 80 % of the cells (axial component, Wörgötter & Eysel, Exp. Brain Res. 76, 307-314, 1989). With small bar stimuli tuning curves with 4 peaks occur because the orthogonally directed orientational and axial components overlap. Strong axial responses are also obtained during repetitive stimulation with long bars using a grating with low spatial frequency. This observation was made in 43 simple and complex cells in area 17 of the anesthetized cat at moderate to fast temporal frequencies. In 8 cells the axial component was substantially stronger than the orientational component. Covering the OFF subfield with a dark mask strongly enhances the axial component while diminishing the orientational component in cells with a clear ON-OFF subfield separation. In these cases the cell's preferred direction turns by 90°. The "barber-pole" phenomenon is described by the effect that an edge will interfere with motion perception. While viewing a moving grating partly obscured by an elongated edge a human observer will in most cases perceive motion along the edge regardless of the actual angle at which the grating is moving. The above described masking effect which shifts the preferred direction of the cells by 90° could be involved in this percept. The r.f.s distant from the edge will all be stimulated identically by the moving grating which will lead to adaptation effects. Thus, they should add little to the precept. On the other hand, r.f.'s overlapping the edge are partly obscured and produce strong responses along the r.f. long axis which is parallel to the axis of perceived motion.

537.7

A SIGNIFICANT PROPORTION OF THE TOTAL INFORMATION IS ENCODED IN A SHORT PERIOD AT THE BEGINNING OF A SPIKE TRAIN OF NEURONS RECORDED IN THE PRIMATE TEMPORAL VISUAL CORTEX. E.T.Rolls*, M.J.Tovee and A.Treves. Univ. Oxford, Dept. Exptl. Psychol., Oxford OX1 3UD, England.

Principal component and information analyses (Optican and Richmond, 1987, J. Neurophysiol. 57: 162-178) were performed on spike trains recorded from single neurons in the temporal cortical areas of macaques performing a visual fixation task during the presentation of static face and non-face stimuli (Rolls, 1992, Phil. Trans. Roy. Soc. 335, 11-21). We subtracted a correction from the calculated information to allow for the small number of trials, and showed that the first principal component, which was highly correlated with firing rate, reflected most of the information available from the spike train. We then showed that the information about the stimulus derived from the firing rate in a short period of 50 ms near the start of the elicited spike train was 0.57 of that which could be obtained using temporal encoding during a 400 ms analysis period. In a period of 20 ms, 0.43 of the information was available. When visual recognition of objects is performed by the visual system, there is time for only about 20-40 ms of processing within each cortical area before the next area must start its processing (see Rolls, 1990, Current Opinion in Neurobiology 1: 274-278). The present results show that a significant proportion of the total available information is present within such short periods of the spike trains of neurons in the visual system, and are consistent with the hypothesis that information from each cortical area is extracted from a short estimate of neuronal firing, rather than a long estimate which allows temporal encoding to be analysed.

537.8

SPATIAL-FREQUENCY-SPECIFIC INTERACTIONS AND THEIR ROLE IN BRIGHTNESS PERCEPTION AND BRIGHTNESS CONSTANCY. M.A. Paradiso*. Center for Neural Science, Brown University, Providence, RI 02912

One can distinguish two major hypotheses which have been proposed to account for brightness (and color) perception. One hypothesis is that surface attributes of objects are explicitly represented in the activities of mechanisms sensitive to luminance contrast over a range of spatial scales. Alternatively some feature, such as luminance contrast at edges, might play a special role in determining brightness. Our experiments demonstrate spatial-frequency-specific interactions which alter the relationship between brightness and luminance contrast depending on stimulus size and complexity. In one experiment contrast discriminations were made with squarewave gratings missing energy at the fundamental spatial frequency. While thresholds with squarewave gratings above about 1 cy/deg are comparable to thresholds for the fundamental sinewave in isolation, at lower frequencies thresholds are significantly elevated. The presence of higher components in the squarewaves decreases sensitivity for the lower frequencies. A similar conclusion is reached from masking experiments. Detection thresholds for sinewave gratings were measured in the presence of high contrast squarewave gratings. The squarewave masks have an asymmetrical effect on thresholds for the sinewaves. Whereas there is a modest effect on the detectability of high frequency gratings, there is a much larger effect on low frequency gratings and this effect covers a broader frequency range. These findings indicate that the representation of areas of uniform brightness becomes increasingly more "edge-based" as these areas get larger (i.e. sensitivity is preferentially lost to the low frequencies). The underlying frequency-specific interactions may be important for understanding brightness constancy and gradient illusions as they support the idea that visible luminance gradients become imperceptible in the presence of other stimuli.

537.9

SPECTRAL SENSITIVITY FOLLOWING CORTICAL HEMISPHERECTOMY IN MAN. P. Stoerig*[1], J. Faubert[2], M.Ptito[3], F.Lepore[3], and A.Ptito[4], Inst. Medical Psychology, Munich University, FRG[1], School of Optometry[2], Dept. of Psychology[3], Université de Montréal and Montreal Neurological Institute[4], Montreal, Canada.

Increment-threshold spectral sensitivity was measured in the normal and blind visual hemifields of three patients who had undergone hemispherectomy 1.5-10 yrs previously. Measurements were taken under photopic and scotopic conditions with the patients monocularily fixating a fixation spot 17^{o} eccentric from the center of the 9x9^{o} 1s stimuli produced on an Apple Macintosh colour monitor. The monitor screen around the stimulus was covered with a mask, and eye movements were monitored with an IScan Eye Movements Monitoring System. Stimuli with the dominant wavelength from 465 to 610nm were presented in random alternation with blanks, and their luminance was increased until the patients' detection reached the 80% correct criterion. The resultant spectral sensitivity curves show that the patients have a normal Purkinje-shift in both hemifields, although sensitivity in the blind field is reduced by 2 to 3 log units. Under photopic conditions, the curves exhibit the discontinuities attributed to colour-opponent interactions, showing that colour-opponent channels subserve the blind field even in the absence of one hemisphere. Unless the patients have abnormal retinal connections to the other hemisphere, this indicates that as yet unknown subcortical colour-opponent pathways survive the removal of a hemisphere with its massive degenerative consequences.

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537.10

VISUAL SUPPESSION WITH THE MAGNETIC COIL (MC): OPTIMAL DIRECTION OF MONOPHASIC CURRENT. P.J. Maccabee* ,V.E. Amassian, R.Q. Cracco, J.B. Cracco, L.P. Eberle, M. Somasundaram and K. Henry. Depts. of Neurology and Physiology, SUNY Health Science Ctr., Brooklyn, NY 11203

We previously demonstrated perceptual suppression of briefly presented horizontal letter trigrams when a Cadwell round MC was energized over calcarine cortex with a polyphasic pulse. Given the topography of the macular projection, this implies a powerful effect at the midline where the induced electric field is maximal. However, classic axonology predicts that excitation occurs near the negative-going first spatial derivative of the electric field, i.e. at least 4cm lateral to the midline. Using a monophasic pulse, recent studies of mammalian peripheral nerve in-vitro showed highest excitability at a bend or abrupt termination of a straight nerve when the induced electric field was maximal there and directed along the nerve toward the bend or nerve ending.

To see if a similar mechanism explains the activation of inhibitory visual processing, the experiments were repeated with monophasic MC pulses. In subjects sensitive to current direction, when the induced current flowed from left to right, the right-most letter was significantly suppressed more, implying preferential inhibition of left calarine cortex, and vice-versa. These findings fit with excitation by induced current directed medially towards the terminations of either geniculocalcarine fibers or of visual cortical neurons.

537.11

DEPTH AND STIMULATION SITE OF MAGNETICALLY INDUCED PHOSPHENES IN THE BRAIN. D. P. Rudiak and E. Marg*. School of Optometry, University of California, Berkeley, CA 94720.

Cortical phosphenes were induced in normals with magnetic stimulator coils over the posterior pole. Peripheral phosphenes (5-50°) along the horizontal meridian are much more common than central ones (<5°) or along the vertical. This pattern is puzzling since: (1) the central representation in visual cortex is much closer to the coil, and (2) there is no obvious reason why one meridian should be favored. Also, when we measured the stimulation depth using 2 coil sizes[1], it was ~4 ± .5 cm from the coil near the midline, or ~2.5 cm below the cortical surface at the pole, regardless of whether the phosphene was central or peripheral. Thus, there seems to be a lowered threshold site for stimulation deep in the occiptal lobe, like that found in the wrist[2]. Such sites, e.g., should lie at sharp bends in the nerve fibers or large tissue conductance changes. We postulate that our site lies near the tip of the posterior horn of the lateral ventricle, because it has: (1) a large conductance change between it and adjacent white matter, (2) about the same depth as our site, (3) the optic radiation fibers surrounding it laterally, the horizontal meridian fibers being adjacent and the vertical fibers more distant, (4) peripherally projecting radiation fibers roughly parallel to induced currents (whereas central fibers are oblique), and (5) the calcarine sulcus (representing the peripheral horiz. meridian in V1) medial and adjacent. This may explain why phosphenes are more common in the horizontal meridian. Fiber tracts adjacent to ventricles elsewhere in the brain may also be sites of preferred stimulation. (Supported in part by the Minerva Foundation, Berkeley, CA.)

[1] Epstein, et. al. (1990), Neurology, 40: 666-670.

[2] Maccabee, et. al. (1990), Neuroscience Abstracts, 16: No. 516.7.

REGULATION OF AUTONOMIC FUNCTION

538.1

EMETIC REFLEX ARC REVEALED BY EXPRESSION OF C-FOS PROTEIN IN CATS. A.D. Miller*and D.A. Ruggiero. Rockefeller Univ. (ADM) and Dept. of Neurology, Cornell Univ. Medical Center (DAR), New York, NY 10021.

In order to investigate the organization of the central neuronal circuitry that produces vomiting, the distribution of c-fos protein (Fos)-like immunoreactivity (FLI) in brainstem and spinal cord was determined in 3 cats receiving multiple emetic drugs (cisplatin, lobeline, protoveratrine, naloxone, apomorphine) and in 3 controls given matched saline injections. Two pairs of animals were decerebrated, paralyzed, and artificially ventilated to avoid sensory feedback. Fictive vomiting was identified in these animals by characteristic respiratory muscle nerve discharge. Tissues were immunoprocessed using an antibody against amino acids 1-131 of FOS (kindly obtained from Dr. T. Curran) and the A.B.C. method. Enhanced nuclear FLI was observed in experimental animals along portions of the entire sensorimotor emetic reflex arc, including the nodose ganglia, area postrema, nucleus tractus solitarii (especially medial subnuclei), intermediate reticular zone, dorsal vagal motor nucleus, nucleus retroambigualis, C2 inspiratory propriospinal cell region, and phrenic motor nucleus. Labeled neurons were also found in the raphe magnus and pallidus, lateral parabrachial nucleus, and spinal dorsal horn. No unique group of labeled neurons that might function as a "vomiting center" has been identified. Emesis is mediated by a localized reflex arc of constituent neurons expressing the immediate-early gene c-fos. Supported by NIH grants NS20585 (ADM) and NS28200 (DAR).

538.2

THE EFFECT OF SUPRANODOSE VAGOTOMY UPON THE VAGALLY EVOKED NON-NICOTINIC INHIBITION OF GASTRIC SMOOTH MUSCLE TONE IN THE RAT. A.J.Carnell, P.J.Roberts* and R.G.Williams, Department of Physiology and Pharmacology, University of Southampton, UK. SO9 3TU.

We have previously described a non-nicotinic vagal inhibitory pathway which modulates gastric smooth muscle tone in the rat (Carnell *et al*, Br.J.Pharmacol. **104**: 290P, 1991). This inhibitory influence might be elicited by vagal efferents or by the antidromic activation of afferents (Delbro *et al*, Acta Physiol.Scand. **114**: 433-440, 1982). Here we establish that this inhibitory pathway involves vagal fibres which have their cell bodies central to the nodose ganglion. Under Hypnorm and diazepam anaesthesia, nine rats (200-500g) received a unilateral supranodose vagotomy. After 7 days, the animals were re-anaesthetised and True blue (TB) was injected into the wall of the stomach at multiple sites. After a further 7 days the animals were anaesthetised with urethane and prepared for the study of the effect of vagal stimulation upon intragastric pressure (IGP). Supramaximal (20V, 20Hz, 20s, 1ms square wave), stimulation of the control vagus elicited an initial contraction to 44.2 ± 9.6% of basal IGP (mean s.e.mean), followed by a long lasting relaxation to 41.7 ± 4.1% of basal IGP. Hexamethonium (30mg/Kg) abolished the excitatory component but a resistant inhibitory component remained, stimulation causing a 33.1 ± 5.0% decrease from basal IGP. Stimulation of the lesioned nerve failed to evoke any change in IGP or mean arterial blood pressure in any animal studied. The success of the supranodose vagotomy was verified by the presence of comparable numbers of TB labelled perikarya within the nodose ganglia of the lesioned and control nerves. Abundant TB labelled perikarya were observed in the dorsal motor nucleus of the control side but none on the lesioned side. These observations indicate that the vagally evoked non-nicotinic inhibition of IGP in the rat is mediated by vagal parasympathetic preganglionic fibres and supports observations that such fibres employ transmitters other than acetylcholine.

Supported by the Wellcome Trust. AJC holds the Adam and Joseph Griffiths Memorial Fund Studentship.

538.3

TWO HYPERPOLARIZATION-ACTIVATED CURRENTS ARE PRESENT IN RAT NEURONS OF THE DORSAL MOTOR NUCLEUS OF THE VAGUS (DMV). R.A. Travagli, Y.M. Hernandez* and R.A. Gillis. Depts. of Pharmacology and Anesthesiology, Georgetown Univ. Med. Ctr., Washington, D.C. 20007.

The whole cell patch clamp recording technique applied to the in vitro brain slice preparation was used to record voltage and current responses from visually identified neurons of the DMV. The majority of DMV neurons (59 out of 85) showed a slowly developing hyperpolarization-activated current (I_H). The time constant of activation was described by a monoexponential equation and proved to be strongly voltage-dependent, decreasing with hyperpolarization. The I_H was K^+- and Na^+-sensitive. Raising the K^+ concentration from 4.1 to 20mM, increased 2-3 fold the peak amplitude of the I_H, while lowering the Na^+ concentration from 146 to 26mM decreased the current to approximately 60% of the peak amplitude. The I_H was completely blocked by externally applied Cs^+ (5mM) but it was insensitive to externally applied Ba^{++} (200μM) and TEA (20mM). A subset of DMV neurons (16 out of 85) exhibited an instantaneous inward rectification but no I_H. The instantaneous inward rectification was K^+- sensitive, increasing with higher extracellular K^+ concentrations, but was Na^+-insensitive, being unaffected by the lowering of the external Na^+ concentration. The instantaneous inward rectification was completely blocked by 5mM Cs^+ and significantly reduced by both externally applied Ba^{++} (0.2mM) and TEA (20mM). Current clamped DMV neurons exibiting I_H were hyperpolarized in a voltage-related manner upon perfusion with Cs^+; at -50mV, Cs^+ (5mM) induced a 7.7 $\pm$ 2.7 mV hyperpolarization while at -65mV, Cs^+-induced hyperpolarization was 30.3 $\pm$ 6.7 mV. We conclude that DMV neurons can be distinguished on the basis of the presence of two hyperpolarization-activated currents. The I_H current is tonically active at resting potentials, and, by increasing the ionic conductance during hyperpolarization, the I_H may be in part responsible for the pacemaker activity of these neurons.

538.4

EFFECTS OF SUBSTANCE P AND TRH MICROINJECTED INTO THE NUCLEUS RAPHE OBSCURUS (NRO) ON GASTRIC TONE AND MOTILITY IN THE RAT. Z.K. Krowicki* and P.J. Hornby. Dept. of Pharmacology and Experimental Therapeutics, Louisiana State University Medical Center, New Orleans, LA 70112.

The caudal NRO is an important site for CNS control of gastric function. Since both substance P (SP) and TRH are co-localized within the same neurons in the NRO we speculate that there should exist a functional interaction between these neurotransmitters in the NRO. To test this, we used seven-barelled glass micropipettes to investigate the effects of SP and TRH on intragastric pressure (IP), pyloric (PM) and greater curvature motility (GCM) in α-chloralose anesthetized rats. SP lowered IP at doses of 135 and 435 pmol [peak change (cm H_2O) $\pm$ SEM(n); -1.9$\pm$0.1(12) and -2.0$\pm$0.1(4), $p<0.001$], but not at a dose of 45 pmol. TRH (6, 15 and 45 pmol) increased IP [4.81$\pm$0.85(4), 7.49$\pm$0.69(6) and 8.98$\pm$1.32(6), $p<0.001$] as well as PM and GCM. Effects of both peptides were inhibited by atropine methyl bromide (1.0 mg/kg i.v.) and abolished with bilateral vagotomy or chlorisondamine (5 mg/kg i.v.). SP (135 pmol), injected into the NRO 30-60 sec before TRH (15 pmol) inhibited the effect of TRH on IP ($p<0.001$), PM ($p<0.01$) and GCM ($p<0.01$). SP (135 pmol) injected into the NRO 30-60 sec after TRH (15 pmol) was no longer able to reduce IP. We conclude that SP and TRH influence the gastric function in the caudal NRO via a vagally-mediated pathway and that SP modulate the effect of TRH on both gastric tone and motility. Supported by PHS grant DK42714.

538.5

CONVERGENCE OF VISCERAL AFFERENT AND SOMATOSENSORY INPUTS ON SINGLE HYPOTHALAMIC NEURONS IN CAT. C.S. Yuan and W.D. Barber*. Department of Anatomy, College of Medicine, University of Arizona, Tucson, AZ 85724

Studies in anesthetized cats evaluated convergent visceral afferent and somatosensory inputs on single neurons in the hypothalamus. Gastric vagal branches, serving the proximal stomach, and the T-9 intercostal nerve were electrically stimulated with pulses 0.3 msec in duration, 300 uA at 0.5 Hz. Unitary responses were recorded in the hypothalamus. Seventy five, of a total of 87 gastric vagal evoked (GVE), hypothalamic units were phasic evoked responses and 12 were tonically active units. The GVE effect was predominantly inhibitory on tonically active units with a mean duration of 493 msec. Eighty-one (93%) of the GVE hypothalamic phasic responses also received input from the T-9 intercostal nerve. When the gastric vagal and T-9 intercostal nerves were stimulated simultaneously the hypothalamic evoked convergent response was characterized by an initial excitation followed by inhibition. The condition-test paradigm evaluated the time course of GVE/intercostal convergent inputs on hypothalamic neurons. There was decreased excitability of the GVE hypothalamic response when the intercostal nerve stimulation occurred within $\pm$ 250 msec of gastric vagal activation. These data identified somatosensory/visceral afferent convergence on single hypothalamic neurons and suggested that the central processing of visceral input can be substantially affected by somatosensory stimuli. (Supported by USPHS Grant NS 27972)

538.6

HEPATIC MECHANORECEPTORS WITH PHRENIC AFFERENTS IN THE DOG. D.R. Kostreva* and S.P. Pontus. Depts. Anesthesiology and Physiology, Med. Col. of Wis. and VA Med. Ctr., Milw., WI 53295.

The objective of this study was to determine if there are mechanoreceptors located in the liver or hepatic vasculature that have phrenic nerve afferents. Mongrel dogs (20-24 kg) were anesthetized with pentobarbital sodium (35 mg/kg i.v.), intubated and placed on positive pressure ventilation. Arterial blood pressure and ECG were monitored. The chest wall was removed on the right side to allow unimpeded access to the phrenic nerve. An upper abdominal incision provided direct access to the inferior vena cava, the hepatic veins and the liver. The phrenic nerve was transected at the level of the first rib. The cut distal end of the nerve was then placed into a nerve dissecting tank filled with warm 37° C mineral oil. The nerve was desheathed and further dissected into fine filaments and laid across a pair of recording electrodes. The electrodes were connected to a preamplifier and a filter amplifier. The nerve activity was recorded on magnetic tape as well as an oscilloscope. Mechanoreceptors with phrenic afferents were then located in the pericardium, inferior mediastinum and the diaphragm as previously reported (FASEB J. 6:1699, 1992). The gall bladder, quadrate and right medial lobes of the liver, the hepatic veins, and the inferior vena cava were then probed for mechanoreceptors. Mechanoreceptors were found primarily in the hepatic veins both on the cranial and caudal sides of the liver. Although these receptors were very sensitive to light touch and probing, they did not respond to increases in venous pressure produced by occlusion of the inferior vena cava above the level of the diaphragm. A few receptors were located on the inferior aspect of the right medial lobe, while some receptors responded to compression of the capsular surface. This study is the first to describe phrenic afferent recordings from mechanoreceptors in the liver and hepatic veins.

538.7

EFFECTS OF CENTRALLY ADMINISTERED CRH AND TRH ON CARDIOVASCULAR CONTROL OF THE ANESTHETIZED RAT C. Schulz, A. Bock, D.H. Hellhammer*, E. Christodulopulu, H. Lehnert Dept. of Endocrinol., Univ. Hospital of Mainz, FRG; *Inst. of Physiol. Psychol., Univ. of Trier, FRG

The icv-injection of TRH and CRH in conscious rats causes increases blood pressure (BP), heart rate (HR) and plasma epinephrine (EPI) and norepinephrine (NE) as well as a rise in locomotor activity. Thus, the question arises whether or not the cardiovascular response to these hormones represents cardiovascular adaption to locomotor activation. To rule out this activation we studied the effect of icv injections of CRH and TRH in urethane anesthetized rats (1.5 g/kg, i.p.). Application of TRH (0.9, 1.8, 2.7, 15 nmol/10 μl, n=7) resulted in a dose-related increase in BP of 8 to 28 mmHg and HR of 50 to 73 beats/min. NE increased from 4.14 $\pm$ 0.50 to 7.04 $\pm$ 1.49 pmol/ml plasma and EPI tended to increase. Injection of 0.57 nmol CRH had no effect on BP but tended to elevate HR as well as EPI and NE. We show that the cardiovascular effects of TRH are expressed in the absence of motor activity whereas the effect of CRH was strongly attenuated. We conclude that the rise in motor activity is not a requisite for pressor and tachycardic effects of TRH and probably of CRH. Supported by DFG (Le 493/2-1).

538.8

CARDIOVASCULAR EFFECTS PRODUCED BY INTRACISTERNAL (IC) INJECTIONS OF S-NITROSOCYSTEINE (SNC) IN CONSCIOUS RATS. S.J. Lewis, H. Ohta, J.N. Bates and A.K. Johnson*. Depts. Pharmacol., Neurol., Anesthesia, Psychol. & Cardiovasc. Ctr., Univ. of Iowa, Iowa City, IA 52242.

The intracerebroventricular injection of the L-isomer (L-SNC) but not the D-isomer (D-SNC) of SNC produce marked changes in regional hemodynamics. This study examined the effects of IC injection of SNC in conscious rats. IC L-SNC (10 - 250 nmoles) produced dose-dependent hypotension, bradycardia and facilitation of baroreflex function (BRF). The higher doses also produced a subsequent hypertension, tachycardia and BRF inhibition. IC D-SNC produced hypertension, tachycardia and BRF-inhibition only. The L-SNC-induced hypotension, bradycardia and BRF-facilitation were abolished by the inhibitor of soluble guanylate cyclase, methylene blue (MB, 100 pmole). The L-SNC- and D-SNC-induced hypertension, tachycardia and BRF-inhibition were abolished by hemoglobin (100 nmole). Biochemical studies showed that L-SNC and D-SNC release equal amounts of nitric oxide (NO). These results suggest that the initial effects of L-SNC may be due to the activation of stereoselective nitrosothiol receptors and that the NO released from L-SNC and D-SNC may produce the hypertension, tachycardia and BRF-inhibition. (Supported by HLB14388 & HLB44546)

538.9

PHENYLEPHRINE INCREASES OPTICAL REFLECTANCE IN THE CAT VENTRAL MEDULLARY SURFACE. R.M. Harper*, D. Gozal, X-W. Dong, and D.M. Rector Brain Res. Inst. and Dept. of Anatomy & Cell Biology, UCLA Sch. of Med.; Div. Neonatology and Ped. Pulmonology, Children's Hospital, USC Sch. of Med., Los Angeles, CA.

We examined regional neuronal activation on the surface of the cat ventral medulla (VM) during phenylephrine-induced blood pressure elevation using large-array optical recording procedures. The VM was exposed through a ventral surgical approach in ten adult cats under pentobarbital anaesthesia. Arterial pressure, end-tidal CO_2, costal diaphragmatic EMG and ECG were monitored. A coherent image conduit was attached to a charge coupled device camera and positioned over the VM. Reflected 700 nm light was digitized continuously at 2-3 sec intervals during a baseline period, and after 10ug/kg i.v. phenylephrine administration. Forty images within each epoch were averaged, and were subtracted from baseline. Regional differences within the image were determined by ANOVA procedures (α=.05). Phenylephrine induced a significant transient elevation of blood pressure associated with diminished respiratory EMG activity. A pronounced increase in optical reflectance (decreased neural activity) was found over widespread regions of the VM surface during the early pressor response with a subsequent return to baseline values. We conclude that pharmacologically induced rapid blood pressure elevations result in a generalized decline in neural activity in areas of the VM traditionally associated with cardiorespiratory control.

(Supported by HL22418 and NIDR DE07212)

CIRCUITRY AND PATTERN GENERATION III

539.1

FLEXIBLE MOTOR PATTERNS IN CHICKS: BI- AND TRIPHASIC ELEMENTS. R. M. Johnston * and A. Bekoff, Department of EPO Biology and Center for Neuroscience, Box 334, University of Colorado, Boulder, CO 80309-0334

There is much evidence which supports the conclusions that the outputs of pattern generating networks are inherently flexible; flexibility and diversity of motor patterns results in part from both intrinsic and extrinsic sources of modulation. With this in mind we have examined the muscle activity patterns in walking, swimming and airstepping in chicks.

Our results show that these three behaviors share the same basic motor output consisting of a biphasic pattern (extensor-flexor alternation) at the hip and ankle coupled with a triphasic pattern (extensor-flexor-extensor alternation) at the knee. Flexibility and the diversity of rhythmic motor patterns results from the modulation of biphasic and triphasic elements. The elements of the biphasic components can be independently modulated. The elements of the triphasic pattern can also be independently modulated, but such modulation affects the interaction among the elements of the triphasic pattern.

Kinematic data show that the hindlimb movements in these behaviors occur within a triphasic framework which consists of one retraction period and two protraction periods per cycle. EMG data synchronized with kinematic data will be presented to establish the relationships between these muscle and behavioral patterns.

Supported by NIH grant NS 20310.

539.2

TIMING *VS.* PATTERN IN CYCLIC MOTOR PATTERNS IN CHICKS. A. Bekoff* and D. L. Nicholl. Department of EPO Biology and Center for Neuroscience, University of Colorado, Boulder, CO 80309-0334.

In previous work we have postulated that there is a timer for episodes of hatching movements in chicks that is separable from the motor pattern generating mechanism (Bekoff and Kauer, 1982; 1984). In the current work, we examine the characteristics of these two features of cyclic motor activity across the perinatal transition.

Spontaneous motor activity is videotaped in late stage chick embryos and continuous videorecordings are made from the time of hatching until walking is well established (6-8 hours posthatching). Quantitative measurements of cycle durations, number of movements per minute, and durations of activity and inactivity periods are made to examine temporal features of motor behavior. Interlimb coordination is analyzed to examine one aspect of the motor patterns.

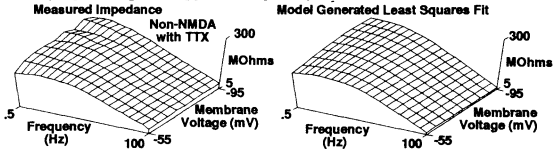
In hatching, synchronous leg movements occur during each hatching episode (Bekoff and Kauer, 1982). Episode duration is approximately 1-3 sec and inter-episode interval is 10-30 sec. Preliminary observations suggest that immediately posthatching, the interlimb coordination pattern switches abruptly to one of alternation of the two legs. Episode durations increase slightly, but inter-episode intervals remain similar to those seen during hatching. Both episode durations and inter-episode intervals gradually increase over the next few hours, while alternation continues to be the primary pattern of interlimb coordination. Thus changes in timing and pattern can occur independently.

Supported by NIH grant HD28247.

539.3

A MODEL OF A LAMPREY NEURON: PARAMETER ESTIMATION ON IMPEDANCE DATA. C.R. Murphey, L.E. Moore* and J.T. Buchanan. Physiology & Biophysics Dept., Univ. of Texas Medical Branch. Galveston, TX 77555.

Fictive swimming can be induced in the isolated lamprey spinal cord by exposing the entire cord to N-methyl-D-aspartate (NMDA) even in the absence of movement-related sensory stimulation. In this study the voltage and frequency dependence of the cell membrane impedance of an individual neuron are measured from the soma by point clamp methods both with and without application of NMDA. The impedance measurements are fitted to a Hodgkin-Huxley model similar to that of Grillner (J. Neurophysiol. 66:473-84, 1991) which characterizes the NMDA sensitive, slow Ca^{2+} activated K^+ and leakage currents using non-linear least squares optimization. The model is based on voltage clamp and frequency domain analysis of the ionic channels activated during fictive locomotion and is capable of reproducing the the membrane potential oscillations observed during fictive swimming. In addition, parameter sensitivity analysis is used to determine the influence of parameters changes on model-generated impedance and membrane potential waveforms. We are extending the model to simulate activity of a network of neurons during fictive locomotion using the Williams and Zipser algorithm (Neural Computation 1:270-280, 1989) to estimate synaptic weights. Supported in part by DHHS-R01-MH45796.



539.4

POSTINSPIRATORY NEURONAL ACTIVITIES IN THE INTACT UNANESTHETIZED CAT. J. Orem* and R.H. Trotter. Physiology Dept., Sch. of Med., Texas Tech Univ. HSC, Lubbock, TX 79430.

Cells that discharge in early expiration and inhibit other respiratory cells purportedly cause a separate phase, postinspiration, of the respiratory cycle (Richter *et al. NIPS* 1:109,1986). Our objective was to study postinspiratory cells in the intact, unanesthetized cat during sleep, wakefulness, and behavioral inhibition of inspiration. Activities of 226 respiratory neurons were recorded extracellularly with tungsten microelectrodes. Of these, 35 were active in early expiration. They were located in the dorsal or ventral respiratory group from behind the obex to the retrofacial nucleus. None of these 35 cells had strong and consistent activity confined to early expiration. Instead, various cell-types were active in early expiration. They included inspiratory-expiratory phase-spanning cells, retrofacial augmenting expiratory cells with bursts in early expiration, retrofacial decrementing expiratory cells, tonic expiratory cells, and cells with variable activity in the early part of expiration. Activities during behavioral inhibition of inspiration and sleep were variable also and depended in part on the strength and consistency of the respiratory signal of the cell, i.e., its η^2 value (Orem and Dick, *J. Neurophysiol.* 50:1089,1983). These results suggest that the state of early expiration is determined by many different cell-types rather than a single class of postinspiratory cells.

Supported by grant HL 21257.

539.5

A HYBRID PACEMAKER - NETWORK MODEL FOR THE RESPIRATORY OSCILLATOR IN MAMMALS. J.C. Smith*, G.D. Funk, S.M. Johnson, and J.L. Feldman. Systems Neurobiology Lab, Dept. of Physiological Science, UCLA, Los Angeles, CA 90024-1527.

Our studies of cellular and synaptic properties of medullary respiratory neurons in *in vitro* neonatal rat brainstem-spinal cord and medullary slice preparations suggest that the respiratory oscillator contains bursting pacemaker neurons (q.v. Smith et al., Science 254: 726-729, 1991) embedded in a complex network. To explore the dynamical properties of this network, we constructed a computational model of synaptically coupled, conditional bursting pacemaker cells which receive synaptic inputs from three other interneuron populations, based on cell mapping experiments (see Funk et al., this volume): two populations of beating (tonic) excitatory or inhibitory cells, and; inhibitory neurons, synaptically driven by the pacemaker cells, that provide phasic recurrent inhibitory inputs. All neurons were modeled as Hodgkin-Huxley type neurons with at least four membrane conductances. Pacemaker cells had eight membrane conductances (e.g. see Johnson et al., this volume), chosen to mimic measured neuronal voltage-dependent oscillatory behavior. Simulations exhibited: i) Synchronization within the pacemaker cell population; ii) Control of voltage-dependent oscillatory behavior of pacemaker cells by tonic inputs; iii) cycle-cycle dynamic resetting of pacemaker cell oscillations by recurrent inhibitory inputs; iv) state-dependent transformation of network oscillatory behavior. With strong excitatory and recurrent inhibitory input, pacemaker cells transformed from bursting to beating behavior, and the oscillatory membrane potential trajectories of pacemaker and follower cells transformed from decrementing to augmenting trajectories. These modes of behavior correspond to different functional states of the respiratory network observed *in vitro* and *in vivo*. Supported by NIH Grants HL40959 & HL02204 to JCS.

539.6

EARLY EXPRESSION OF THE RESPIRATORY PATTERN IN THE FETAL LAMB. I.R.C. Cooke*, D.J. Gould and P.J. Berger. Dept. of Biological Sciences, Deakin University, Geelong, Vic. 3217, Australia and Monash University Centre for Early Human Development, Clayton, Vic. 3168, Australia.

We have previously shown that episodes of stereotyped rhythmic bursting activity which occur in the diaphragm of the fetal lamb at 65 days gestation (G65; term = 147 days) are produced by a neural system which exhibits the major organizational features of the mature respiratory pattern generating system (Cooke and Berger, 1990, Brain Res. 522: 333-336). We have now examined the ontogeny of patterns of activity in the diaphragm of the fetal lamb over the period from G44 to G70. Using maternal general anaesthesia, fetal lambs at G43 were instrumented for the chronic recording of EMG activity in the right and left sides of the costal diaphragm (Di) and from the longissimus dorsi (Lo) muscles. Spontaneous EMG activity was recorded daily for 4-hour periods until G60, then at 2-day intervals until G70. Sustained discharges lasting up to 1min occurred coincidentally in both the Di and Lo as early as G44: this activity persisted after spinal cord transection at C1. Rhythmic bursting activity occurred only in the Di: these bursts occurred synchronously in the left and right sides of the Di and were abolished after cord transection at C1. Di bursting at G44-G50 was much less stereotyped than at G65, with considerable burst-to-burst variability in the shape, duration and intensity of individual bursts. Furthermore, rhythmic Di bursting and sustained discharge activity at G44-G50 did not exhibit the temporal segregation characteristic of G65 fetuses. Instead, episodes of Di bursting usually occurred at the end of, or during, sustained discharges. Our results suggest that the respiratory pattern generating system is active as early as G44 and that a control system develops between G44 and G65 which regulates the expression of the respiratory pattern of the Di.

539.7

SCP-CONTAINING RADULA MECHANOAFFERENT NEURONS IN THE BUCCAL GANGLION OF *APLYSIA*: RESPONSE PROPERTIES AND BIOCHEMICAL CHARACTERIZATION. M.W. Miller*, S.C. Rosen, E.C. Cropper, S. L. Schissel, I. Kupfermann, and K.R. Weiss. Ctr. for Neurobiol. & Behav., Columbia Univ., and NYS Psychiat. Inst., NY, NY 10032; Dept. Physiol. & Biophys., Mt. Sinai Sch. of Med., 1 Gustave Levy Pl., NY, NY 10029.

A cluster of neurons located on the rostral surface of each buccal hemiganglion of *Aplysia* has been studied using electrophysiological, immunocytological, and biochemical techniques. This cluster exhibits SCPb-like immunoreactivity and corresponds to the 'rostral group' previously identified in juvenile specimens (Lloyd et al., 1985). In mature animals (300 - 400 g), this cluster consists of 30 to 50 electrically-coupled cells ranging in size from 15 to 150 um. These neurons exhibit considerable diversity of size and form, with smaller spherical cells located laterally and larger ovoid cells positioned closer to the buccal commissure. Axons from these neurons exit the buccal ganglion via the radula nerve, typically branching at the initial radula nerve bifurcation. These axons project to specific regions of the membranous sheath underlying the radula surface, where they ramify extensively and appear to end in terminal specializations. Brief application of von Frey hairs (0.1 - 1.0 g) to the radula surface produces rapidly adapting trains of impulses in these cells. They are not responsive to chemical stimulation (NaCl, seaweed) of the radula. Their receptive fields exhibit considerable overlap, with the highest density occurring at the grasping surface of the radula. Radiolabelling and fractionation by HPLC of the peptides synthesized by physiologically and functionally identified radula mechanoafferent neurons demonstrated that these cells produce authentic SCPa and SCPb. The presence of sensorin-A-like immunoreactivity (see Brunet et al., 1991) was not detected histologically in these cells, indicating that they represent a novel class of mechanosensory cells in *Aplysia*. Their receptive fields, relatively low threshholds to tactile stimuli, and synaptic connections (Rosen et al., this vol.) suggest that these neurons may play an important role in the generation or modulation of feeding-related behaviors.

539.8

SCP-CONTAINING RADULA MECHANOAFFERENT NEURONS IN THE BUCCAL GANGLION OF *APLYSIA*: SYNAPTIC CONNECTIVITY OF IDENTIFIED CELLS. S. C. Rosen*, M. W. Miller, K. R. Weiss, and I. Kupfermann. Ctr. Neurobiol. & Behav., NYS Psychiat. Inst., NY, NY 10032; Dept. Physiol. and Biophys., Mt. Sinai Sch. of Med., NY, NY 10029.

A rostral cluster of SCPb-containing cells is comprised of diverse radula mechanoafferent neurons (Miller et al., this vol.). Two of these cells (designated B21 and B22, each immunopositive in 30 of 30 cases) were identified by morphological and electrophysiological criteria. Similar properties of B21 and B22 include spike amplitude, spike duration, sensory threshold (low), and receptive field location (grasping surface of radula). They differ however, in morphology and synaptic connectivity. B21 is a bipolar neuron (80-120 μm) that projects axons to the ipsi- and contralateral buccal hemiganglia. The contralateral axon sends peripheral branches, via the radula nerve, to the membrane underlying the chitin of the radula, near the sites of insertion of the I4 and accessory radula closer (ARC) buccal muscles. Its central axons have short tufted processes. B22 is a smaller cell (50-80 μm) with a similar distribution of axons. Its central axons have long filamentous processes. B21 receives a monosynaptic IPSP from neuron B4, whereas B22 does not. B21 and B22 make various monosynaptic chemical and electrical synaptic connections to motor neurons and interneurons. All the known connections to motor neurons are to those (B8a, B8b, B15, B16) that innervate muscles (I4, ARC) controlling radula closure. B21 produces a slow chemical EPSP in neuron B15 that can modulate B15 excitablity. Sensory evoked excitability changes in B15 might explain increases in B15 activity seen in intact animals when food is drawn into the buccal cavity (Cropper et al., 1990). Both B21 and B22 make electrical connections to buccal-to-cerebral interneuron B19. B19 regulates the actions of command-like interneuron CBI-2. Stimulation of B21 modulates the feeding motor program driven by CBI-2. The sensory properties and connections of B21 and B22 suggest that they provide peripheral sensory information that modulates the strength and timing of radula closure during food ingestion.

539.9

NETWORK MODEL OF A CENTRAL PATTERN GENERATOR IN THE BUCCAL GANGLIA OF *APLYSIA*. I. Ziv*, D.A. Baxter and J.H. Byrne Dept. of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77225

A pattern of neural activity (pattern 2) in the buccal ganglia is associated with a depolarization of identified neurons B31/32 and B35 and is followed by activation of B4 and by repolarization of B31/32 and B35 (Susswein & Byrne, 1988). Several neurons have been identified that are believed to be elements of the central pattern generator (CPG) that produces pattern 2: including B31/32, B35, B51 and B52 (Susswein & Byrne, 1988, Plummer & Kirk, 1990). The aim of the present study is to determine whether a network model of these neurons can exhibit pattern 2.

The network was simulated with SNNAP (Ziv et al., 1991), in which membrane conductances are simulated by H-H type equations and chemical synaptic conductances by second-order differential equations. Our strategy in simulating the network was first to simulate the characteristic firing properties of the individual neurons, then to simulate the features of the synaptic connections and finally to incorporate the components into the network. At each step, parameters were adjusted to match experimental data and then fixed.

Although we simulated the intrinsic properties of the neurons and their interconnections, the network failed to exhibit pattern 2. We then added an additional neuron that provided feedback inhibition to B31/32 and B35 and excitation to B4. With this modification, the network exhibited pattern 2, and exhibited rhythmic bursts of activity in B31/32 and B4 in response to sustained depolarization of B31/32. Preliminary recordings from buccal ganglia indicate the presence of such an additional neuron. As this cell is characterized further and incorporated into the network model it will be possible to determine the quantitative contribution that individual neurons and synaptic connections make to the different phases of pattern 2.

539.10

AN IDENTIFIED MODULATORY NEURON EXHIBITS DIFFERENT ACTIVITY PATTERNS IN SPATIALLY SEPARATE ARBORS. M.J. Coleman* and M.P. Nusbaum. Neurobiology Research Center and Dept. of Physiology & Biophysics; Univ. of Alabama at Birmingham; Birmingham, AL 35294.

The modulatory axon SNAX 1 excites the pyloric and gastric mill networks and also receives local rhythmic synaptic feedback from these networks in the stomatogastric ganglion (STG) of the crab, *Cancer borealis* (Nusbaum et al., J. Neurosci. 12:In Press). SNAX 1 recordings are made intra-axonally, at the entrance to the STG. We have now recorded intracellularly within the commissural ganglion (CoG) from the SNAX 1 cell body, which we have named Modulatory Commissural Neuron 1 (MCN 1).

Intra-somatic stimulation of MCN 1 mimics the effects of intra-axonal stimulation of SNAX 1 on the STG networks. Furthermore, like SNAX 1, MCN 1 receives both pyloric- and gastric mill-timed synaptic feedback. However, the feedback to these two regions of the same neuron is different. For example, the gastric mill neuron LG synaptically inhibits SNAX 1 in the STG, but does not influence MCN 1 in the CoG. Thus, when the gastric mill rhythm is driven by SNAX 1 stimulation, SNAX 1 activity is suppressed during each LG neuron impulse burst. In contrast, during MCN 1 stimulated gastric mill rhythms, there is no suppression of MCN 1 activity during LG impulse bursts. Similarly, while the pyloric-timed feedback onto SNAX 1 occurs in the STG, the pyloric-timed feedback onto MCN 1 occurs in the CoG. We are now attempting to make paired recordings from MCN 1 and SNAX 1, to directly determine whether this identified neuron simultaneously produces distinct activity patterns in its spatially separate arbors.

Supported by NS29436 and HFSP (MPN).

539.11

THE USE OF GENETIC ALGORITHMS TO EXPLORE NEURAL MECHANISMS THAT OPTIMIZE RHYTHMIC BEHAVIORS: QUASI-REALISTIC MODELS OF FEEDING BEHAVIOR IN *APLYSIA*. I. Kupfermann, D. Deodhar*, S.C. Rosen, and K.R. Weiss. Center for Neurobiology and Behavior, Columbia University, New York, NY 10032 and Fishberg Center, Mt. Sinai Schl. Med., New York, NY 10029.

We have begun to explore how behavior may be optimized by means of simple neural models, in which the effects of parameters in the model are systematically studied. As a beginning we have attempted to define the simplest model of the process by which a strip of food is rhythmically drawn into the buccal cavity and swallowed. The operation of a 2 neuron, 2 muscle model was determined using an integrate-and-fire algorithm (Perkel and Mulloney; Getting). As a first approximation, the fitness of our model was defined as the difference between the energy expended by the muscles and the energy gained as the radula pulls in seaweed. The parameters of 4 synapses in the model were evolved using a genetic algorithm (Goldberg), and this produced a number of "fit" solutions that generated rhythmic behavior that resulted in a net gain of energy. The defined models lack any modulatory mechanisms, and therefore, as predicted, their fitness was dramatically reduced when the system was challenged by even small variations of the evolved parameters. Current work is extending this approach, utilizing genetic algorithms to increase the number of variables that can be explored, and to study how the models improve by including sensory feedback and modulatory mechanisms that can dynamically alter relevant parameters according to the environmental conditions, as we have postulated to occur in the *Aplysia* feeding system.

539.12

MODELING A HALF-CENTER OSCILLATOR THAT TIMES HEARTBEAT IN THE MEDICINAL LEECH.
Ronald L. Calabrese, Erik De Schutter and Ted W. Simon*
Dept. of Biology, Emory University, Atlanta, GA 30233

Heartbeat in the leech, *Hirudo medicinalis*, is timed by half-center oscillators comprising pairs of reciprocally inhibitory heart interneurons. Oscillation is sustained through the interaction of synaptic and voltage-gated membrane currents. Physiological experiments (J Neurosci 9:2846, 1989; J Neurosci 11:746, 1991) indicate: a) a hyperpolarization-activated inward current (I_h) is crucial in pacing oscillation. b) inhibitory interactions involve a strong graded component in addition to spike-mediated transmission. c) low-threshold Ca^{2+} currents [I_{CaS} (slowly inactivating) and I_{CaF} (rapidly inactivating)] underlie graded transmission.

We have developed a model of graded transmission in this system in which postsynaptic conductance is dependent on presynaptic Ca^{2+} entry, accumulation and removal. This model has permitted us to study the interaction between synaptic transmission, low threshold Ca^{2+} currents and I_h in sustaining oscillation. The model was created with Nodus software (Comput Bio Med 19:71, 1989) and all voltage gated currents were represented as Hodgkin-Huxley equations derived from biophysical data. Our results indicate that the transition from the inhibitory to the burst phase of the oscillation is determined by voltage gated-conductances in the inhibited interneuron, not by postsynaptic conductance and that the period of the oscillation depends critically on g_h-max. A primary interaction between synaptic and voltage-gated conductances occurs when I_h limits deinactivation of I_{CaS} that sustains graded synaptic transmission. We are also studying the role of action potentials in generating oscillation. (NIH NS24072).

BLOOD-BRAIN BARRIER II

540.1

ASSESSMENT OF BLOOD-BRAIN BARRIER IN THE MAGGOT (Delia platura; Insecta, Diptera).
J. L. Juang, S. D. Carlson and C. Chi*, Department of Entomology; Neuroscience Training Program, University of Wisconsin, Madison, WI 53706. *Nicolet Instrument Corp., 5225 Verona Rd. Madison, WI 53711

Insects are one of only several classes of invertebrates that possess a blood-brain barrier (BBB). Very little knowledge of the BBB exists for immature insects, yet larvae undergo sweeping changes in CNS development and are the most metabolically energetic form. Because of this plasticity and other positive experimental attributes, dipteran larvae may be a useful ('throwaway') first animal model for BBB studies involving higher animals. A fully formed BBB exits for the earliest postembryonic larva. The BBB remains intact for the duration of larval life only to be compromised in early pupal life. From TEM data the BBB consists of a widespread array of septate junctions that bind perineurial cells overlying ventral ganglion and abdominal nerves. Ionic La^{3+} accumulates in the (pleated sheet) septate junctions and does not gain access to neuronal surfaces. No tight junctions have been found in the larval CNS. X-ray microanalysis indicates absence of La^{3+} in TEM sections where La^{3+} is not detected. The BBB can be manipulated using various osmotic gradients. Supported by NSF BNS89080881.

540.2

BLOOD-BRAIN BARRIER GLUCOSE TRANSPORTER IN DEVELOPING RABBITS: REGULATION OF GENE EXPRESSION.
K.J. Dwyer, A. Yuwiler*, and W.M. Pardridge. Departments of Medicine and Psychiatry and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA, 90024.

Physiological studies have described developmental changes in blood-brain barrier (BBB) glucose transporter activity. Recent studies have shown that the GLUT1 isoform is the predominant BBB glucose transporter. The present studies examine molecular mechanisms underlying the developmental modulation of BBB GLUT1 gene expression. Initially, a capillary depletion technique was performed to demonstrate selective localization of brain GLUT1 mRNA levels at the microvasculature of rabbit brain. BBB GLUT1 mRNA levels were measured by Northern analysis of poly A+ mRNA, and were generally stable in the postnatal period despite marked downregulation of brain mRNA levels for cytoskeletal proteins such as actin or tubulin. In parallel to the postnatal stabilization of GLUT1 mRNA levels, immunoreactive GLUT1 protein, as detected by quantitative Western blotting employing a purified human erythrocyte GLUT1 protein as standard, demonstrated initial downregulation of immunoreactive GLUT1 at 14 days followed by upregulation by 28 and 70 days in isolated brain capillaries. The concentration of immunoreactive GLUT1 in 70-day rabbit brain capillaries was 87 ± 1 pmol/mg capillary protein. Immunocytochemistry of developing rabbit brain sections demonstrated differential regulation of choroid plexus and microvascular GLUT1 with overexpression of choroidal GLUT1 at 28 days relative to the microvascular GLUT1. The opposite regulation of GLUT1 mRNA and GLUT1 immunoreactive protein provides support for the hypothesis that a principal mechanism of GLUT1 gene expression in the developing BBB is post-transcriptional.

540.3

DEVELOPMENTAL MODULATION OF BLOOD-BRAIN-BARRIER GLUCOSE TRANSPORT. E. M. Cornford, S. Hyman, E. M. Landaw and A. V. Escueta*. Dept. of Neurology, UCLA School of Medicine, and West Los Angeles VA Medical Center, Los Angeles, CA 90073.

Blood-brain barrier (BBB) glucose transport has been characterized in newborn, 14-day-old suckling, 28-day-old weanling and adult rabbits. A polyclonal antiserum immunocytochemically identified the GLUT-1 isoform in rabbit brain capillaries. Cerebral blood flow rates increased from 0.19 and 0.26 ml/min.gm (neonates and sucklings) to 0.51 (28-day) and 0.70 (adults) ml/min.gm ($p < 0.05$). BBB Extraction (E) of water decreased with age. The Permeability-Surface Area product (PS) of water was 0.80 ml/min.g in neonates, decreased to 0.39 ml/min.g in sucklings and increased in weanlings (0.47 ml/min.g) and adults (0.75 ml/min.g). The Km (half-saturation constant) for glucose transport (13-19 mM) was not significantly different at any of the ages examined. Maximal velocities (Vmax) of glucose transfer (an indicator of the activity and relative number of transporter proteins) increased significantly ($p < 0.05$) with age. In neonates Vmax = 0.61, in sucklings = 0.68, in weanlings = 0.88, adults = 1.01 umoles/min.g; indicating developmental up-regulation of the BBB glucose transporter.
Supported by NIH Grant NS 25554.

540.4

EXPRESSION OF Na,K-ATPase AT THE BLOOD-BRAIN INTERFACE IN RATS. B.V. Zlokovic*, J.B. Mackic, L. Wang, C. Magyar, J.G. McComb and A.A. McDonough. Depts. Neurol. Surg., Physiol. & Biophys.*, and Divn. Neurosurg. CHLA, USC Sch. Med., LA, California

Regulation of ionic composition of brain extracellular fluid (ECF) and cerebrospinal fluid (CSF) is essential for normal neuronal functions, and for control of brain volume and intracranial pressure. The blood-brain barrier (BBB) and choroid plexus (CP) play major roles in Na^+ and K^+ homeostasis in brain ECF and CSF. Na,K-ATPase is a key enzyme responsible for active transport of these ions between blood, ECF and CSF. The pumps are composed of αß heterodimers. Three α and two ß isoforms have been characterized in brain. In this study we utilized isoform specific antibodies to test for Na,K-ATPase subunits α_1, α_2, β_1 and β_2 in cerebral microvessels, capillary-depleted (CD) brain tissue and CP of rats. Microvessels isolated from cerebral cortical mantles by dextran density centrifugation, and CPs from lateral ventricles were prepared for SDS-polyacrylamide electrophoresis Western blot analysis as described [Am.J.Physiol. (1985) 248: C247-C251], and quantitated by scanning densitometry. α_1, α_2, β_1 and β_2 subunits were all detected in CD-brain tissue, as previously reported in brain. The CP expressed α_1 and β_1, while β_2 signal was weak and α_2 was absent. In contrast, microvessels expressed α_1, α_2, β_1 and β_2. When comparing subunits levels in different samples relative to a constant amount of protein, α_1 was expressed the highest in CP. Expression of α_1 and α_2 in BBB was higher in comparison to CD-brain tissue. The data support the concept [Soc. Neurosci. Abstr. (1991) 17: 240] that BBB and blood-CSF barrier regulate brain ionic composition of brain ECF and CSF by different mechanisms. (Supported by TRDRP grant 2RT0071. Antibodies to rat Na,K-ATPase subunits are provided by Dr. K. Sweadner).

540.5

CONFORMATIONALLY CONSTRAINED PEPTIDES AND THE BLOOD-BRAIN BARRIER. S.J. Weber and T.P. Davis. Department of Pharmacology, University of Arizona College of Medicine, Tucson, AZ 85724.

The present study examined structural modifications that should enhance peptide blood-brain barrier (BBB) penetration. Halogenation at the Phe[4] position of DPDPE has been previously shown to increase lipophilicity. Therefore, the intent of this study was to examine halogenation's effect upon BBB penetration using an *in vitro* BBB model and the whole animal (*in vivo*). The *in vitro* BBB model consisted of a monolayer of confluent primary bovine brain microvessel endothelial cells (BMEC) grown on 3μm polycarbonate filters. Permeability coefficients (P) were calculated based on the diffusion of DPDPE or [ρ-ClPhe[4]]DPDPE (0-120 min) across the BMEC. *In vivo* BBB penetration was determined by tail-vein injection of [^{3}H]DPDPE or [^{3}H][ρ-ClPhe[4]]DPDPE, saline perfusion via the left ventricle of the heart at a specified time (5-40 min postadministration) followed by removal, solubilization, and counting of the brain. The BMEC permeability coefficient of DPDPE (P=0.0021 cm/min) was significantly less ($p<0.01$) than that of [ρ-ClPhe[4]]DPDPE (P=0.0031 cm/min). Data from the *in vivo* study showed the amount of [^{3}H][ρ-ClPhe[4]]DPDPE crossing the BBB (0.166-0.188%) was significantly greater ($p<0.01$) than that of [^{3}H]DPDPE (0.059-0.067%). The data from the *in vitro* and *in vivo* studies provide strong evidence that the halogenation of DPDPE at the Phe[4] position increases BBB penetration and that the BMEC model may be useful in predicting *in vivo* BBB penetration. Supported by U.S.P.H.S. grant DA-06284, MH-42600 and HD-26013.

540.6

A VASOACTIVE INTESTINAL PEPTIDE ANALOGUE COUPLED TO A BRAIN DRUG DELIVERY VECTOR INCREASES CEREBRAL BLOOD FLOW. T. Yoshikawa, U. Bickel, S. Diamond*, and W.M. Pardridge. Departments of Medicine and Neurology and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

An in vivo CNS pharmacological effect is demonstrated for the first time by systemic administration of small doses of a neuropeptide (12 μg/kg) employing a blood-brain barrier (BBB) transport vector for drug delivery. An analogue of vasoactive intestinal peptide (VIP) was synthesized, and monobiotinylated using a cleavable biotin linker. The VIP analogue (I) and the biotinylated (II) and des-biotinylated (III) derivatives showed retained affinities to the VIP receptors in a binding assay with rat lung membranes. K_i values for the high affinity binding site were 3.160 nM (I), 0.999 nM (II) and 1.635 nM (III), compared to 0.260 nM for native VIP. Recent studies have shown that a monoclonal antibody to the transferrin receptor, OX26 may serve as a potential brain drug-transport vector (Friden et al., P.N.A.S., 88, 4771, 1991). A covalent conjugate of avidin and OX26 was prepared for brain delivery of the biotinylated VIP analogue through the BBB. Brain uptake of ^{125}I-labeled biotinylated peptide was investigated in rats with an internal carotid artery perfusion/capillary depletion technique. The transport vector, avidin/OX26, mediated the transcytosis of the VIP analogue through the BBB. Effect of biotinylated VIP analogue coupled to avidin-OX26 conjugate on cerebral blood flow rate in rats was measured from the tissue uptake of ^{3}H-diazepam. Rats were immobilized and ventilated with 70% N_2O and 30% O_2 to keep blood pCO_2 level constant. Cerebral blood flow in rats was not changed by intracarotid infusion of the biotinylated peptide. However, a 65% increase in cerebral blood flow was elicited when the peptide was coupled to the BBB transport vector.

540.7

AN EXCEPTION TO THE CONCEPT THAT A GRAFT DETERMINES ITS TYPE OF VASCULARITY. S. Ishihara, L. Chang and M. Brightman*. N.I.H., Bethesda, Md. 20892.

The hypothesis, that the type of vessel supplying an organ is determined by the organ rather than the vessel's source, (Stewart and Wiley, 1981) has been verified, by others, for brain. An exception is reported here in muscle grafted to mature rat hosts. In autografts of mature skeletal muscle, inserted into the IV ventricle of adult rats, some of the vessels were of the fenestrated type, like that of adjacent choroid plexus and area postrema, rather than of the muscle type. Two days, one and four weeks after pieces of neck muscle had been inserted into the IV ventricle, the grafts became vascularized by fenestrated blood vessels (FBV) that lay among muscle cells. The intact cerebral cortex of rats contain FBV up to a fetal age of 17 days. In order to see whether brain tissue responded as did muscle, pieces of 18 day old fetal brain were grafted to the rats' ventricle. These grafts came to contain only barrier vessels. Thus, the components of mature skeletal muscle did not normalize the morpholgy of its vessels whereas the components of a brain tissue graft, presumably its astrocytes, permitted the expression of its endothelial barrier properties.

540.8

A STUDY OF MICROVESSELS DURING HUMAN BRAIN DEVELOPMENT. L. Wang*, W.X. Tang, D.C. Liu, D.Y. Hong and Y.R. Liang. Dept. of Pathology, and Lab of Electron Microscopy, Hunan Medical University, P.R.China, and *Dept. of Neurosurgery, USC School of Medicine, LA, CA 90033.

Kinetic studies have demonstrated that fetal blood-brain barrier (BBB) is more permeable to large and /or small polar molecules than adult BBB. However, the structural basis of increased permeability of fetal BBB is still not fully understood .In this paper, an ultrastructural examination was performed on cerebral capillaries of human fetuses aged from 5 to 9 months, and several structural parameters were determined (Brain Res. 1987; 429: 271-281). The results were as follows: (1) endothelial tight junctions of fetal BBB became longer and tortuous; clefts were found within the junctions and there was no an significant change between 5 and 9 months; (2) plasmalemmal vesicles were larger in 5th month fetuses than in 9th month fetuses, and the density of vesicles was low (<2/μm^2 cytoplasm); (3) capillary diameter was decreased ($P< 0.01$) while the thickness of capillary wall was increased with the fetal age; (4) no significant difference was observed in the nucleo-cytoplasmic ratio and in surface area of pericyte per vessel profile; the basal laminae became thicker and denser obviously, while perivascular space narrowed gradually. The results indicate that capillaries of human fetuses between 5 and 9 months are immature. It is suggested that incomplete development of the tight junctions may play more important role in fetal BBB permeability than plasmalemmal vesicular transport.

540.9

CELLULAR LINE OF DEFENSE UPON BREACHMENT OF THE BLOOD-BRAIN BARRIER (BBB). B. Baker and R. Broadwell*. Div. Neurosurgery, Univ. Maryland Sch. Med., Balto., MD 21201

Rats administered native HRP, the lectin WGA-HRP or the ligand transferrin-HRP into the blood exhibit CNS populations of perivascular and pial surface phagocytes labelled with each of the blood-borne macromolecules. These probes enter the CNS by transcytosis through the BBB and/or by extracellular pathways circumventing the BBB. The labelled cells likely represent pericytes, macrophages, or micoglia. Immunostaining for macrophages (ED2 antibody) revealed such cells on the pial surface, within Virchow-Robin spaces and perivascular clefts throughout the brain parenchyma, and in all circumventricular organs (CVOs) of the adult CNS; similar cells on the pial surface and in CVOs also stained immunohistochemically for major histocompatibility complex class II expression (OX6 antibody). In the 2 day neonatal CNS, macrophages were on the pial surface, in CVOs and white matter; in the 20 day fetal CNS, macrophages occupied the pial surface and CVOs. Staining for microglia (OX42 antibody and the lectin Griffonia simplicifolia) revealed microglia throughout the CNS, including the pial surface, CVOs, and perivascular clefts. The data suggest that phagocytic and potential antigen-presenting cells exist everywhere in the CNS where the BBB is breached either by transcytosis through the barrier or by extracellular circumvention. The phagocytic/antigen-presenting cells represent a cellular component of the BBB and are the first line of defense in the brain once the BBB is breached. Supported by NIH grant #NS18030.

540.10

TISSUE FACTOR EXPRESSION IN ASTROCYTES *IN VITRO* AND *IN VIVO*. M.P. Eddleston*, J.C. de la Torre, M.B.A. Oldstone. Div. of Virology, Dept. of Neuropharmacology and N.Mackman. Dept. of Immunology, The Scripps Research Institute, La Jolla, CA 92037.

Tissue factor (TF) or tissue thromboplastin is a transmembrane glycoprotein that functions as the initiator of the coagulation protease cascades. The widespread distribution of TF in the body is consistent with it forming a 'haemostatic envelope' around the vascular system. The brain contains very high levels of both TF mRNA and functional protein and is the major source of commercial tissue thromboplastin used in clotting assays. However, until this report the localisation of TF in a specific brain cell was unclear. Initial *in situ* hybridisation studies suggested that astrocytes may express TF mRNA. Therefore, TF expression in astrocytes *in vitro* and *in vivo* was examined. TF mRNA was expressed constitutively in both primary mouse astrocytes and a mouse astrocyte cell line, termed JCT. mRNA was also present in one rat (C6) and two human (U373,CCF) glioma cell lines. JCT cells expressed high levels of procoagulant activity in a single-stage clotting assay. Lipopolysaccharide (LPS) and serum are known to induce TF in other cell types; we noted that serum induced a 4-6 fold increase in both TF mRNA levels and procoagulant activity in JCT cells, whereas stimulation with LPS did not alter the mRNA level. Use of a mouse TF ^{35}S riboprobe and antibody to glial fibrillary acidic protein (GFAP) on consecutive sections of mouse brain indicated that astrocytes express high levels of TF mRNA in both normal and gliotic brain. After damage to the blood brain barrier by needle injury, the highest levels of TF mRNA were in GFAP nil cells, presumably representing either activated microglia or infiltrating macrophages. Our results indicate that astrocytes express functional TF *in vitro* and are a major source of TF *in vivo*. We propose that astrocytes form the 'haemostatic envelope' in the CNS. Moreover the very high level of TF expression in astrocytes and its induction by serum raises the possibility of further roles for TF in the brain during trauma and development.

540.11

DIFFERENTIAL EXPRESSION OF 5-LIPOXYGENASE TRANSCRIPTS IN HUMAN BRAIN TUMORS. R.J. Boado, W.M. Pardridge, and K.L. Black*. Departments of Medicine and Surgery and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

In addition to the important role of leukotrienes as mediators in allergy, inflammation and neurotransmitter release; these compounds have also been linked to pathophysiological events in the brain including cerebral ischemia, cellular and vasogenic brain edema and increased permeability of the blood-brain barrier in brain tumors. Although brain tumors have been shown to secrete leukotrienes, no studies to date have provided evidence for the tumor expression of genes encoding enyzmes involved in leukotriene production. Therefore, the present study determined the abundance of the 5-lipoxygenase (5-LO) transcript (limiting step of leukotriene synthesis) in a series of human brain tumors. Macrophage/monocyte infiltration was evaluated by measuring the abundance of the phagocyte-specific transcript cytochrome b heavy chain (gp91-phox). A single 2.8 kb 5-LO transcript was observed in bovine brain. In human brain tumors and in the dimethyl sulfoxide-induced promyelocytic human leukemic HL-60 cells, the 5-LO gene is expressed as a multitranscript family (2.7, 3.1, 4.8, 6.4, 8.6 kb). The relative abundance of these transcripts in HL-60 cells was 40.4, 30.5, 25.3, 2.5, and 1.4%, respectively. The abundance of 5-LO transcripts, the expression of the larger transcripts, and the 5-LO/gp91-phox ratio were found to be correlated with the tumor malignancy.

Conclusion: the present study supports the hypothesis that the 5-LO gene product may play an important role in human tumor-induced brain edemas and provides evidence for a differential tumor-specific expression of high size 5-LO transcripts in human brain tumors.

CELL MIGRATION AND MOTILITY II

541.1

THYMOSIN $\beta 4$ IN BRAIN CELLS OF HIV INFECTED (HIV^+) INDIVIDUALS. L.C.Stanley*, R.E.Mrak, L.J. Perrot, M.+Morrison-Bogorad, and W.S.T.Griffin. Departments of Anatomy, Pathology, and Pediatrics, UAMS, Little Rock, AR 72205 and +Departments of Biochemistry and Neurology, UTSWMC, Dallas, TX 75235.

Thymosin $\beta 4$ ($\beta 4$) is a member of the β thymosin actin-binding protein family. Cellular levels of these proteins are sufficient to account for actin sequestration which regulates actin polymerization. $\beta 4$ is present in immune cells, *e.g.*, macrophages, and in non-astrocyte glial cells in rat and human brain. Presuming that activation and mobility of the numerous microglia-macrophages in brain from HIV^+ individuals involves changes in actin polymerization, we compared $\beta 4$-immunoreactive cells in formalin-fixed, paraffin-embedded temporal lobe sections from HIV^+ individuals (n=8) with analogous cells from controls (n=6) to determine if expression of $\beta 4$ is altered in HIV^+ individuals. $\beta 4$ was present in glial cells uniformly distributed in grey and white matter. These GFAP-negative cells appeared to be either activated microglia or enlarged oligodendrocytes. In the microglial nodules commonly observed in brain of HIV^+ individuals, $\beta 4$ was found in the microglia-macrophages that contained elevated amounts of interleukin-1α. Since the number of $\beta 4$ immunoreactive cells in each of these cell types in HIV^+ individuals was greater than that in controls, we conclude that elevated expression of $\beta 4$ in brain is associated with HIV infection and may reflect an alteration in actin metabolism in specific glial cell types. Supported in part by NS27414.

541.2

MIGRATORY MECHANISMS OF FRESH HUMAN GLIOBLASTOMA MULTIFORME. J.J. Bernstein*, E.R. Laws, E. Hattwick, and W.J. Goldberg, G. Tadvalkar, Lab. CNS Inj. Regen., DVA Med. Ctr. Wash., DC 20422, & Dept. Neurosurg. George Wash. Univ. Wash., DC, & Univ. VA Sch. Med., Charlottesville, VA.

Fresh human glioblastoma multiforme (GM, high grade malignant astrocytoma) xenografted into rat brain migrate upon basement membrane lined surfaces. This migratory capacity is now studied *in vitro* using hydrated gel wafers (16.0 mm diameter, 11.0 mm thick) of extracellular matrix components (artificial basement membrane [ABM, Matrigel]; or collagen I [Col I, Vitrogen]). Fresh GM astrocytomas were mechanically disrupted and a heavy cell suspension seeded on hydrated gel wafers. Cultured wafers were prepared for scanning electron microscopy over 7 days. Cells that migrated into both ABM and Col I gels were immunohistochemically positive for GFAP and $p185^{c\text{-}neu}$, the *c-neu* proto-oncogene encoded transmembrane tyrosine kinase receptor protein. $p185^{c\text{-}neu}$ is overexpressed after astrocyte transformation. The fresh tumors were immunohistochemically stained for the presence of plasminogen activators as an index of migratory capacity. Both tissue (tPA) and urokinase (uPA) plasminogen activators were observed as well as guanidinobenzoatase, a serine protease associated with migrating cells. These data demonstrate that migration of the GM malignant astrocytoma cells into ECM hydrated gels is correlated with the expression of plasminogen activators and proteases which can either activate or function as collagenases. Supported by DVA and NIH, NCI 48956 (JJB).

541.3

GABA AND NGF INDUCE EMBRYONIC RAT SPINAL CORD CELLS TO MIGRATE. T. N. Behar*, A. E. Schaffner, C. Colton[1], and J. L. Barker. Lab. of Neurophysiology, NINDS, NIH, Bethesda, Md. 20892 and [1]Dept. of Physiology and Biophysics, Georgetown University School of Medicine, Washington, D.C. 20007.

Chemotactic and chemokinetic migration of acutely dissociated spinal cord cells derived from 12 to 15 day-old rat embryos (E12-E15) was analyzed in vitro using a chemotaxis chamber. Beginning at E13, embryonic cells migrated within 4 hours toward nanomolar concentrations of γ-amino butyric acid (GABA) and picomolar concentrations of nerve growth factor (NGF) and muscimol, a $GABA_A$ agonist. Bicuculline, a $GABA_A$ receptor antagonist, completely blocked the muscimol-induced migration, suggesting that the chemokinetic effect of GABA is specific and involves a bicuculline-sensitive receptor. Cellular migration was also inhibited by pre-incubation of GABA or NGF with specific antibodies. Extending the length of the migration period to 18 hours did not result in a significant increase in the total number of migrated cells, suggesting that all of the cells capable of responding to the chemoattractants responded within the first 4 hours. A modified "checker-board" analysis indicated that GABA exerted a chemokinetic effect on cells, while NGF was predominantly chemotactic, inducing cells to migrate along a chemical gradient.

E13 and E14 spinal cords contained the greatest number of responding cells, which were located primarily in the ventral half of the cord. Immunolabelling indicated that most migrating cells were postmitotic neurons. The majority (>75%) of migrating cells expressed neurofilament protein (NF) and none of the migrating cells incorporated BrdU during the course of an 18 hour assay. These results suggest that both GABA and NGF are capable of directing the migration of newly generated neurons during early spinal cord development.

541.4

ELECTRON MICROSCOPIC OBSERVATION OF MIGRATING GRANULE CELLS BY PERPENDICULAR CONTACT GUIDANCE IN THE CEREBELLAR MICROEXPLANT CULTURE. K.Ono[1]*, N.Nakatsuji[2] and I.Nagata[3], [1]Dept. of Anat., Okayama Univ. Med. Sch., Okayama 700. [2]Natl. Inst. of Genetics, Mishima 411, [3]Tokyo Metropo. Inst. Neurosci., Fuchu 183, Japan.

We have previously found that cerebellar granule cells migrated not only parallel but also perpendicular in cerebellar microexplant cultures (Development 106, 442-447, '89). In the present study, we examined the fine structure of granule cells with special reference to the cytoskeletal architecture at their transitional stage. On the 1st day of culture, asymmetrical bipolar cells migrated out radially from the explant. Microspikes (filopodia) protruded from their perikarya and radial neurites, which frequently contacted the neighboring cells and parallel neurites. Microspikes from perikarya contained microtubules (MT), suggesting that they initiate the perpendicular processes. After 2-3 days in culture, the cells changed orientation perpendicularly to the radial migration. Centrioles (CT) were frequently observed at the base of the perpendicular process, and MT extended from CT. These findings suggest that; 1) microspikes seem to play important roles to determine the initial orientation of perpendicular processes, 2) CT act as MT organizing centers and may be related to formation of perpendicular processes.

541.5

THE ROLE OF THROMBOSPONDIN IN GRANULE CELL MIGRATION IN THE DEVELOPING CEREBELLUM. L.J. Boyne*, K.S. O'Shea, V.M. Dixit. University of Michigan School of Medicine, Ann Arbor, MI 48109.

The role of TSP in cell migration, process outgrowth and fasciculation was assessed using cerebellar explants. Glass coverslips were coated with polyLlysine followed by either 20 ug/ml laminin (LN) or 10 ug/ml TSP. Cerebella were dissected from postnatal day 8 mice and the external granule and Purkinje layers removed and plated onto the coverslips and defined medium added. When the distance processes extended and cells migrated from the explants were measured, growth on LN exceeded that on TSP. Immunocytochemical localization of anti-neurofilament (NF) antibodies (Ab) and anti-glial fibrillary acidic protein Ab showed that initial outgrowth on LN was NF positive. The initial outgrowth on TSP, however, was glial with neuronal outgrowth occurring secondarily. Addition of anti-TSP strikingly inhibited granule cell migration on explants growing on LN. When DiI labelled, NGF-primed PC12 cells were added after 3 days in culture, 53.9% of the PC12 cells extended neurites onto the granule cell processes growing on LN. In the presence of anti-TSP Ab only 30.3% of the PC12 cell processes fasciculated. TSP promotes early glial cell differentiation and consistent with its deposition in association with migrating cells and their processes in the developing cerebellum *in situ*, TSP plays a crucial role in granule cell migration and fasciculation *in vitro*. Supported by Foundation for Physical Therapy (LJB), HD-07273 (LJB) and HD23867 (KSO).

541.6

Identification of Polypeptides that Potentially Comprise the Plasmalemmal Microdomain Between Migrating Neuronal and Radial Glial Cells. R. Cameron and P. Rakic*, Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510.

Detachment of postmitotic neurons from other cellular elements in proliferative zones and their subsequent movement along a scaffold of radial glial cell processes is a basic tenet of cortical development. Although several neuronal cell adhesion components have been described, glial cell counterparts have remained elusive. We have initiated studies to identify glial cell polypeptide components that may participate in the selective affinity for migrating neurons. Of several heterologous polyclonal antisera developed against plasmalemmal fractions obtained from primary cultures of neonatal rat Type-I glial cells, one (D4) immunolabels the surface of astroglial cells in a patchy pattern. Immunofluorescent analyses demonstrate that the plasmalemmal microdomains are present on both Type-I astroglial and radial glial cells but are absent from oligodendrocytes and Type-II astrocytes. The microdomains do not co-localize with vinculin, actin, or integrin subunits. In many cases, immunostaining appears to localize to sites of cell-cell contact, both glial-glial and glial-neuronal. For example, for migrating neuronal cells in dissociated cell cultures, D4 immunoreactivity is detected at sites where the somal region and the leading process are in contact with an elongated glial cell fiber. The integrity of the microdomains is maintained in the absence of extracellular Ca and Mg ions and following the disruption of the actin cytoskeleton, but appears to require the presence of an intact microtubule cytoskeleton. Biochemical analyses suggest that the plasmalemmal microdomains are composed of several antigenic components and the isolation of these microdomains is being pursued. Thus far, we have focused on the characterization of a polypeptide of 70-72 kD. Preliminary glial-neuronal cell co-culture analyses suggest that this glial cell polypeptide undergoes extensive N-glycosylation in the absence of neuronal cell contact. These data suggest that the polypeptide(s) identified may contribute to the neuronal-glial cell migration junction but definitive proof will require analyses ultilizing immunological-based perturbation of cell-cell adhesion and cell migration events. Supported by NS22807.

541.7

N-METHYL-D-ASPARTATE (NMDA) RECEPTORS MODULATE NEURONAL MIGRATION: A LASER MICROSCOPIC STUDY IN A CEREBELLAR SLICE PREPARATION. H. Komuro* and P. Rakic, Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510

The majority of postmitotic neurons in the brain migrate to their final positions. Recently, we found that Ca ion influx, regulated specifically by N-type calcium channels, is essential for this movement, but the mechanism of regulation was not understood. Here, we report that inhibition of the NMDA receptor-ion channel in a cerebellar slice preparation also slows down the granule cell migration by reducing the Ca ion influx. Cerebellar slices (400-800 μm thick) from postnatal 10 day-old mice (CD-1) were stained for 30 min with the carbocyanine dye-DiI (10 μg/ml) and then maintained in a tissure culture medium for up to 6 hours at 37 C. Use of a confocal laser microscope allows the estimate of the migratory rate by measuring the change in distance between the place of neuron origin in the external granular layer and the front of the labeled cell soma in the molecular layer. Under these conditions the mean rate of granule cell migration in slice preparation of 10-15 μm/hr, is comparable to that measured in vivo. Blockade of the nonNMDA receptors or GABA receptors by their specific antagonists did not change appreciably the rate of granule cell migration. In contrast, blockade of the NMDA receptor by addition of 100 μM D-AP5 or 10 μM MK801 to the culture medium resulted in a significant slowdown in cell movement. On the other hand, the enhancement of NMDA receptor activity by the removal of the Mg ion or addition of 10 μM glycine to the culture medium increased the rate of cell migration. Likewise, the rate of cell migration could be also enhanced by the slight increase in extracellular glutamate produced by addition of p-CMSP which inhibits glutamate uptake by adjacent glial cells. These results indicate that NMDA receptors and extracellular glutamate play an important role in neuronal migration by regulating Ca ion influx in cytoplasm of postmitotic cells. Supported by NS22807.

541.8

SUBSTRATES FOR DIVERSE MIGRATORY PATHWAYS IN THE DEVELOPING CORTEX. Nancy A. O'Rourke*, Christine E. Kaznowski, & Susan K. McConnell, Dept. of Bio. Sci. , Stanford Univ., Stanford, CA 94305

In previous time-lapse imaging studies, we examined the migration of DiI-labeled cells through the intermediate zone of cortical brain slices. A number of migratory patterns were revealed, including radial migration directly toward the pial surface, migration orthogonal to the radial direction, and migration in almost all angles in between. Non-radial migration patterns may underlie some of the spread of clonally-related cortical neurons recently revealed in retroviral lineage studies. In addition, the diversity of migration routes suggests that the substrates for cortical migration may be more complex than previously suspected. To examine the most likely cellular substrate, the radial glia, slices were stained in whole mount with antibodies to vimentin. Staining revealed dense palisades of glia extending directly radially or in shallow sinuous curves from the ventricular to the pial surface. These patterns closely resemble those seen in the intact developing cortex and suggest that cortical slices retain normal distributions of radial glia. Additionally, their orientation suggests they could support migration in the radial or near-radial directions. The 3-D morphologies of individual glia, labeled in fixed slices with DiI, were examined with confocal microscopy. While most glia extended radial processes, occasional glia were found with processes that made right angle turns within the intermediate zone, and thus could conceivably serve as substrates for orthogonal migration. While radial glia may provide a viable substrate for the various patterns of migration, other possible substrates could include axons and ECM. To identify definitively the substrates of the individual migrating cells observed in the time-lapse studies, their fluorescent label was photoconverted into a DAB reaction product for future identification. Slices were then either stained with vimentin antibodies or processed for EM. The results confirmed that at least some radially migrating cells use radial glia as their substrate. Similar methods can be used to identify the substrates of orthogonally migrating cells. Supported by NIH EY06314, NSF PYI , and PEW Scholars.

541.9

Development and Trajectory of Chick Olfactory Nerve and Its Relationship to GnRH Neuronal Migration. K.A. Sullivan* and A.J. Silverman, Dept. Anat. & Cell Biol, Columbia Univ., NY,NY 10032.

Gonadotropin releasing-hormone (GnRH) neurons originate in the olfactory placode in the chick and migrate across the nasal septum within the olfactory and/or terminal nerve. The importance of these nerves in directing the extracranial course of GnRH neurons and their entry into the CNS was suggested by an analysis of a Kallmann's syndrome fetus (Schwanzel-Fukuda et al., Mol. Brain Res.6:311, '89). The factors involved in targeting GnRH neurons to their final destinations in the forebrain are unknown. The current investigation was undertaken to follow the ontogeny of the olfactory nerve in the chick forebrain and to ask if GnRH neurons use axon branches to migrate within the CNS. Embryonic chicks at E6, E9 and E12 were fixed, the lipophilic dye, DiI (Bioprobes D-3886) applied to the placode [E6] or to the olfactory nerve [E9 and E12] and tissue maintained at 37^{0} C for 5 to 10 days. DiI was observedin 30um cryostat sections in the olfactory nerve and in branches of the trigeminal. Sections containing DiI were immunostained for chicken neurofilament or cGnRH I to verify the axonal character of the DiI^{+} structures and to examine the relationship of olfactory derived axons to GnRH neurons, respectively. DiI was retained by omitting any detergent treatment. Preliminary results indicate that migrating GnRH neurons are closely associated with the olfactory nerve along its entire course on E6 and E9. HD 10665 T32HD07093.

541.10

OBSERVATIONS REGARDING NEURAL CREST CELL MIGRATION IN NEURULATING CHICK EMBRYOS. K.R. Shankar, C.M. Chuong, T. Jaskoll* and M. Melnick. Graduate Program in Craniofacial Biology, University of Southern California, Los Angeles, CA 90089-0641.

Craniofacial abnormalities frequently observed in association with neural tube (NT) defects may be related to altered neural crest (NC) migration. Cephalic NC contributes extensively to mesenchyme from which most of the craniofacial structures develop. The migratory fate of NC appears to depend on the control of initial adhesion of crest cells between themselves and to the extracellular matrix. We have designed experiments to pertubate these interactions in chick embryos with excess retinoic acid (RA) administered in ovo. Immunocytochemistry and 3-D reconstruction of developing NT's so exposed reveal decreased NC associated with a persistent up-regulation of N-CAM as compared to unexposed controls. Direct observations of progressive NT/NC differentiation in unexposed live specimens using high definition, time-lapse microcinematography demonstrates that normal chick cranial NC, unlike trunk NC appear not to migrate in large numbers or as a sheet. Further, our in vitro coculture experiments of head and trunk NT explants support the filmed observations in live specimens; cranial NC migrate in significantly smaller numbers than trunk NC and over shorter distances per unit time. The spatiotemporal immunohistochemical distribution of N-CAM in the NC of these cocultured explants, as well as explants exposed to exogenous RA confirms our observations in ovo. Supported by NIH DE 07006-16

541.11

A LONG-DISTANCE CUE FROM EMERGING DERMIS STIMULATES NEURAL CREST MIGRATION. K.W. Tosney *. Biology Department, University of Michigan, Ann Arbor, MI 48109.

Neural crest cells delay entering the dorsolateral path (DLP) between the ectoderm and somite until ventral migration is virtually complete. This delay may be imposed by a temporal regulation of both inhibitory and stimulatory cues. An early inhibition has been documented using surgeries: when the dermatome is absent, markers for inhibition are absent and crest cells enter the DLP precociously (Lasky et al., N.S. Abs., 1991). However, dermatome deletions fail to abolish the delay fully, suggesting a second cue is also needed for crest cell entry.

To test whether crest cells also require a stimulatory cue from dermis which first emerges from distal dermatome as crest cells enter the DLP, I grafted older dermatome from quail embryos to the distal DLP of chick embryos and fixed the embryos after 20-24 hr. I identified quail cells (MAB 275.7B, B. Carlson) and crest cells (DiI label or MAB HNK-1, C. Erickson) in serial frozen sections. When older tissue is grafted and forms dermis, chick crest cells enter the DLP precociously. Larger grafts recruit more crest cells into the DLP and also recruit crest cells from the ventral path into the DLP. Stimulation is specific to dermis since crest cells enter on schedule when younger dermatome or posterior sclerotome are grafted. Dermis stimulates migration at a distance since grafts lie 100-200 μm from the entry to the DLP. Grafts do not alter the distribution of a marker for inhibitory function (peanut agglutinin-binding) in the proximal DLP. The emerging dermis thus provides a putative chemotactic cue that can stimulate neural crest cells to enter the DLP. Supported by NS-21308.

541.12

PERIPHERAL CONNECTIONS OF AUTONOMIC MOTOR NEURONS ARE NOT REQUIRED TO SUSTAIN NORMAL MIGRATION PATTERNS *IN VITRO*. R.P. Barber,* P.E. Phelps and J.E. Vaughn. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

The phenotypic differentiation of spinal autonomic motor neurons (AMNs) may be modulated by epigenetic factors encountered during the histogenic migrations of these cells. The dorsal translocation of AMNs from the primitive motor column of embryonic rat spinal cord occurs between embryonic days 14 and 16 (E14 and 16), after AMN axons have reached their peripheral targets. Since this movement also occurs in organotypic slice culture, the present experiments were conducted to determine if the connection of AMNs with their peripheral targets is necessary to sustain the dorsal translocation of these cells. The ventral roots and paravertebral ganglia of E14 spinal trunk slices were injected with DiI and cultured for 4 h. Subsequently, the injection sites were microsurgically removed, and the remaining part of the slice was cultured for a total of 72 h. Confocal microscopy revealed AMN migration patterns that were virtually the same as those observed in control slice preparations. Thus, continuous connection to peripheral targets does not appear to be necessary to sustain the migratory patterns of AMNs in developing rat spinal cord, as is also the case for the less complex histogenic movements of AMNs in chicks (Prasad, A. and Hollyday, M., *J. Comp. Neurol.* 307:237, 1991). *Supported by grants NS25784 and NS18858 from NINDS.*

PROCESS OUTGROWTH, GROWTH CONES AND SPROUTING VI

542.1

EFFECTS OF STAUROSPORINE AND CALPAIN INHIBITORS ON NEURITE OUTGROWTH IN CULTURED PC12 NEURONS. J.B. Denny* Division of Life Sciences, The University of Texas at San Antonio, San Antonio, TX 78249.

PC12 cells were cultured in either DMEM or RPMI-1640 medium containing 10% horse serum and 5% fetal calf serum. Neurite extension was induced by the addition of NGF (100nM) to these media. Some cultures were pretreated for 1 hr at 37°C with either 2 uM staurosporine, 13 uM calpain inhibitor I (N-acetyl-leu-leu-norleucinal), or the vehicle DMSO. Calpain inhibitor II (N-acetyl-leu-leu-methioninal) was also used. NGF was then added and the morphology of the cells, and particularly the growth cones, was observed using phase contrast microscopy 1 hr, 24 hrs, and 3 days later. The staurosporine-treated cells produced growth cones, as did the DMSO and calpain inhibitor-treated cells, but the growth cones were distorted morphologically. The same average number of growth cones appeared per cell in the case of all pretreatments. By contrast, the growth cones of the DMSO and calpain inhibitor-treated cells were indistinguishable from those seen in cells receiving no pretreatments. After 24 hrs the staurosporine-treated cells disintegrated, while the other cells continued to extend neurites. When the above treatments were applied to cells that had been allowed to extend neurites for 7 days, staurosporine was found again to induce cell disintegration after 24 hrs, while the other treatments produced no effect.

542.2

EFFECTS OF CALMODULIN INHIBITORS ON NGF-DEPENDENT NEURITE OUTGROWTH AND ON MAINTENANCE OF NGF-DEPRIVED NEURITES BY ELEVATED K^+. *KENNETH K. TENG* AND LLOYD A. GREENE.* Department of Pathology and Center for Neurobiology and Behavior, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

We previously showed that elevated K^+ exhibits a stabilizing, but not growth-promoting, effect on neurites in NGF-deprived cultures of PC12 and PC12-C41 cells and that this effect is mediated via both N- and L-type Ca^{+2} channels. The present work investigated the role of Ca^{+2} on neurite stabilization by elevated K^+ and on the underlying molecular mechanism of NGF-promoted neurite elongation and stabilization. The calmodulin inhibitors TFP or calmidazolium (R 24571), but not TPA-induced down-regulation of PKC, abrogated neurite-maintenance by elevated K^+. The role of calmodulin on NGF-induced neurite outgrowth was tested by treating PC12-C41 cells in the presence of NGF with or without R 24571 (1μM). While R 24571 did not affect generation or maintenance of the neurite networks, the neurites of cells pre-treated with NGF plus R 24571 could not be maintained by elevated K^+, even after wash out of the drug. This suggests that stabilization of neurites by elevated K^+ requires the activation by NGF of a calmodulin-dependent system. Finally, although neither dBtcAMP nor elevated K^+ alone elicits neurite outgrowth, both treatments together yield the formation of neurite-like processes and partially mimic the biochemical effects of NGF on the cytoskeleton. Taken together, these results suggest that neurite outgrowth can be experimentally dissected into at least several discrete components, one of which is calmodulin dependent.

542.3

EFFECTS OF EXOGENOUS (NGF) AND ENDOGENOUS TROPHIC FACTOR (S) ON THE REINNERVATON OF TARGET REGIONS WITHIN FETAL AND ADULT SPINAL CORD BY ISOLATED DORSAL ROOT GANGLIA (DRG) IN ORGANOTYPIC CO-CULTURES. R. Evans and H. Yip*. Dept. of Anatomy, University of Utah, Sch. of Med., Salt Lake City, UT 84132.

The effects of exogenous and endogenous neurotrophic factors on the reinnervation of fetal or adult spinal cord were studied by co-culturing DRG explants. Clusters of 4 DRGs from either 15-day old fetal rat or young adult rats (100 g) were co-cultured in close proximity (0.5 mm) to spinal cords from the same age or different age. The DRG and spinal cord co-cultures are of 4 types (all stripped of DRGs and meninges): (1) fetal DRGs with spinal cord explants from the same age; (2) fetal DRGs with spinal cord explants from adult animals; (3) adult DRGs with spinal cord explants form the same age; (4) adult DRGs with spinal cord explants from fetal animals. The cultures were grown on collagen-coated glass coverslips in the modified polystrene Nunclon 4-well multidishes and maintained in Eagle's minimum essential medium with Earle's balance salt solution. To visualized and trace neurite outgrowth within the spinal cord, DRGs were incubated with carbanocyanine dye (DiI) before they were co-cultured with the spinal cord. The cultures were observed under fluorescence microscopy. Eighty percent of fetal DRG co-cultured with fetal cord had neuritic outgrowth with or without NGF within the first week. NGF seems to increase the number and rate of neuritic outgrowth from the DRG explants. Addition of anti-NGF antiserum to the cultures blocked about 50% of the outgrowth. Intensely fluorescent neurites can be found both in the dorsal and ventral gray matter of the spinal cord. Retrograde HRP tracing confirmed the source of neurites was from the nearby DRG. Most of the neurites tend to avoid the white matter. Little or no neurites outgrowth could be elicited from the adult DRG explants with or without NGF. Neurites outgrowth from the fetal DRGs co-cultures with the adult spinal cord did not grew into the spinal cord.

542.4

NGF AND cAMP INDUCE NEURITE GROWTH IN PANCREATIC B-CELLS R. Vidal-Tamayo, T. Martínez, C. Sánchez, M.C. Ramírez-Medeles and M. Hiriart* Inst. Fisiología Celular, Dept. Bioenergética, UNAM, México D.F. 04510, MEXICO.

The embryological origin of pancreatic B-cells is unclear. B-cells share several features with neurons, which include their ability to extend neurites in vitro. We studied whether neurite development by adult and fetal (18 i.u.) rat B-cells is promoted by neural growth factor (NGF and dibutyryl 3'-5'cyclic adenosine monophosphate (DBc-AMP). Dissociated pancreatic islet cells were cultured for 14 days in the presence of 50 ng/ml NGF or 5 mM DBcAMP or both, and stained for insulin. Fetal cells responded better to treatment than adult ones. DBcAMP induced cell flattening and the production of short cytoplasmic extensions.

The overall morphological changes of a population was estimated by the neurogenization index (NI, % neurite-bearing cells times average neurite longitude). NGF and DBcAMP clearly induced phenotypical changes in the cells, showing positive synergism in promoting neurite extension in pancreatic B-cells.

FETAL CELLS	%MC*	NI	ADULT CELLS	%MC	NI
CONTROL	7	44	CONTROL	37	340
NGF	43	843	NGF	41	635
DBcAMP	90	896	DBcAMP	53	1015
BOTH	94	1829	BOTH	94	1766

*%MC: Modified cells

Supported by DGPA grant IN206291, UNAM, MEXICO

542.5

MICROGLIA AND ASTROCYTE PROLIFERATION AND TROPHIC FACTOR GENE EXPRESSION DURING TERMINAL DEGENERATION AND CHOLINERGIC SPROUTING. A.M. Fagan* and F.H. Gage. Dept. Neurosci., Univ. of Calif., San Diego, La Jolla, CA 92093.

Transection of the perforant path (PP) leads to terminal degeneration in the dentate gyrus molecular layer (ML), rapid glial responses and subsequent neuronal sprouting in the denervated zone. Reactive gliosis, characterized by glial hypertrophy and/or hyperplasia, may be a determinant of the degree and/or quality of neural repair. Using combined immunocytochemistry and 3H-thymidine autoradiography, we demonstrate hypertrophy and proliferation of microglia and astrocytes in the denervated ML. Inhibition of glial proliferation with anti-mitotic drug administration does not affect cholinergic sprouting in this model system. Hypertrophic changes, suggesting functional activation, proceed independently of proliferation. Activated microglia and astrocytes produce cytokines and growth factors in vitro, which if induced in vivo, may be involved in neural reorganization. Using riboprobe in situ hybridization, we demonstrate 1) IL-1B mRNA localized to cells surrounding the wound but not in reactive glial cells in the denervated ML, 2) changes in GFAP mRNA in the ML, indicating upregulation of astrocytic structural proteins, and 3) no evidence for NGF or bFGF mRNAs in hypertrophic glial cells. These data demonstrate that glial proliferation is not required for cholinergic sprouting in this model system. Furthermore, upregulation of gene expression for IL-1B, NGF and bFGF by reactive glial cells does not appear to play a role in initiating these sprouting events.

542.6

STIMULATORY EFFECTS OF RETINOIC ACID ON NEURITE OUTGROWTH FROM CHICK SENSORY GANGLIA EXPLANTS. L. Hsu.*Department of Biology, Seton Hall Univ. S. Orange, NJ 07079.

Explants of sensory ganglia from chick embryos ranging from 9-13 days of age developed neurites upon exposure to trans-retinoic acid. Fine outgrowths were detected extending from the periphery of the explants within 20 hrs after as short an exposure period as 5 hrs. Retinoic acid was an effective neurite-promoting agent in growth medium which included insulin but was unable to induce ganglia differentiation in unsupplemented F12 medium or F12 medium with progesterone. Dose concentrations above 5 x 10^{-5}M were cytotoxic to the cultures and caused explants to detach from the collagen substratum. The stimulatory effects of retinoic acid on neurite outgrowth were synergistic with those of 12-0-tetradecanoylphorbol 13-acetate (TPA). When the two agents were combined, the treated explants produced morphologically distinct neurites which formed short, thick fascicles. Explants of central nervous tissue including embryonic brain and spinal cord were unresponsive to treatments of either retinoic acid alone or combinations of retinoic acid and TPA.

542.7

OPTIMIZED SURVIVAL OF HIPPOCAMPAL NEURONS IN B27, A NEW DEFINED SERUM-FREE MEDIUM, G.J. Brewer*, J.R. Torricelli and P. Price+,Southern Illinois Univ. Sch. Med.,Springfield, IL 62794, +GIBCO/BRL Life Technologies,Grand Island, NY 14072.

In B27 (GIBCO 680-7504AB), the concentration of each of the 25 components of a previously developed serum-free medium (Brewer & Cotman, Brain Res. 494:65-74) was optimized. Optimization was assessed by growth of embryonic rat hippocampal neurons at 160 cells/mm^2 for 4 days at 37^{0}C, in a 5% CO_2, 9% O_2 incubator without a glial feeder layer. Viability was determined by staining with fluorescein diacetate and propidium iodide. Above 400 plated cells/mm^2, survival was 70-80% in B27, independent of plating density. Less than 10% of the cells stained as glia with anti-GFAP antibody. At lower cell densities, survival was significantly lower but could be rescued to the 60% level at 40 cells/mm^2 by simply applying a cover slip on top of the cells. This suggests that medium deficiencies or trophic factors affect survival at low cell densities. By optimizing glutamine supplementation, neuron growth could be selected over glial growth. B27 was found to at least double the survival produced by DME/F12 + 10% fetal bovine serum or DME/F12 + Bottenstein's N2 supplements. B27 also supports acceptable survival for as long as one month. This defined medium should be useful for studies of individual and small networks of neurons, their development, plasticity and responses to pharmacologic, toxic and trophic factors.

PROCESS OUTGROWTH, GROWTH CONES AND SPROUTING VII

543.1

PROTEIN SYNTHESIS AND mRNA IN GROWTH CONES FROM DIFFERENTIATED SH-SY5Y NEUROBLASTOMA CELLS. G. Meyerson[1]*, V. Parrow[1], H.H. Pfenninger[2] and S. Påhlman[1]. [1]Department of Pathology, University Hospital, S-751 85 Uppsala, Sweden, [2]Department of Cellular and Structural Biology, Denver, Colorado, USA.

The human SH-SY5Y neuroblastoma cells differentiate into neuron like cells when treated with the phorbolester 12-0-tetradecanoylphorbol-13-acetate (TPA). Phenotypical changes of the differentiated cells include growth inhibition, formation of neurites with growth cones and varicosities containing dense core granules. In this study we have characterized a growth cone and a cell body fraction from differentiated SH-SY5Y cells. The characterization is based on both ultrastructural (EM-analysis) and biochemical (protein markers as GAP-43, synaptophysin and MAP-2) criteria. Ribosomes were commonly found in intact growth cones of differentiated SH-SY5Y cells. By ^{35}S-methionine labeling of fractionated material, local protein synthesis were shown to occur in growth cones. Northern blot analysis shows that both dendritic- (MAP-2) and axon-(GAP-43) specific mRNA are present in the growth cone fraction, as well as mRNA coding for neuropeptide tyrosine. However, mRNA coding for the nuclear proteins c-**jun** and N-**myc** are detectable at very low levels in the growth cone.

543.2

A PHOSPHORYLATION EPITOPE ON MAP 1B THAT IS EXPRESSED IN GROWING AXONS IN THE DEVELOPING RAT CNS. P. R. Gordon-Weeks*, S. G. Mansfield, C. Alberto and F. Moya. Dev. Biol. Res. Cen., King's College London, London WC2R 2LS, U.K. and Dpto. Gen, Mol. Micr., Univ. Alicante, Alicante, Spain.

We are interested in the role of the cytoskeleton in neurite outgrowth. We have isolated a monoclonal antibody (mAb 150) that recognises a phosphorylation epitope on the microtubule-associated protein (MAP) 1B. Immunoblot analysis of developing rat central nervous system (CNS) shows that mAb 150 is directed against a protein of approximately 325 kDa (MAP 1B) that stoichiometrically co-polymerizes with microtubules through successive cycles of temperature-dependent assembly and disassembly. Removal of phosphate from blotted proteins using alkaline phosphatase abolishes the binding of mAb 150 to MAP 1B indicating that the epitope is phosphorylated. In the developing rat nervous system, immunohistochemistry with mAb 150 shows that the phosphorylation epitope on MAP 1B is transiently expressed in growing axons and growth cones but not in dendrites. For instance, in the neonatal rat cerebellum, parallel fibres are stained only during elongation and not after synaptogenesis. Similarly, in the embryonic spinal cord, commissural and other axons express the mAb 150 epitope only while extending. The mAb 150 epitope is also transiently expressed in radial glial fibres and in certain cell nuclei. Immunostaining of sections with mAb 150 was completely abolished by alkaline phosphatase treatment. Immunoelectron-microscopy in the floor-plate region of the embryonic spinal cord confirmed that mAb 150 stains growing axons and their growth cones. These observations and previous ones made by us in cell culture (Mansfield, *et al. J. Neurocytol.* **21**, 1007, 1991), suggest that a developmentally regulated phosphorylation epitope on MAP 1B recognised by mAb 150 may be important in axonogenesis.

543.3

ENTRY OF MICROTUBULES INTO DEVELOPING NEURITES DOES NOT REQUIRE POLYMERIZATION OF TUBULIN. C.L. Smith* and S.A. Walters. Laboratory of Neural Control, NINDS, NIH, Bethesda, MD 20892.

Our previous work suggested that neurite formation by chick sympathetic neurons grown *in vitro* involves bulk flow of cytoplasm from the neuronal cell body into a filopodium. The simultaneous entry of microtubules and cytoplasm into neurites suggested that assembled microtubules move into the neurite, and that this movement does not require tubulin polymerization. Here, we show that microtubules move into neurites formed in the presence of nocodazole, an inhibitor of tubulin polymerization. Cultures of dissociated sympathetic neurons were exposed to medium containing 1μM taxol for 30 min to stabilize microtubules and then transferred to medium with 2μg/ml nocodazole. This dosage of nocodazole blocked polymerization of tubulin, as assessed by immunolabelling with a monoclonal antibody specific for recently assembled microtubules. Filopodia in living cultures examined by time-lapse video-microscopy were observed to fill with cytoplasm from neuronal cell bodies, thereby transforming into processes resembling neurites. Immunolabelling showed that these neurites contained microtubules, whereas other filopodia that had not filled with cytoplasm did not. The presence of microtubules in neurites formed during inhibition of tubulin polymerization indicates that microtubules assembled in neuronal cell bodies are translocated into filopodia.

543.4

HETEROGENEOUS DISTRIBUTION OF ALPHA1 AND BETAII TUBULIN mRNAs IN ADULT RAT BRAIN REVEALED BY IN SITU HYBRIDIZATION. C.M. Paden*, J.A. Watt, X. Zhou, J. Pickett[1], and M.M. Oblinger[1]. Dept. of Biology, Montana State Univ,. Bozeman, MT 59717 and Dept. of Cell Biology and Anatomy[1], The Chicago Medical School, North Chicago, IL 60064.

Northern blot analysis of rat brain extracts has revealed that expression of various tubulin isotypes is developmentally regulated. Alpha1 and 6II tubulin mRNAs are present in higher levels in the immature brain, where their expression is believed to be correlated with axonal growth. In addition, their expression is upregulated in adult neurons undergoing axonal regeneration or sprouting. Because the capacity for axonal growth may vary in different areas of the adult brain, we sought to determine whether some mature brain regions retain heightened expression of the α1 and 6II tubulin isotypes. Male rats 65-70 days of age were sacrificed by decapitation under ether anesthesia and the brain rapidly removed and frozen. Sets of serial coronal cryosections were collected at 0.5mm intervals and immersion-fixed for in situ hybridization using ^{35}S-labeled cDNA probes specific for 6II tubulin (RBT1) and α1 tubulin (Mα1) mRNAs. Film as well as emulsion autoradiographs were prepared. Initial inspection revealed a marked regional heterogeneity in the density of film autoradiographs, with similar patterns apparent for both Mα1 and RBT1 probes. Areas of greatest density included: piriform cortex, parts of the septal nuclei, nucleus of the diagonal band, hippocampal formation; paraventricular, supraoptic and ventromedial hypothalamic nuclei; anterodorsal and paraventricular thalamic nuclei; habenula, substantia nigra pars compacta, red nucleus, optic nerve layer of the superior colliculus, parabrachial nucleus, dorsal raphe and locus coeruleus, parts of the olivary complex, and numerous cranial nerve nuclei. Further analysis is required to determine to what extent the apparent regional differences in tubulin expression reflect differences in mRNA expression by individual neurons versus differences in neuronal density.

543.5

INCREASED EXPRESSION OF ALPHA1 AND BETAII TUBULIN mRNAs DURING COLLATERAL SPROUTING OF CNS NEURONS. J.A. Watt*, C.M. Paden, X. Zhou, J. Pickett[1], and M.M. Oblinger[1]. Dept. of Biology, Montana State Univ,. Bozeman, MT 59717 and Dept. of Cell Biology and Anatomy[1], The Chicago Medical School, North Chicago, IL 60064.

Increased expression of the α1 and 6II tubulin isotypes is known to accompany the axonal growth that occurs during regeneration of peripheral nerves and during collateral sprouting of sympathetic efferents. To our knowledge, however, their possible role in collateral sprouting of central neurons has not been previously investigated. We have utilized a new model of collateral sprouting by peptidergic neurons of the rat supraoptic nucleus (SON) to address this question. Collateral sprouting of neurons in the contralateral SON was induced via a unilateral knife cut of the hypothalamo-neurohypophysial tract (Watt and Paden, *Exp.Neurol.* 111, 9-24,'91); the knife was stopped dorsal to the hypothalamus in sham-operated controls. Rats were sacrificed 10-30 days later by decapitation under ether anesthesia; the brain was rapidly removed, frozen, and cryosectioned. Sections were immersion-fixed for in situ hybridization using ^{35}S-labeled cDNA probes specific for 6II tubulin (RBT1) and α1 tubulin (Mα1) mRNAs. Film as well as emulsion autoradiographs were prepared. Initial results from densitometric analysis of films indicate that, compared to sham-operated controls, expression of both mRNAs is significantly increased in the intact contralateral SON by 20 days post-lesion, coinciding with the period of axonal sprouting. Conversely, expression in the lesioned SON is significantly reduced by 10 days post-lesion, consistent with the effects of axotomy on expression of α1 and 6II tubulins seen in other CNS systems. While these results must be confirmed by grain counting over individual neurons, they suggest that mature CNS neurons retain the capacity to upregulate tubulin expression as required for axonal growth under appropriate conditions, even though they may fail to do so following axotomy.

543.6

ANTIBODIES AGAINST MICROTUBULE-ASSOCIATED PROTEINS FROM *DROSOPHILA MELANOGASTER* CROSS REACT WITH DEVELOPING AND ADULT RAT BRAINS. A.A. Alcantara*, A.R. Reilein, K.A. Chaney, T.M. Pazdera, T.L. Karr and W.T. Greenough. Depts. of Psych., Biochem., and Cell & Struct. Biol., Neuroscience Program, Beckman Institute, Univ. of Illinois, Urbana, IL 61801.

We have examined the patterns of immunoreactivity with monoclonal antibodies raised against in vitro purified *Drosophila* microtubule proteins (MTP) (described in the adjacent abstract—Srinivasan et al.). The potential role of microtubule-associated-proteins (MAPs) in neuronal differentiation makes it relevant to study their expression in developing and adult animals. P2C11 expression was examined in rats between postnatal days P1 and P25 and adult ages P97 and 2 yrs. In cerebral cortex, P2C11 was expressed in the marginal zone and in fibers in the cortical plate and underlying layers at P1. Cortical pyramidal cell dendritic labelling remained in adults. Hippocampal mossy fibers, which synapse with CA3 pyramidal cells, were P2C11 positive at P5. Labelling in CA3 was still evident in 2 yr. old adults. The cerebellum revealed intense P2C11 labelling in the lower molecular layer at P5 and became increasingly wide at subsequent ages during dendritic growth, parellel fiber extension and synapse formation.

A subset of adult rat cerebellar lobules exhibited differential staining with these antibodies. Purkinje cells were P2C11 immunoreactive, whereas inhibitory interneurons in the molecular layer were P2C6 positive. P1C5 staining was present in Purkinje cells, basket cells, and other inhibitory interneurons. A similar pattern of *c-fos* expression has previously been reported in rats during forelimb reach training (Alcantara et al., 1991). We are pursuing a possible correlation between the spatio-temporal expression of Fos and these MAPs.

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543.7

BIOCHEMICAL ANALYSIS OF MICROTUBULE-ASSOCIATED PROTEINS IN *DROSOSPHILA MELANOGASTER* EMBRYOS. S.Srinivasan, A.R.Reilein,M.A.Canady ,W.T. Greenough* and T. L. Karr Dept. of Biochem., and Neuroscience Program, Beckman Institute, Univ. of Illinois, Urbana, IL 61801

Microtubules are involved in a variety of cellular processes such as meiosis, mitosis, cell shape determination, and cytoplasmic transport. Several proteins associate with microtubules and are termed microtubule associated proteins (MAPs). We have taken a biochemical approach, using a well defined genetic organism, *Drosophila melanogaster*, to study the function of MAPs during the development of the embryonic nervous system. We have characterized microtubule proteins (MTP) from different embryonic stages in *Drosophila* and quantitated the average percent yield of MTP during development and noticed a dramatic increase in average MTP yield from 8 to 12 hours. This increase corresponds to the onset of neurogenesis whereby the microtubules are assembled and stabilized in order to form an elaborate set of neurons needed for the formation of the ventral nerve cord. One dimensional and two dimensional SDS-PAGE gels revealed differences in MAP profiles at different developmental stages. As a first step in trying to identify these different MAP species, we have used purified MTP from 16 hour *Drosophila* embryos as antigens and obtained three monoclonal antibodies (MAb), P2C11, P2C6, and P1C5. P2C11 (~55-60 kD) is associated with mitotic spindles of the pre-cellular blastoderm in 2-3 hour *Drosophila* embryos. The same antibody shows a less systematic diffuse pattern in 16 hour embryos. MAb P1C5 (~66 kD) revealed ubiquitous staining which excluded the nerve cord in 16 hour embryos. MAb P2C6 (~46 kD) stained the ventral nerve cord of 16 hour embryos with high specificity. In addition, these MAbs (P2C11, P2C6, and P1C5) cross react with developing and adult rat brains as described in the adjacent abstract (Alcantara et al.).

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543.8

"WAVES": GROWTH CONE-LIKE STRUCTURES THAT TRAVEL DOWN THE PROCESSES OF CULTURED HIPPOCAMPAL NEURONS. G. Ruthel* and G. Banker. Department of Neuroscience, University of Virginia School of Medicine, Charlottesville, VA 22908.

By using time-lapse video microscopy to examine the development of isolated hippocampal neurons in dissociated culture, we have observed heretofore undescribed motile structures that we refer to as "waves". Waves are growth cone-like lamellipodial extensions that form at the base of both axons and the processes that will become dendrites and travel toward the tips of the processes at rates of 150-250 μm/hr. Typically, waves form on the axon every 15-25 minutes. Arrival of a wave at the tip of a process commonly results in a growth spurt which lasts five to ten minutes. Axons in hippocampal cultures grow intermittently and sometimes lack growth cones when they are not elongating. When a wave reaches the tip of such an axon, it often appears to become the new growth cone and the axon begins to grow again. To determine whether the molecular composition of waves is similar to that of growth cones, waves were identified by time-lapse video microscopy, then fixed and stained. Like growth cones, waves stain intensely with rhodamine-phalloidin, indicating that they contain a high concentration of filamentous actin. Waves also stain with an antibody against the actin-associated protein ezrin, which has previously been shown to preferentially stain growth cones (Goslin *et al.* 1989. J. Cell Biol. 109:1621). The similarity between waves and growth cones raises the possibility that waves may represent a mechanism by which materials found in growth cones are delivered to the tips of developing processes.

This research was supported by NIH grant NS17112.

543.9

GROWTH CONE FILOPODIA AS SENSORS OF THEIR ENVIRONMENT: ISOLATED FILOPODIA RESPOND TO SPECIFIC STIMULI. P. Dou*, R.W. Davenport, V. Rehder, R.E. Lee, S.B. Kater, Program in Neuronal Growth and Development, Colorado State University, Fort Collins, CO 80523.

Filopodia have been regarded as the sensory extensions of neuronal growth cones, and they are implicated in directing growth cones towards their targets. Therefore, to study how filopodia themselves react to environmental cues, it is necessary to study them in isolation. We developed a technique to isolate filopodia from growth cones on identified neuron B19 of the snail *Helisoma*. The cells were grown in conditioned culture media (L-15) for 18-24 hours. Subsequently, filopodia were mechanically transected from the growth cone with a fine tip glass micropipette. Many isolated filopodia retained their original shape for times exceeding one hour. In order to test the ability of filopodia to act as sensory structures, we asked whether isolated filopodia could respond to environmental stimuli known to affect growth cone morphology. Previous work has demonstrated that focally applied electric fields and elevated extracellular K^+ concentrations dramatically alter growth cone morphology. Isolated filopodia show striking morphological changes in response to these stimuli: they either retracted or beaded, while a few showed no response. Also in accord with whole growth cone responses, isolated filopodia respond to the neurotransmitter serotonin. Additionally, freeze fracture results demonstrate a high density of intramembrane particles on growth cone filopodia. Taken together, these results suggest that filopodia possess a complement of surface molecules including ion channels and specific neurotransmitter receptors that allow them to act as sensors of their environment.

543.10

GROWTH CONE FILOPODIA AS SENSORS OF THEIR ENVIRONMENT: INTRACELLULAR CALCIUM AS A SECOND MESSENGER R.W. Davenport*, P. Dou, V. Rehder, S.B. Kater Prog in Neuronal Growth and Development, Colorado State University, Ft Collins, CO 80523.

Neuronal growth cones extend filopodia into their environment. If filopodia act as environmental sensors for growth cones, then filopodia must be capable of responding to stimuli in a manner which allows direct signalling to the growth cone proper. In the previous abstract we addressed the capability of isolated filopodia to respond morphologically to stimuli known to affect growth cone behavior. Here we show that stimuli which affect both growth cone calcium levels and filopodial disposition also affect calcium levels within isolated filopodia. Identified, cultured *Helisoma* neurons were iontophoretically loaded with Fura-2 prior to filopodial transection. Fluorescent images were obtained with a 100X objective and a chilled CCD camera and digitally stored. Signal to noise ratios for isolated filopodia ranged from 3 - 10 for each excitation wavelength (350, 380 nm), thus allowing standard dual wavelength estimates of $[Ca^{++}]_i$ to be made. Focally applied electric fields (10 mV/μm) caused a rapid (≤ 10 sec) rise in $[Ca^{++}]_i$ to approximately twice rest levels. Elevated KCl (10 mM) caused a transient increase in $[Ca^{++}]_i$ (> 100 nM). These results suggest the presence of both voltage dependent calcium channels and some calcium efflux mechanisms on isolated filopodia. Furthermore, the presence of neurotransmitter receptors is suggested by the ability of 5HT (50 μM) to raise $[Ca^{++}]_i$ (up to 15 fold) and ACh (50 μM) to reverse this effect. These results suggest that neuronal growth cone filopodia can independently respond to environmental stimuli due to functional signal transduction machinery. Thus, filopodia which can contact environmental signals up to 2 hours prior to the advancing growth cone proper can convey relevant guidance information via intracellular calcium to the growth cone.

543.11

Depolarization-Induced Calcium Responses in Cytoplasm, Nucleus, and Nucleolus of Cultured Adult Rat DRG Neurons. M.N. Rand*, D.L. Eng, S.G. Waxman & J.D. Kocsis, Dept. of Neurology and Sect. Neurobiology, Yale Medical School, New Haven, CT 06510; and VAMC, West Haven, CT 06516.

Adult rat DRG neurons were dissociated and maintained in culture for up to one week. Relative intracellular calcium levels $[Ca^{2+}]_i$ were imaged and measured using the calcium indicator dye fluo-3AM and confocal scanning laser microscopy (CSLM), at various times after plating (AP), either before, during, or after process regeneration and outgrowth. Cells were loaded in 5 μM fluo-3AM for 35 minutes, transferred to a perfusion bath of oxygenated normal Krebs' solution (with 2 mM Ca^{2+}), and resting levels of $[Ca^{2+}]_i$ were imaged with the CSLM in frame-scan mode. Depolarization of cells via high K^+(60 mM) in the bath resulted in elevated levels of $[Ca^{2+}]_i$, with a proportionally greater fluorescent signal seen in the nucleus and nucleolus 24 hours AP (during neurite outgrowth), compared to 5 hours AP, or more than 100 hours AP (after extensive process outgrowth). The nuclear signal was greater than that of the cytoplasm immediately after plating, but attenuated over time in culture. The nucleolar signal was less than the nuclear signal immediately after plating, increased and surpassed the nuclear signal from 12-48 hours AP, and then diminished again at times greater than 100 hours AP. Local bipolar electrode stimulation at 50 Hz, 1 second duration resulted in transient, reversible elevations in $[Ca^{2+}]_i$ in the three cellular compartments. These electrically-evoked changes could be repeated several times sequentially in most cells; occasionally cells responded with chronically-elevated $[Ca^{2+}]_i$ levels to the initial depolarizing current. High-resolution imaging of the nucleus and nucleolus 24 hours after plating revealed elaborate heterogeneity of the intranuclear fluorescent signal, suggesting the involvement of Ca^{2+} in nuclear mechanisms related to process regeneration and outgrowth.

Supported in part by the NIH and Department of Veterans Affairs.

543.12

PATTERNS OF SPONTANEOUS TRANSIENT ELEVATIONS OF INTRACELLULAR CALCIUM IN EMBRYONIC SPINAL NEURONS PRIOR TO NEURITE EXTENSION. X. Gu*, E. Olson and N. C. Spitzer. Dept. of Biology & Center for Molecular Genetics, UCSD, La Jolla CA 92093.

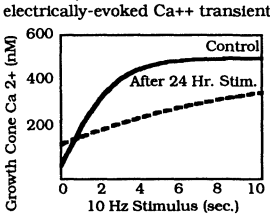
Ca^{++}-dependent action potentials are normal features of early development of *Xenopus* spinal neurons. Prevention of Ca^{++} influx alters several aspects of subsequent neuronal differentiation of postmitotic cells dissociated from neural plate stage embryos. Elevations of $[Ca^{++}]_i$ assessed with fura-2 or fluo-3 occur spontaneously during this period. We have used confocal microscopy to image $[Ca^{++}]_i$ at 5 sec intervals in cultured cells over one hr periods using fluo-3 AM. Active cells were scored as those showing spontaneous increases above baseline in fluorescence and thus $[Ca^{++}]_i$. Cell counts indicated that imaging does not promote cell death.

Neurons exhibit distinct signatures of elevation of $[Ca^{++}]_i$ during this early period of development when they are undergoing primary morphological differentiation. Of 65 differentiated neurons, 18 of 26 (69%) and 26 of 39 (67%) were active at 6-7 and 7-8 hr *in vitro*, respectively. Of 15 latent neurons, without neurites at the time of imaging but differentiated at 1 day, 6 of 8 (75%) and 5 of 7 (71%) were active during the same periods. 40% of both differentiated and latent neurons exhibit spikes with a rapid rise to >150% of baseline in <15 sec, followed by double exponential decay with τ's of 10 sec and 2.8 min. Overall spike frequency ranged from 1-7/hr; latent and differentiated neurons showed 2.0 and 2.4 spikes/hr. Only 26% of morphologically undifferentiated cells exhibited elevations of $[Ca^{++}]_i$; this activity was of variable form, including putative neuronal signatures. However elevations were generally of longer duration and showed single exponential decay. Characteristic patterns of Ca^{++} spike activity may direct neuronal differentiation. Supported by NIH NS15918.

543.13

KINETICS OF ELECTRICALLY EVOKED CALCIUM TRANSIENTS IN MOUSE DRG GROWTH CONES. R.D. Fields* and J.T. Russell, Lab. Dev. Neurobiol. NICHD,NIH. Bethesda, MD 20892.

Growth cone motility is exquisitely sensitive to changes in intracellular calcium and electrical stimulation. We measured the kinetics of electrically-evoked Ca++ transients in mouse DRG growth cones and homeostasis of Ca++ during and after 15 min. trains of action potentials (see Fields et al., *J. Neurosci.*10: 2950 for methods). Changes in FURA-2 fluorescence ratio followed exponential kinetics. 10 Hz stimulation induced a rapid increase in Ca++ within the growth cone, with a time constant (τ) of 1.36s. In contrast, in neurons stimulated chronically (> 24 hrs.), electrically-evoked Ca++ transients were slowed (τ = 6.00s).



Intracellular Ca++ recovered during stimulation (τ=90.4s) and after terminating a 15 min. period of stimulation (τ=8.50s). These kinetics were not altered by chronic stimulation. These results suggest that Ca++ homeostasis is regulated by the state of electrical activity of neurons. This may involve decreased Ca++ influx into neurons, or increased activity of a rapidly acting Ca++ removal or buffering mechanism. Activity-dependent regulation of Ca++ homeostasis would make growth cones from electrically active circuits less vulnerable to electrically-induced collapse. In addition, activity-dependent changes in Ca++ homeostasis could have effects on other neuronal processes that are dependent on calcium.

543.14

CHRONIC EXPOSURE TO PICROTOXIN INCREASES AXOSOMATIC SYNAPSES AND FUNCTIONAL INHIBITION OF PURKINJE CELLS IN MOUSE CEREBELLAR CULTURES. R. Drake-Baumann [1, 2], A. L. Leiman [4], R. M. Herndon [2, 5], K.L. Tiekotter [5] and F. J. Seil* [1, 2, 3] Neurology Research, VA Medical Center [1] and Depts. of Neurology [2] and Cell Biology & Anatomy [3], Oregon Health Sciences University, Portland, OR, Dept. of Psychology [4], University of California, Berkeley, CA and Good Samaritan Hospital [5], Portland, OR.

Organotypic cerebellar cultures of newborn mice were continuously exposed either to picrotoxin (PTX) or to penicillin (PEN) in nutrient medium from explantation until 13-16 days in vitro (DIV) to examine whether chronic enhancement of neuronal activity would induce specific morphological or physiological changes. The spontaneous electrical activity of explants exposed to PTX by DIV 13-16 revealed numerous units with slow discharge rates. The overall mean discharge rate for PTX treated explants was lower than for controls, while the overall mean discharge rate for PEN treated explants was slightly higher than controls and often revealed a tendency to bursting activity. Cortical stimulation produced a transient inhibition of spike discharge in all groups but the inhibitory effect was significantly enhanced in PTX treated explants. Electron micrographs at DIV 14-15 of explants exposed to PTX revealed an increased number of synapses on Purkinje cell (PC) somata (5/PC), most of which appeared to be with basket cell terminals, while the number of axosomatic synapses present on Purkinje cells of PEN treated explants did not differ from controls (2.2 /PC). The mechanism by which chronic exposure to PTX enhances synapse formation by basket cells may be independent of its inhibitory action on GABAergic transmission.

544.1

ROLE OF NERVE GROWTH FACTOR (NGF) IN THE EARLY DEVELOPMENT OF QUAIL DORSAL ROOT GANGLIA (DRG). L. Yao, J. Speight, and P. Bernd.* Department of Anatomy and Cell Biology, State University of New York, Health Science Center, Brooklyn, NY 11203.

We have previously shown that ^{125}I-NGF binding to DRG, *in situ*, is first seen at embryonic day 3.5 (E3.5; stage 23) in cryostat sections of quail. Physiologically important high affinity NGF receptors appear to be present, because specific binding was seen at a low ^{125}I-NGF concentration (2 ng/ml; 80 pM) and cross-linking with HSAB reveals that the high molecular weight ^{125}I-NGF-receptor complex is present. These results are surprising since other labs have shown that avian DRG are unresponsive to NGF, prior to E5, at least with respect to neurite outgrowth. Our recent studies have examined the effects of NGF on neuron-enriched cultures prepared from E3.5 DRG. Cells were grown at a density of 2500/20 mm well in complete medium (MEM with 15% fetal bovine serum and 5% chick embryo extract) on a polyornithine/laminin substrate. Preliminary results have shown that there were approximately 30% more neurons in the presence of exogenous NGF (100 ng/ml) after 24 hrs *in vitro*, as compared to cultures grown in complete medium in the presence anti-NGF (100 ng/ml, Boehringer Mannheim). The effect of NGF was also be blocked by anti-NGF. We have also shown that anti-NGF has no toxic effect on the cultures; cell number remains the same when anti-NGF has been pre-absorbed with NGF. Cultures grown in complete medium with or without exogenous NGF have a similar number of neurons, suggesting the presence of NGF or NGF-like substances in complete medium. Our current studies are aimed at determining whether the increase in cell number is due to a survival or mitogenic effect. Supported by a grant from the Deafness Research Foundation.

544.2

OPIOID RECEPTOR BLOCKADE DIFFERENTIALLY MODIFIES NGF AND NGFRs LEVELS DURING RAT BRAIN DEVELOPMENT. E. Pérez-Navarro, J. Alberch*, E. Arenas and J. Marsal. Laboratori de Neurobiologia Cel.lular i Molecular. Dept. de Biologia Cel.lular i Anatomia Patològica, Hosp. Bellvitge, Univ. Barcelona, Casanovas 143, 08036 Barcelona, Spain.

The present work deals with the relationship between naltrexone (NTX), an opioid antagonist, and nerve growth factor (NGF) and its receptors (NGFRs) in the regulation of the central nervous system development.

It has been described that opioid antagonists exert an influence on the growth of neural tissues that is dependent on the duration of opioid receptor blockade (Zagon and McLaughlin, 1986). Thus, we have studied the long term effect of NTX on the content of nerve growth factor (NGF) in the main cholinergic regions of the brain: cortex, hippocampus, septum and neostriatum, and on NGFRs levels in cortical membranes. Chronic NTX treatment, during 14 days (starting P5), induces changes in NGF content or in NGFRs depending on the dose used. 50 mg/kg NTX treatment induced a decrease in the number of ^{125}I-NGF high-affinity binding sites, without detectable changes in NGF levels. However, low doses of NTX (1 mg/kg) produced a decrease in NGF levels in hippocampus, septum and neostriatum with no differences in ^{125}I-NGF binding sites.

Our results suggest that changes in neuronal activity induced by opioid receptor blockade could influence neuronal development through modifications of NGF and NGFRs content.

This work was supported by DGICYT and CIRIT.

544.3

OVEREXPRESSION OF NERVE GROWTH FACTOR IN EPIDERMIS OF TRANSGENIC MICE CAUSES SKIN HYPERINNERVATION AND HYPERTROPHY OF TRIGEMINAL GANGLION. K. Albers*, D. E. Wright, B. M. Davis. Departments of Pathology and Anatomy and Neurobiology, University of Kentucky School of Medicine, Lexington, KY 40536.

Nerve growth factor (NGF) is a target derived neurotrophic factor that plays a critical role in the survival and differentiation of neurons in the developing vertebrate nervous system. NGF deprivation in the embryo results in sensory degeneration whereas in neonatal mice, lack of NGF leads to degeneration of neurons in the sympathetic nervous system. In culture, NGF significantly enhances the survival of sensory and sympathetic neurons and neural crest derived cells. We have examined the role of NGF in neuronal survival by targeting the expression of an NGF cDNA (a gift from R. Edwards, UCLA) to the epidermis of transgenic mice. Mouse NGF cDNA expression was driven using the human keratin K14 promoter and enhancer sequences. K14 is an intermediate filament protein expressed in the basal cell layer of the epidermis and other stratified squamous epithelium. The increased levels of NGF resulting from expression of the K14-NGF transgene led to an increase in the density of innervation in the epidermal target tissue, hypertrophy of the trigeminal ganglia, and an increase in the number and size of primary afferents. These results demonstrate that *in vivo* the level of NGF in the target tissue controls cell survival and growth of primary afferents. Supported by NIH NS25617 to BMD & AR40873 to KMA.

544.4

PD 90780: A SELECTIVE NON-PEPTIDE LIGAND FOR THE p75 NGF RECEPTOR. R.E. Davis*, M. Dickerson, J. Fergus, T. Hepburn, J. Hopkins, H. Levine, J. Marks, K. Spiegel, J. Jaen, W. Moos. Parke-Davis Pharmaceutical Research, Warner-Lambert Co., Ann Arbor, MI 48106

A series of novel nonpeptide ligands have been discovered that selectively displace NGF from its p75 binding protein (BP). PD 90780 is characteristic of this series. It displaces ^{125}I-NGF from the extracellular domain of p75 in a cell free system with an IC_{50} between 100-200 nM. PD 90780 also inhibits DSS crosslinking of ^{125}I-NGF to p75 but not to gp140^{trk}, suggesting that PD 90780 does not inhibit the binding of NGF to gp140^{trk}. Two ^{125}I-NGF binding components were seen in whole PC12 cells in the presence of PD 90780. A large proportion of ^{125}I-NGF binding was blocked by PD 90780 with an IC_{50} of approximately 200 nM. A smaller component of ^{125}I-NGF binding was not displaced by PD 90780. The relative proportion of PD 90780 sensitive and resistant binding is similar to the proportion of p75 and gp140^{trk} BP immunoprecipitated from native PC12 cells. Therefore, the PD 90780 sensitive binding of ^{125}I-NGF in whole PC12 cells may represent binding of NGF to p75 while the PD 90780 resistant binding may reflect NGF binding to gp140^{trk}.

Despite the ability to completely displace ^{125}I-NGF from the p75 BP, PD 90780 does not block the ability of NGF to stimulate ChAT and MAP kinase activity in PC12 cells. PD 90780 also does not block the ability of NGF to promote survival of rat fetal SCG neurons in culture or inhibit the retrograde transport of NGF from the eye to the SCG in adult rats.

544.5

DISTRIBUTION OF ^{125}I NGF BINDING SITES IN ADULT AND DEVELOPING RAT NERVOUS TISSUE THAT ARE NOT DISPLACED BY PD90780. B. D. Shivers*, P.D. Doyle, C. Raby and R.E. Davis. Neuroscience Pharmacology, Warner-Lambert/Parke-Davis, Ann Arbor, MI 48105.

Nerve Growth Factor (NGF) binds to p75 and p140^{trk} receptor proteins, signalling through the latter. PD90780 is a small organic molecule that displaces NGF binding from the extracellular domain of recombinantly-expressed p75 in a cell-free system. *In vitro* autoradiography was performed using 100pM ^{125}I mNGF in the presence or absence of 100μM PD90780 and the distribution of binding sites determined from film and emulsion autoradiograms. In adult rat brain sections, NGF binding that was not displaced by PD90780 was observed on cell bodies in olfactory tubercule, nucleus accumbens, caudate-putamen, medial septum, diagonal bands, midline thalamic nucleic, cochlear nuclei, interpeduncular nucleus and spinal trigeminal nuclei. Unlabelled, lightly-labelled or heavily-labelled cell bodies were intermingled in all subdivisions of the trigeminal ganglion. Other ganglia were not examined. Neuropil labelling was observed in regions harboring labelled cell bodies as well as presumptive terminal fields of these cell bodies, *i.e.*, cerebral cortex, basolateral amygdala, hippocampal formation, the spinal trigeminal tract and substantia gelatinosa. Spinal intersegmental rootlets and a tract ventral to the central canal were also labelled. On postcoital day (p.c.d.) 12-16, labelled structures included trigeminal nuclei, superior cervical and dorsal root ganglia, spinal cord and regions surrounding whisker follicles. By p.c.d. 20, basal ganglia and forebrain labelling were apparent. In early cerebellar development, transient labelling of the molecular layer was observed. These observations are consistent with the hypotheses that NGF specifies target field innervation and maintains the differentiation state of neuronal subpopulations, *e.g.*, cholinergic neurons. Work is in progress to determine if NGF binding sites that are not displaced by PD90780 represent p140^{trk}. We appreciate the contributions of our Parke-Davis colleagues, J. Marks, K. Spiegel and T. Hepburn, who identified and characterized PD90780.

544.6

NERVE GROWTH FACTOR INCREASES CHOLINE ACETYLTRANSFERASE ACTIVITY IN THE NORMAL AND DAMAGED ADULT RAT BRAIN. W. J. Lipinski*, M. J. Callahan, S. L. Fisher, T. Hepburn and R.E. Davis. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

Nerve Growth Factor (NGF) is an endogenously expressed protein that is responsible for survival and maintenance of select neuronal populations. NGF has been shown to protect basal forebrain cholinergic neurons from atrophy due to age and damage. The effects of NGF on central cholinergic neurons, however, has not been rigorously studied. We report that continuous intracerebroventricular infusions of NGF into otherwise undamaged male rats increases choline acetyltransferase (ChAT) activity in regions of the brain rich in cholinergic neuronal cell bodies: striatum, basal forebrain, and medial septum. Intentional damage to the striatum enhanced the ability of NGF to increase ChAT activity in forebrain cholinergic neurons. This effect of NGF developed gradually over the course of the infusion, reaching a peak following 7 to 10 days of administration. Additionally, intentional damage to the brain shifts the dose-effect curve leftward further suggesting that the damaged brain exhibits heightened responsiveness to NGF. A marked loss or atrophy of forebrain cholinergic neurons is one characteristic of Alzheimer's disease (AD). These data support the cautious therapeutic use of NGF in AD as a means to prevent or reverse cholinergic neuronal atrophy. Because NGF does not travel far from the site of injection, however, procedures must be developed to ensure adequate delivery of NGF to target neuronal populations in the human brain.

544.7

REGULATED EXPRESSION OF NERVE GROWTH FACTOR (NGF) IN BRAIN IMPLANTS: USE OF METALLOTHIONEIN PROMOTER-DRIVEN CONSTRUCTS IN TRANSFECTED GLIAL CELLS. V. Nipper,[1] C.A. Machida[1,2] and G.Nilaver[3]*. [1]Div. Neurosci., Oregon Regional Primate Res. Ctr., Beaverton, OR 97006, [2]Dept. Biochem. & Mol. Biol., and [3]Dept. Neurol., OR Hlth. Sci. Univ., Portland, OR 97201.

Genetically modified cultured cells have been employed to express NGF in brain. We sought to determine if NGF expression in such cells could be modified by regulatory effectors. An 820 bp Pst I fragment containing the entire coding region of mouse NGF gene plus 5′ flanking DNA was directionally inserted into the human metallothionein IIA promoter-driven expression vector pMEP 4 (Invitrogen). This vector contains the zinc-inducible IIA promoter and SV40 polyadenylation signal, and utilizes the hygromycin B gene as the selectable marker. Recombinants were transfected into the rat glioma C6 cell line (calcium phosphate transfection). Transfectants were selected with hygromycin B (1000U/ml; 10-14 days) and colonies isolated using glass cloning cylinders. The transfection efficiency is 7.5 x 10^{-7}. Clonal isolates will be analyzed to determine gene dosage (genomic Southern blot), basal and induced levels of NGF mRNA (Northern blot), and intracellular and secreted NGF (ELISA). Transfectants screened to identify cell clones with minimal leakiness of the IIA promoter will be used as donor cells for transplantation into rat brain. Pilot studies of C6 cell implants in rat brain do not show evidence of metastatic seeding or graft rejection.

544.8

PRODUCTION AND ANALYSIS OF TRANSGENIC MOUSE LINES OVEREXPRESSING NGF IN CNS. A. Roghani, T. Farris, J. D. Oh, G.D.Ellison*, L.L. Butcher, D. Hanahan[1] and R.H. Edwards. Depts. of Neurology and Psychology, UCLA School of Medicine, LA, CA 90024 and [1]Dept. of Biochemistry, UCSF, San Francisco, CA 94143.

The basal forebrain cholinergic system is involved in memory processes and the pathogenesis of Alzheimer's disease. NGF is normally produced in small quantities by the target cells to which this system projects. To determine whether or not the expression of NGF in targets affects the synaptic contacts of afferent cholinergic neurons, we placed the mouse NGF cDNA under the control of the regulatory region of the somatostatin gene and used this construct to generate three lines of transgenic mice. S1 nuclease protection assays performed on the total RNA from the adult brain, liver, and kidney of these mouse lines, as well as those of normal C57BL/6 mouse, demonstrated that only one of the three lines (#4) expressed the NGF transgene in the brain, but not in the liver or kidney. We are currently in the process of immunohistochemically analyzing the morphologic response to the expression of this transgene. Among the antibodies we have tested so far, our NGF antibody clearly stains clusters of neurons in various regions of basal forebrain in both normal and transgenic tissues. Preliminary quantitation of cross-sectional area of neurons in the olfactory tubercle of the basal forebrain showed that the cholinergic somata of the #4 transgenic line were increased in size compared to the nontransgenic mouse brain.

544.9

CONSEQUENCES OF INCREASED NGF GENE EXPRESSION IN THE HEARTS OF TRANSGENIC MICE. A. Hassankhani#, M. Steinhelper, L. Field, M. Geschwind#* and H.J. Federoff#. #Albert Einstein College of Medicine, Bronx, N.Y. 10461 and Krannert Institute of Cardiology, Univ. of Indiana School of Medicine

To examine the role of nerve growth factor (NGF) in determining the extent of innervation of sympathetic neurons in a target organ, a transgenic mouse model has been created. The cardiac specific α-myosin heavy chain (α-MHC) promoter has been used to drive the expression of an NGF minigene (α-MHC-NGF). α-MHC is activated prior to the ontogenic development of cardiac innervation, becoming the predominant myosin heavy chain expressed in the adult rodent heart. Five independent α-MHC-NGF founders were initially obtained, all of which expressed the transgene exclusively in the heart as demonstrated by RNAase protection and reverse transcription PCR. These lineages all exhibited an interesting albeit variable phenotype consisting of gross cardiomegaly and the presence of immature neural cells which appear to be infiltrating the epicardial region and particularly the atrio-ventricular groove. We have observed death (possibly due to arrythmia) in all transgenic lineages. Increased NGF would be expected to cause sympathetic hyperinnervation and an attendant increase in cardiac catecholamine content. Examination of all transgenic lineages reveal a significant, 20-fold rise in the level of cardiac tissue catecholamines. The extent of cardiomegaly has been best correlated with the level of total catecholamines. During propagation of the α-MHC-NGF lineages, we observed a decline in the penetrance of phenotype and expression of the transgene. Available data indicate that methylation of the transgene, within CpG rich regions is responsible for the diminished expression of the transgene. Studies are currently underway to further characterize physiological and neurobiological issues related to this model.

544.10

A RHOMBOTIN PROMOTER 1-NGF CONSTRUCT FOR THE PRODUCTION OF TRANSGENIC MICE

K. STALEY*, S.J. PICKERING, J.B. UNEY, M.H. JOHNSON, T.H. RABBITTS[1] AND M.V. SOFRONIEW. Department of Anatomy, University of Cambridge and [1]MRC Laboratory of Molecular Biology, Cambridge, U.K.

Nerve growth factor (NGF) influences transmitter-associated enzyme expression in developing basal forebrain cholinergic neurons, but it is not known if NGF regulates developmental cell death in this system. This question could be addressed by altering the levels of NGF produced in the hippocampus or neocortex, the target regions of these neurons. Towards this end, we have generated a rhombotin (rbtn) promoter 1-NGF fusion construct for production of transgenic mice. We have previously found that mice transgenic for a rbtn promoter 1-lac Z construct show promoter 1 activity in the CA1 and CA2 regions of the hippocampus, layers 3 and 5 of the cerebral neocortex, and the cerebellum, which are also major sites of NGF production. NGF cDNA was obtained from mRNA isolated from mouse submaxillary glands and selectively amplified by PCR. The PCR product was cloned and sequenced, and the biological activity of the recombinant protein confirmed. The NGF sequence was subcloned into a plasmid containing the rbtn promoter 1 and an SV40 polyadenylation sequence. Mice generated by injection of a gel-purified 10 kb Not 1 fragment into fertilised MF1 eggs are currently being examined for insertion and activity of the transgene. Analysis of developing basal forebrain cholinergic neurons in these transgenic mice should allow a direct investigation of the putative neurotrophic role of NGF in the septo-hippocampal system and may provide clues as to the function of NGF in other brain regions such as the cerebellum.

544.11

HISTIDINE AND CARBOXY-TERMINAL MUTANTS OF MOUSE ß-NERVE GROWTH FACTOR (NGF) Sang B. Woo, Yuling Luo, and Kenneth E. Neet*. Dept. of Biological Chemistry, UHS/ Chicago Medical School, N.Chicago.

Histidine residues 75 or 84 of mouse NGF were replaced with an alanine residue by site-directed mutagenesis. Mutant genes were transiently expressed in COS-7 cells and proteins were partially purified with an NGF-specific, N60-monoclonal immunoaffinity column. The bioactivity of each His mutant was compared with authentic NGF from mouse submaxillary glands by neurite outgrowth in PC12 cells. H75A showed about 5-fold less activity, while H84A showed far less activity than ß-NGF. These results indicate that His 75 and 84 may be involved in the interaction of NGF with its receptor(s) and support previous chemical modification studies of His residues with diethylpyrocarbonate. However, the lower activity of these mutants could result from incomplete protein folding when transiently expressed in COS-7 cells.

Recombinant mouse NGF (rNGF) was isolated from insect cells infected with a recombinant baculovirus containing a prepro-NGF insert. Three purified forms of rNGF were separated on Mono-S chromatography and had distinct binding and biological activity in DRG and PC12 cell assays. Each form of rNGF differed from mature submaxillary gland mNGF in that the C-terminal Arg-Gly dipeptide had not been processed. Removal of the C-terminal dipeptide by γ-NGF peptidase treatment converted the three forms into a single form, identical to mature mNGF in structure and bioactivity. Thus, a single polypeptide of rNGF can exhibit three distinct biological activities due to the C-terminal dipeptide. We suggest that C-terminal processing of NGF may be physiologically important. (Supported by NIH grant NS24380)

544.12

NEUROTROPHIN EXPRESSION IN THE DEVELOPING CHICK RETINA. X.-Y. Xie*, A.S. Garner, J.M. Voci and T. H. Large. Dept. of Neuroscience, Case Western Reserve University, Cleveland, OH 44106.

The biological actions of the neurotrophins in the developing nervous system are not limited to survival, but now appear to include the regulation of stem cell proliferation and commitment to a phenotype by post-mitotic cells. We have begun to examine these potential roles using the embryonic chick retina as a model system.

Northern analysis of RNA from embryonic chick retina indicates that neurotrophins and their receptors are expressed during the period of cell proliferation (E3-7) and the subsequent formation of retinal layers. Transcripts for *trk*B (a receptor for BDNF, NT3 and NT4) are present at E5, the earliest age tested. An increase in *trk*B mRNA levels is observed at E13 and corresponds to the period of ganglion cell (RGC) dependence on target-derived factor(s) and maximal expression of BDNF in the tectum. In the retina, NT3 mRNA is present at E7 and is maximal by E14, but BDNF mRNA remains relatively low throughout development. NGF mRNA has previously been shown to be present as early as E6. Thus NGF and NT3 may be "intrinsic" regulators of retinal development while BDNF is a candidate "extrinsic" factor supporting RGC survival.

In order to further examine neurotrophin actions, we have begun to express recombinant neurotrophins in baculovirus. The coding sequence for the precursors of chicken NGF, BDNF and rat NT3 (differs from chick by one K-R substitution) were subcloned into the Bluebac transfer vector for color selection of recombinants. The use of linearized baculovirus DNA improved the efficiency of recombination to 30-40%. Finally, the adaptation of Hi-5 cells to suspension culture in serum-free medium yielded higher expression of recombinant factors compared to Hi-5 monolayer or Sf9 suspension cultures. Bioassays using PC12 cells and chick DRGs indicate Hi-5 cells properly process the precursor proteins to the active, mature factors. In addition, the 13 kD mature chicken NGF protein has been identified on Western blots using an antibody against mouse NGF. These recombinant neurotrophins and the ongoing production of factor-specific, function blocking antibodies will allow us to manipulate neurotrophin levels and to test neurotrophin functions in the developing retina.

Supported by grants from the American Heart Association to XYX, MSTP to ASG and NIH grants AG00533 to JMV and EY08885 to THL.

544.13

DEVELOPMENTALLY REGULATED EXPRESSION OF THE NEUROTROPHIN HIGH AFFINITY RECEPTORS. T. Ringstedt[1,2], H. Lagercrantz[2] and H. Persson[1]*.1/ Department of Medical Chemistry, Laboratory of Molecular Neurobiology, Karolinska Institutet, 2/ Department of Pediatrics, Karolinska Hospital, Stockholm, Sweden.

Tyrosine protein kinases trk, trkB and trkC are essential components of the high affinity receptors necessary to mediate the biological effects of the neurotrophins NGF, BDNF, NT-3 and NT-4. Here we report on their expression during neonatal and perinatal development in the rat brain. Cells expressing mRNAs encoding different members of the trk family were identified by in situ hybridization using oligonucleotides complementary to their respective mRNA. In septum, striatum and brainstem, higher levels of trk mRNA was detected at 2 and 4 weeks than at 1 weeks of age. In certain thalamic nuclei, trkB and trkC mRNA were highly expressed at P1 to P7, but the expression declined gradually in 2 and 4 weeks old animals. TrkB was not detected in tenia tecta and piriform cortex until 2 weeks of age. In P1 and P4 animals a high labeling was seen for trkC mRNA in the deeper parts of neocortex, while in 2 and 4 weeks old animals the highest labeling was seen over the outer neocortical layers. Several brainstem nuclei showed a higher labeling for trkC mRNA at P1 to P7 than in animals of older age. These data suggest that high-affinity neurotrophin-receptors mediate a transient response to neurotrophins in many regions during brain ontogeny.

544.14

EXPRESSION OF THE TRK PROTO-ONCOGENE IN THE DEVELOPING RAT. S. Elkabes*, C. F. Dreyfus, W. J. Friedman and I. B. Black. Department of Neuroscience and Cell Biology, Robert W. Johnson Medical School, UMDNJ, 675 Hoes Lane, Piscataway, NJ 08854.

The trk proto-oncogene has recently been identified as an important component of the nerve growth factor (NGF) receptor and has been implicated in high affinity binding of NGF. To begin investigating the regulation of the trk proto-oncogene (trk) during development, we have studied prenatal and postnatal expression in the rat, by in situ hybridization. Our results indicate temporal and spatial regulation of trk expression. At early embryonic stages (E13.5 and E14.5), trk mRNA was exclusively localized to spinal and cranial ganglia. In the central nervous system trk expression occurred at later developmental stages. During the first postnatal weeks (P1 and P15), highest levels of trk mRNA were observed in the hippocampus. However, in the adult CNS, expression of trk in regions such as the basal forebrain and piriform cortex was comparable to that in hippocampus. Our studies suggest that actions of NGF on various cell types during development may be modulated by temporal and spatial regulation of trk proto-oncogene expression.
(Supported by NS 10259-Javitz Award and NIH: HD23315)

544.15

NEUROTROPHIN RECEPTOR EXPRESSION IN THE DEVELOPING RAT EMBRYO. N.G. Carri*, S. Söderström and T. Ebendal. Department of Developmental Biology, Uppsala University, Biomedical Center, S-751 23 Uppsala, Sweden and IMBICE (*), CC 403, 1900 La Plata, Argentina.

Expression of neurotrophin receptors was studied using antisense oligonucleotides specific for the low-affinity NGF receptor (LNGFR) and for the neurotrophin high-affinity receptors *trk*, *trk*B and *trk*C. *In situ* hybridization was performed on sections of rat embryos at days E16, E20 and E22. The hybridization patterns were quantitatively analyzed in contact-exposed X-ray films using video-based image processing. The percentage of neuron area covered by silver grains in emulsion-dipped microscopic slides was also studied using this system. The results show distinct patterns of distribution for mRNA for each of the receptors. In the E16 embryo, LNGFR mRNA was highly expressed in the trigeminal and dorsal root ganglia, in the ventral part of the spinal cord. Weaker labelling was found also in the retina. The *trk* oligoprobe intensively labelled the trigeminal and dorsal root ganglia but only marginally the retina and spinal cord. *trk*B was highly expressed in the telencephalon, retina, trigeminal ganglion and was abundant throughout the spinal cord. *trk*C expression was intense in the hippocampus at E16. The same pattern was found at E20, although *trk*B labelling in the retina. Also at E22 similar patterns were found but with an increased labelling of retinal neurons with the LNGFR probe. Our results show that neurotrophin receptors are expressed in neurons in a developmentally regulated pattern. The temporo-spatial expression of neurotrophin receptors may regulate the trophic response of a given neuronal population such as the retinal ganglion cells to a particular neurotrophic factor during development. (Supported by the Swedish Natural Science and Medical Research Councils, the Swedish Environmental Protection Agency and Conicet-TWAS BC 90-099).

544.16

REGULATION OF $p75^{NGFR}$ AND $p140^{trk}$ EXPRESSION IN DEVELOPING SENSORY NEURONS IN RELATION TO INNERVATION AND CHANGES IN NEUROTROPHIC FACTOR RESPONSIVENESS Sean Wyatt[1], Luis Parada[2], Susan O. Meakin[3] and Alun Davies *[1] [1]St. George's Hospital Medical School, London SW17 ORE, UK; [2]NCI, Fredrick, MD21701; [3]Stanford University, CA94305.

When embryonic mouse trigeminal neurons are extending axons to their targets they survive independently of neurotrophic factors. As their axons approach their targets they display a transitory survival response to BDNF and NT-3 which is lost as the neurons become dependent on NGF for survival. Before acquiring NGF dependence, these neurons express low levels of $p75^{NGFR}$ and $p140^{trk}$ mRNAs. At this stage, BDNF and NT-3, but not NGF, increase the level of $p75^{NGFR}$ mRNA in cultured neurons. With the acquisition of NGF dependence, the levels of $p75^{NGFR}$ and $p140^{trk}$ mRNAs increase markedly. At this stage, NGF, but not BDNF or NT-3, up-regulate $p75^{NGFR}$ mRNA. We are currently studying the effects of neurotrophic factors on $p140^{trk}$ mRNA expression in this system.

544.17

IMMUNOHISTOCHEMICAL DETECTION OF $p75^{NGFR}$ IN NEONATAL AND ADULT OLFACTORY NEUROEPITHELIUM OF RAT. C.P. Turner* and J.R. Perez-Polo, Human Biological Chemistry and Genetics, Univ. Texas Med. Branch, Galveston, TX 77555-0652.

We have shown expression of the low affinity receptor for NGF, $p75^{NGFR}$, using monoclonal antibody 192. In neonates, we have observed $p75^{NGFR}$-immunoreactivity ($p75^{NGFR}$-ir) in the olfactory neuroepithelium (ONE) that was confined to a well defined band, superficial to the lamina propria (LP). A stain-free, single cell layer between the LP and band of $p75^{NGFR}$-ir was very obvious at all neonatal ages. The absence of $p75^{NGFR}$-ir in this stem cell layer suggests that only immature and/or mature olfactory receptor neurons (ORNs) express $p75^{NGFR}$. In adults, staining of the ONE was now confined to the deepest layer, indicating that stem cells now express $p75^{NGFR}$. However, the profiles displayed in neonates versus adults may be a reflection of the change in cell dynamics that takes place in the ONE as the animal matures. Within the LP, we observed staining of the olfactory fascicles at all ages. We believe glial cells are responsible for this staining. Not all fascicles were stained, perhaps because there is a mixed population of mature and immature ORN axons. The mammalian ONE is capable of continuous regeneration throughout life and expression of $p75^{NGFR}$ in both adults as well as neonates implies that neurotrophic support is required at all times. Supported in part by NINDS Grant #18708.

544.18

THE p75 NEUROTROPHIN RECEPTOR IN HUMAN RETINA. I. M. Hopkins*, N. Kleitman†, and K. Spiegel. Parke-Davis Pharmaceut. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48106 and †The Miami Project to Cure Paralysis, Univ. of Miami, Miami, FL 33136.

The p75 neurotrophin binding protein (low-affinity NGF receptor) is a transmembrane receptor for NGF, BDNF, and NT3, with no known signal transduction activity. In previous studies of rat and primate retina, p75 immunoreactivity appeared to be confined to Muller glia, with rare p75-positive retinal ganglion cells (RGC) in adult rat. One report of p75 in the optic nerve fiber layer of developing primate retina suggested p75 involvement in the growth of RGC axons. As part of a study of neurotrophin receptors in the human retina, we have examined the distribution of p75 in normal embryonic human retina, normal adult human retina, and regenerating adult human retina. Adult human donor eyes were a generous gift of the Mid-America Eye and Tissue Bank, St. Louis, MO. Retinas were either cultured on a monolayer of transformed rat Schwann cells or prepared for cryostat sectioning. Sections of embryonic human retina were prepared as part of an approved study at the University of Miami. Sections and cultures were immunostained with a monoclonal antibody (ME20.4) directed against human p75. p75 appeared to be confined to Muller glia in the adult human retina. In embryonic retina at 6 weeks gestational age, p75 was evenly distributed through the retina, with no enhancement of p75 staining in the RGC fiber layer. ME20.4 staining did not detect p75 on adult human retinal neurites regenerating in vitro, due to unexpected staining of the underlying monolayer of transformed rat Schwann cells. We conclude that human retinal ganglion cells and their axons do not express significant amounts of p75 protein in the normal adult or the early embryo..
(Supported by Warner-Lambert Co. and The Miami Project.)

544.19

RAS p21 PROTEIN PROMOTES SURVIVAL AND NEURITE OUTGROWTH OF NGF-DEPENDENT HUMAN EMBRYONIC NEURAL CREST DERIVATIVES *IN VITRO*.

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In previous studies we demonstrated dose-dependent survival and neurite outgrowth induced by the ras p21 protein after microinjection into cultured chick embryonic neurons. To determine whether similar effects could be induced in human cells, we introduced the oncogenic form of ras p21 into the cytoplasm of cultured human neural crest derivatives (11th to 12th gestational week): adrenal chromaffin cells, dorsal root ganglion (DRG) and sympathetic neurons. We verified that these cells are dependent on nerve growth factor (NGF) for survival and neurite outgrowth *in vitro*. In DRG neurons, ras p21 (30 mg/ml) promoted survival and neurite outgrowth comparable with NGF (84% vs. 92%, respectively). Sympathetic neurons in the chick embryo were demonstrated to be unresponsive to ras p21. Intriguingly, this molecule promoted survival in human sympathetic neurons, albeit less strongly than NGF (67% vs. 94%). The effect of ras p21 on purified human chromaffin cells was indistinguishable from NGF (41% vs. 40% neurite outgrowth). These results are compatible with the involvement of ras or ras-like proteins in the intracellular transduction of NGF in human neural crest derivatives and suggest species-specific differences in neurotrophic signal transduction.

544.20

DISTRIBUTION OF VGF mRNA IN THE POSTNATAL RAT BRAIN.

S. E. Snyder*, R. D. Streck, J. E. Pintar, and S. R. J. Salton.
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VGF is a neural immediate-early gene product which is rapidly and selectively induced by neurotrophic factors (e.g., nerve growth factor, NGF) relative to nonneurotrophic factors in PC12 cells. We have determined the anatomical distribution of VGF mRNA in the P5 rat brain by *in situ* hybridization using [^{35}S]-labeled antisense or sense control VGF cRNA probes. Selective labeling of limbic and paralimbic cortex, including cingulate, retrosplenial, entorhinal, and piriform cortices, was noted in contrast to a distinct lack of signal in frontoparietal, occipital or temporal regions. Strong labeling was also seen in olfactory bulb, the hippocampal formation (particularly CA1 and dentate), amygdala, basal forebrain, striatum, nucleus accumbens, lateral geniculate, gelatinosus and ventroposterolateral/ventroposteromedial thalamic nuclei, septal nuclei, inferior colliculi, cerebellum, and portions of the pons and medulla. A previous study of whole brains found VGF mRNA to first be detectable at embryonic day 18 (E18), peak at P4, and decrease to intermediate levels in the adult [Salton *et al.*, 1991]. Also, preliminary studies have shown high levels of VGF mRNA in developing cerebellum but very low levels in adult. Ongoing *in situ* hybridization studies of animals from embryogenesis through adulthood will determine the ontogeny of VGF expression in specific brain structures.

544.21

NEUROTROPHINS AFFECT THE ISTHMO-OPTIC NUCLEUS IN CHICK EMBRYOS: CELL DEATH *ENHANCED* BY NGF IN VIVO.

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The isthmo-optic nucleus (ION) is the source of centrifugal innervation of the retina. Neurons in the ION express p75 neurotrophin receptors during the period of normal developmental cell death (E13-17, von Bartheld et al., 1991, J. Comp. Neurol. 310: 103-129). To determine which neurotrophin supports ION neurons, we tested NGF, BDNF, and NT-3 (1-10 ng/ml) on ION neurons in primary culture. ION neurons were identified by intraocular injection and retrograde transport of the fluorescent tracer DiI, and labeled cells were plated from embryos at ages E11, E12 and E13. BDNF increased survival and neurite extension of ION neurons. The survival effect was most pronounced for neurons from E11 embryos, but still significant for ION neurons from E12/13 embryos. Neither NGF nor NT-3 increased survival rates. To determine effects of neurotrophins on ION neurons in vivo, we injected 75-900 ng NGF in the eye daily from E10-15. This treatment enhanced cell death in the ION by 25-72% in the embryos, but not at later ages. Injections of vehicle only did not increase cell death. The negative effect of NGF on developing ION neurons may be due to competition at the level of the neurotrophin receptor. NGF may act as a competitive antagonist, possibly to BDNF, thus preventing trophic action. Competitive interactions among the neurotrophins may play a role in the specificity of target innervation and the regulation of neuronal survival. **Supported by NIH grants NS 08990, 23343 and 29582.**

544.22

RECOMBINANT HUMAN NGF, ITS PRECURSOR AND THE FORMATION OF A "7S NGF"-LIKE COMPLEX.

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We investigated whether purified alpha and gamma subunits from murine 7S complex could form a complex with either mature rhNGF or precursor forms of rhNGF. The mature and precursor forms of rhNGF were isolated by classical purification techniques (Schmelzer, et al. (1992) J. Neurochem., in press and unpublished experiments, respectively). Multiple forms of the precursor were observed as two major groups of bands on SDS-PAGE: ~26 kDa and ~18 kDa. A more complete characterization of the precursor forms will be presented. We observed complex formation with several forms of mature rhNGF as well as precursor processing to mature rhNGF after the addition of the alpha and gamma subunits. For precursor, both limited tryptic digestion and the formation of a 7S NGF-like complex caused each form of the precursor to be reduced to a band migrating in the region of 13 kDa on SDS-PAGE, corresponding to a mature NGF. The activity of the processed precursor and its resulting processed product was compared using PC-12 cell-based neurite extension and chick dorsal root ganglion cell survival assays. Processed precursor yielded mature rhNGF showing full activity while precursor itself exhibited minimal activity.

OTHER FACTORS AND TROPHIC AGENTS: GLIA

545.1

ASTROCYTES PROTECT DOPAMINERGIC NEURONS AGAINST TOXICITY PRODUCED BY 1-METHYL-4-PHENYLPYRIDINIUM (MPP$^+$) AND 6-HYDROXYDOPAMINE (6-OHDA) IN CULTURE.

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We studied the trophic effect of astrocytes upon dopamine (DA) neurons to examine whether or not glia can modify the toxicity produced by the dopaminergic neurotoxins, MPP+ and 6-OHDA. Glial cultures, enriched in astrocytes, were prepared from the striatum or mesencephalon of newborn rats in serum containing medium and 24 hrs before use were switched to chemically defined medium. Mesencephalic cells from embryonic day 14 rats were plated on either polyornithine coated 35mm dishes or on dishes with a confluent layer of astrocytes. MPP$^+$ (5μM) was applied for 48 hrs at 4 days in vitro (DIV) and 6-OHDA (100μM) for 45 min at 6DIV. Neurotoxicity was assessed at 7DIV by ^{3}H-DA uptake and morphometric analysis of TH immunostained cells to measure the extent of neuritic damage and neuronal survival. We found that the presence of either striatal or mesencephalic glia modified the extent of neuronal toxicity produced by both MPP$^+$ and 6-OHDA. ^{3}H-DA uptake in pure neuronal cultures was approximately 5% of control after treatment with either MPP$^+$or 6-OHDA, while DA uptake was ~15% of control after MPP$^+$ and ~60% of control after 6-OHDA treatment. Similarly, the number of surviving TH+ cells was between 20-40% of control after MPP$^+$ or 6-OHDA treatment in neuronal cultures, compared with ~90% of control in mixed cultures. Our results show that the presence of glia can protect DA neurons against uptake reduction and cell loss produced by both toxins. However, the glial protective effect against 6-OHDA toxicity is greater than against MPP$^+$. (Supported by NIH grants and by the United Parkinson Foundation).

545.2

TYPE 1 ASTROCYTE CONDITIONED MEDIUM PROTECTS SUBSTANTIA NIGRA DOPAMINE NEURONS AGAINST EXCITOTOXIC CELL DEATH.

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Previous work from our laboratory has indicated that Type 1 astrocytes from the substantia nigra selectively enhance the survival of substantia nigra dopamine neurons in dissociated cell culture. Further, conditioned medium from Type 1 astrocytes (CM) is able to elicit dopaminergic survival (O'Malley et al., 1992). To determine whether the CM effect on survival could be extended to protection against neurotoxic insult, we established an *in vitro* model of neurodegeneration using two well known neurotoxins, glutamate and 6-hydroxydopamine (6-OHDA). Substantia nigra from E16 rats were dissociated and plated in either serum-free medium (SFM) or CM. After 7 days *in vitro*, cells were exposed to 100 µM glutamate for 90 min. The cultures were returned to original plating medium for two additional days. Dopamine neurons were visualized by immunostaining for tyrosine hydroxylase (TH), the rate limiting enzyme in dopamine synthesis. Exposure of SFM cultures to glutamate resulted in cell death. However, CM rescued dopamine neurons from excitotoxic death. To determine whether this protective effect was specific for excitotoxins, or whether it could be generalized to toxicants with different mechanisms of action, we treated cultures with 100 µM 6-OHDA. In contrast to the experiments with glutamate, CM did not protect nigral dopamine neurons against cell death caused by 6-OHDA. These data indicate that a factor elaborated by nigral Type 1 astrocytes protects nigral dopamine neurons from cell death caused by an endogenous excitotoxin. (Support: NIH HD23315, NS20788; NIDA DA05132, DA05403-01)

545.3

SUBSTANTIA NIGRA TYPE I ASTROCYTES ELABORATE A SOLUBLE FACTOR THAT AUGMENTS DOPAMINERGIC NEURON SURVIVAL.
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We have previously demonstrated that local glia, specifically Type I astrocytes, selectively increase substantia nigra (SN) dopaminergic (DA) neuron survival. However, the mechanism of action remains unclear. To determine whether effects are elicited through diffusible agents, partially purified Type I astrocyte conditioned medium (CM) was tested on embryonic day 16 rat SN dissociates. After 3 days of exposure to CM, DA neuron number was monitored immunocytochemically with antibody to tyrosine hydroxylase (TH), the DA biosynthetic enzyme. CM increased TH+ cell number 2-fold, suggesting that a soluble factor(s) promoted neuron survival.

Neurons cultured in serum free medium are known to contain few, but detectable numbers of glia. To determine whether CM affected neurons directly, or indirectly through glia, cultures were labelled with antibody against the glial marker, glial fibrillary acidic protein (GFAP). CM increased GFAP+ cells 2-fold, implying that CM may affect DA neuronal survival indirectly. To test this possibility, cultures were exposed to a concentration of the gliotoxin α-amino adipic acid that eliminated detectable GFAP+ cells. Under these conditions, CM still elicited a 2-fold increase in TH+ cells. Our observations suggest that CM directly enhances substantia nigra DA neuron survival, in culture. (Support:NIH HD 23315, Javits:NS 10259.)

545.4

TGFα SELECTIVELY INCREASES DOPAMINERGIC CELL SURVIVAL IN VENTRAL MESENCEPHALIC CULTURES.
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Transforming growth factor α (TGFα) mRNA and protein have been localized to rodent brain (Wilcox and Derynck 1988; Fallon et al., 1990). Based on its high expression in the striatum, TGFα has been proposed as a trophic factor for dopaminergic neurons. Therefore, we characterized the actions of TGFα on E15 fetal rat cultures of dopaminergic cells. Addition of TGFα (10ng/ml) for 1 day to low-density mesencephalic cultures selectively promoted dopaminergic cell survival after 4 days as measured by an increase in the number of tyrosine hydroxylase (TH) immunopositive cells. These cells represent only 1% of the total neuron number. In contrast, the total number of neurons (neuron specific enolase immunopositive) was unaffected. The number of vimentin immmunopositive astrocytes was elevated by 175% above control. Dopamine (DA) uptake and TH activity were also enhanced approximately 100% and 45% above control, respectively. In cultures lacking a poly-cationic substrate, TGFα dramatically amplified neuronal adhesion to astrocytes. This adhesion was blocked by the anti-mitotic agent, cytosine arabinoside. Two other growth factors, epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1), stimulated DA uptake to a lesser degree than TGFα. EGF shares the same receptor with TGFα, and in combination the two did not show additive effects, while the IGF-1-induced elevation was additive with TGFα. Inhibition of possible actions of endogenous neurotrophins with K252b did not alter TGFα's effects on DA uptake or neuron-astrocyte adhesion. Ciliary neurotrophic factor was ineffective in altering control or TGFα-induced DA uptake. These findings indicate that TGFα selectively promotes survival of dopaminergic neurons and that this effect is probably mediated by glial cells.

545.5

DEVELOPMENTAL ASPECTS OF THE PRIMARY CULTURE OF MESENCEPHALIC DOPAMINERGIC NEURONS.
T. Takeshima¶, K. Shigematsu*, K. Shimoda§ and J.W. Commissiong†. Tattori West Hosp.¶ and Div. Neurol., Inst. Neurol. Sci.§, Tattori Univ. Sch. Med., Yanago 683, Japan. Dept. Neurol. 2*, Nagasaki Univ. Sch. Med., 12-4 Sakamoto-machi, Nagasaki 852, Japan. LMCN-NINDS-NIH†, Bldg. 10/5N214, Bethesda, MD. 20892.

We have recently described a primary culture of mesencephalic dopaminergic cells from the E14 rat brain that is 20% TH+ between 4 hr and 10 days in culture (Soc. Neurosci. Abs 17.: 982, 1991; Brain Res. In press). Progressive neuronal death occurs, and by 14 days in culture (DIV14), <10% of all neurons survive. Those TH+ cells that survive, however, develop extensive, elaborate, neurofilament positive (NF+) dendritic arborizations, at DIV14 when the astrocytes (GFAP+) become confluent, providing an astrocyte feeder layer (AFL) for cell growth. When the cells were grown on poly-D-lysine, and seeded at 5.0 x 10^4 cells/cm^2, or less, 99% of the neurons died by DIV5. However, when grown on an AFL, even at 1000 cells/cm^2, neuronal survival was >95% even at DIV14. The results suggest that confluent astrocytes produce a factor that promotes dopaminergic cell survival in culture. Preliminary results suggest that the conditioned medium from the DIV10 to DIV14 cultures also promotes dopaminergic neuronal survival. Extracts from astrocytes of DIV14 to DIV21 are also being tested. Experiments are in progress to identify and isolate the putative neurotrophic factor(s) (NTF) for dopaminergic neurons. Antibodies to several proposed NTFs for dopaminergic neurons did not inhibit dopaminergic cell rescue by the AFL or the conditioned medium. Isolated pockets of cells develop in some cultures, all of which are TH+, suggesting that TH+ cell clones may develop in the cultures spontaneously. In addition, in some cultures, some TH+ cells developed elongated neurites that were devoid of varicosities, suggesting the existence of a specific, varicosity-inducing factor.

545.6

CHARACTERIZATION OF GLIA-DERIVED CHOLINERGIC NEUROMODULATORY ACTIVITY INDUCED BY EPIDERMAL GROWTH FACTOR (EGF) IN SEPTAL CULTURES.
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We have demonstrated that EGF in the nanomolar range can decrease the activity of choline acetyltransferase and the number of acetylcholinesterase positive neurons in cultures from the fetal rat medial septal area without affecting cholinergic or general neuronal cell survival. The effects of EGF on these cholinergic cells first became evident after 5 days of continuous treatment with this factor and was indirectly mediated as it depended upon active glial cell proliferation. Both astroglia and macrophage/microglia were induced to proliferate in these mixed culture preparations following EGF application, while the number of oligodendrocytes remained unchanged. By combined immunocytochemistry and autoradiography we found the astroglia to actively incorporate ^{3}H-thymidine transiently between 2 to 4 days of EGF application, while the microglia only began to proliferate after 4 days of factor treatment and continued to increase thereafter. Consequently, changes in the number of these 2 glial cell types either preceded (astrocytes) or occurred concommitantly (microglia) with the changes seen in cholinergic cell expression in EGF-treated cultures thus rendering both cell types potential sources of cholinergic neuromodulatory activity. In order to assess whether astroglia or microglia are implicated in the cholinergic cell response to EGF, the microglial cells were eliminated by treating cultures with l-leucine methyl ester. Elimination of the microglia did not affect astroglial cell proliferation nor the changes observed in the cholinergic neurons following EGF treatment. These data suggest that the astroglia represent the cell source of the cholinergic neuromodulatory activity, the properties of which are currently under investigation.

545.7

IN VIVO EXPRESSION OF PDGFRα IN PHENOTYPICALLY DEFINED GLIA.
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Growth factors such as basic fibroblast growth factor and platelet derived growth factor (PDGF) can modulate oligodendrocyte (OL) development influencing migration, proliferation and differentiation. Recent studies have demonstrated the presence of PDGFα receptors (PDGFRα) in the rodent brain (Schatteman et al.,Dev. in press, 1992; Reddy et al., J. Neurosci. Res. 31;1992) and on cells of the OL lineage *in vitro* (McKinnon et al. Neuron 5; 1990; Hart et. Dev. 105;1989). No study, however, has demonstrated the presence of PDGFRα on phenotypically defined cells *in vivo*

We show using combined immunocytochemistry and in situ hybridization, PDGFRα mRNA in cells in both the gray and white matter of the neonatal rat cerebral cortex. We carried out a spatial and temporal analysis of PDGFRα mRNA expression in the rat forebrain on postnatal days 1,3,6, and 9. PDGFRα message is detected in cells which are immunoreactive for vimentin, GD3 or O4. In contrast, cells which are galactocerebroside + (GC), glial fibrillary acidic protein + (GFAP), or 68 Kd neurofilament protein + (NF) do not express a detectable message. *In vivo* studies are important not only to verify *in vitro* data, but also to understand the cell-cell interactions which occur only when the cytoarchitecture is maintained. Our results support the *in vitro* data which suggest that: 1) PDGFRα is expressed by immature OL, 2) GD3+ and O4+ immature OL have the potential to respond to PDGF, and 3) GC+ OL, GFAP+ astrocytes, and NF+ neurons are not responsive to PDGF. Supported by NICHD and DOE.

545.8

GRAFTED SCHWANN CELLS AND INFUSION OF A SCHWANN CELL-DERIVED GROWTH FACTOR (DNTF) ENHANCE MORPHOLOGICAL RECOVERY IN THE DAMAGED ADULT RAT DOPAMINE SYSTEM.
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We have previously demonstrated that co-cultured Schwann cells, or a partially purified fraction of conditioned medium from peripheral nerve or a Schwann cell line, denoted as dopamine neurotrophic factor (DNTF), promote survival and neurite outgrowth of cultured embryonic rat tyrosine hydroxylase (TH)-positive ventral mesencephalic neurons. In the present studies, we have begun to characterize the in vivo effects of grafted Schwann cells and DNTF infusion in rats with varying degrees of damage to the nigrostriatal dopamine (DA) system. Implantation of Schwann cells from primary cultures derived from neonatal rat sciatic nerve into the striatum of rats with partial lesions of the DA pathway yielded effects on amphetamine-induced rotational behavior and TH-positive fiber staining in a pattern that varied with the level of striatal denervation: implantation of 60,000 Schwann cells at each of two locations in the striatum of rats with partial lesions yielded good recovery; similar implants in rats with near-complete lesions had no effect. In a second experiment, we infused DNTF via osmotic minipump (0.5ul/hr for 2 weeks) into the lateral ventricle of adult rats with large unilateral lesions of the nigrostriatal DA pathway. At 3 weeks following completion of the infusion, rats receiving DNTF exhibited a marked increase in staining of TH fibers present in the lateral septal area proximal to the intraventricular infusion, corresponding to fibers of midbrain DA neurons spared by the lesion. This enhanced fiber staining was not observed in the hemisphere contralateral to infusion, or in control rats receiving comparable infusion of culture medium which did not contain DNTF. Thus, grafting of cultured rat Schwann cells or intraventricular infusion of DNTF produce morphological effects consistent with possible stimulation of DA fiber proliferation in adult rats. Supported by AG08133, NS24032 (TJC), AG08969 (JES).

545.9

An Assay for Detecting Schwann Cell Secretory Activity *In Vitro*. Daniloff, J.K.*, K. Crossin#, A. Smith, T. Yoes, S. Gibson and M. Powell, Department of Veterinary Anatomy, Louisiana State University, Baton Rouge, Louisiana and Department of Neurobiology, Scripps Research Institute, LaJolla, CA.

Schwann cells have broad contributions to development, regeneration, and tumor formation. In many situations, activity involves the coordinated production and secretion of molecules. Our goals were 1) to select *in vitro* conditions that mimicked Schwann cell reactions to development, nerve injury and tumor formation *in vivo* and 2) to establish methods to detect, quantify and localize these molecules *in vitro*. The neural adhesion molecule, N-CAM, is a membrane glycoprotein of M_r 180,000, 140,000 and 120,000; it is responsible for homophilic neural cell binding. The substrate adhesion molecule, cytotacin, is an extracellular glycoprotein of M, 200,000 and 220,000; its distribution in the brain is highly specialized. The specific source of these molecules under the conditions we have described has not been established. Our rationale was to verify Schwann cells as a source of these molecules. *In vitro* conditions involved cultures of Schwann Cells isolated from neonatal nerves, nerves recovering from transections, and a Schwannoma cell line. Sciatic nerves from neonatal (2-4) old Sprague Dawley rats were sacrificed 10 days post surgery for aseptic removal of injured nerves. All tissues were minced and digested in 0.25% trypsin containing 0.03% collagenase. Washed cells were cultured in a petri dish with Minimum Essential Medium 2-4 hrs for attachment of fibroblast. Suspension cells were then transferred to a flask and re-incubated for 12 hrs. Supernates were analyzed via SDS polyacrylamide gels (PAGE), immunoprecipitation and immunoblots. Schwann cells were capable of producing both molecules. Variabilities in quantity, and modulations of N-CAM form, will be described. Although this assay was used to test the secretion of adhesion molecules, its use is not restricted to these proteins. Supported by NIH grant #R29-NS-25102-05 and in part by a grant from Sentron Medical Inc., Cincinnati, Ohio.

545.10

TRANSFORMING GROWTH FACTOR ALPHA IS PRESENT IN DEVELOPING BRAIN AND A POTENT ASTROCYTE MITOGEN. Kenneth R. Huff* and Qi-Xiao Yuan. Harbor/UCLA Medical Center, Dept of Pediatrics. Torrance, California.

Transforming growth factor alpha (TGFa) regulates the growth and replication of nontransformed cells. Epidermal Growth Factor (EGF)-like proteins may have critical morphogenic functions in the developing brain as mutant homeotic genes encoding for homologous peptides produce lethal anomalies. In non brain tissues TGFa binds competitively to the EGF receptor (EGFR) and no other receptor has been found for it. The majority of the EGFRs in the brain are on astrocytes. We have measured TGFa in developing rat brain and investigated a potential role of TGFa as a ligand for the EGFR on purified developing astrocytes.

A specific radioimmunoassay was developed and the antisera displayed no immunoblot reactivity with EGF. The antisera was not competed by over 100 times the concentration of rat TGFa by several species of EGF, NGF, or human TGFa. This RIA measured whole brain levels of TGFa to be 160 pg, 119 pg, and 55 pg per gram at ages 7 days, 15 days, and 120 days (adult) respectively. Thyroid hormone treatment of the animals for 6 days starting at birth reduces the level at day 7 from 160 pg to 64 pg. Astrocytes grown in primary culture were studied for TGFa mitogenicity and binding activity with 125-Iodine labelled TGFa. Increased thymidine incorporation occurs at 5-10 nM after 16 hours. Maximum cellular binding is reached by 60 minutes. A scatchard analysis of saturability data indicates a dissociation constant of 330 pM and 6200 receptors per cell assuming the high affinity receptor class. These calculations correspond well with similar affinity and receptor number data obtained respectively using the A431 cell line with an amplified EGFR and using astrocytes with EGF as the ligand.

We conclude that TGFa is present and may be developmentally regulated in the brain. This growth factor binds to the EGF receptor with high affinity in purified astrocytes. These findings may have implications for astrocyte mediated structural development of the brain or glial proliferation disorders such as gliosis or glioma.

545.11

ESTRADIOL REGULATES S-100ß mRNA EXPRESSION IN CULTURED RAT ASTROCYTES. D.A. Hinkle, D.C. Hilt, P.J. Yarowsky, S.R. Max*, and P.M. Wise. Department of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201.

Estradiol is implicated in reproductive senescence of the hypothalamus, a brain region which becomes markedly gliotic with increasing age and in response to treatment with estrogens (Biol. Repro. 42:21-8, 1990). S-100b, a glia-derived protein which moderates the growth and development of neurons and glial cells, is reported to be elevated in reactive astrocytes in gliotic brains. The aim of this study was to determine if and to what extent estradiol regulates S-100ß mRNA expression in cultured rat astrocytes. Primary astrocytes were cultured from various brain regions of 1 day old rats, grown to confluence in serum-containing medium, then passaged and treated in steroid-free medium with 0, 50 nM, 100 nM, or 1 µM estradiol for 3 weeks. Astrocyte RNA was subsequently harvested, hybridized to radiolabelled antisense riboprobes to S-100ß and cyclophilin mRNAs, and subjected to RNase protection. We report that, while cyclophilin mRNA levels remain constant regardless of treatment, estradiol increases S-100ß mRNA expression in cortical astrocytes. We are currently analyzing the effect of estradiol on hippocampal, cerebellar, and hypothalamic astrocyte S-100ß mRNA. We speculate that estradiol regulation of S-100ß mRNA expression may be involved in the mechanism of estrogen- and age-related gliosis in reproductive senescence.

545.12

IN VITRO DIFFERENTIATION OF THE BASAL FOREBRAIN CHOLINERGIC NEURONS FROM THE TRISOMY 16 MOUSE. P. Corsi*, S.M. Cozza, M. Troia, T. Lettini, V. Del fino Pesce. Ist. di Fisiologia Umana, Fac. di Medicina, Univ. di Bari, Italy, 70124; Consorzio di Ricerca DIGAMMA, Bari, Italy, 70100.

The trisomy 16 mouse (Ts16), which shares genetic and phenotypic homologies with Down syndrome, exhibits impaired development of the basal forebrain (BF) cholinergic system with significant reductions of the choline acetyltransferase activity. In the present study the BF obtained from Ts16 fetuses and their euploid littermates (EUPL.) at 15 days of gestation, were enzimatically dissociated and cultured either in defined medium with trophic factors or in the presence of conditioned medium from astrocites cultures in order to rescue the developmental impairments of the cholinergic neurons (ChN) from the Ts16 brains. Another group of cultures have been obtained by plating the ChN from Ts16 BF on normal glial cultures and the "in vitro" differentiation of these neurons have been compared to that of the ChN from EUPL. BF plated on Ts16 glial cultures. In these experimental conditions we considered the following parameters: neuronal survival, neuronal morphology, number of ChN, length of processes and activation of a set of genes, c-fos and c-jun, mediating neuronal differentiation. Our preliminary observations suggest that the differentiation of the Ts16 ChN "in vitro" depends on the degree of maturation of the glial cells and on cell to cell contact interactions between neurons and glia.

545.13

ASTROGLIA REACTING TO EXPERIMENTAL SPINAL CORD INJURY EXPRESS INSULIN-LIKE GROWTH FACTOR I (IGF-I) AND VIMENTIN. D.-L. Yao, N. West, C. Bondy, M. Brenner, G.H. Collins*, and H.deF. Webster. LENP, DEB, LMB, NIH, Bethesda, MD 20892 & Dept. Path., SUNY Hlth. Sci. Cntr., Syracuse, NY 13210.

To study astrocytic responses associated with spinal cord regeneration in adult rats, the procedure of Collins and West (Brain Res. Bull., 22:71-79, 1989) was used to produce cryogenic posterior column lesions. After 3d, lesions contained cells which expressed IGF-I mRNA; the cells were identified as immature astroglia by their vimentin immunoreactivity, their failure to stain with anti-GFAP and their electron microscopic appearance. At 7d, IGF-I mRNA levels in these cells were significantly higher and by 14d had decreased to 50% of the 7d levels. Hypertrophic astroglia around the lesions did not contain detectable IGF-I mRNA at these early intervals; instead they expressed high levels of GFAP mRNA and peptide. EM observations 20-60d after similar cryogenic lesions have shown that regenerating neurites and myelin sheaths are present (West and Collins, J. Neuropath. Exp. Neurol. 48:94-108, 1989). Studies of IGF-I expression at these intervals are in progress.

545.14

NEUTRALIZING ANTISERUM TO ACTIVITY-DEPENDENT NEUROTROPHIC FACTOR PRODUCES NEURONAL CELL DEATH IN MAMMALIAN CNS CULTURES

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Activity-dependent neurotrophic factor (ADNF) is a glia-derived protein survival factor that is released by vasoactive intestinal peptide. ADNF has been purified to homogeneity and has been shown to increase the survival of neurons derived from spinal cord, cerebral cortex and hippocampus at concentrations <1 pM. Physical properties (16 kD, pI 8.1) and partial sequence data indicated ADNF is novel. Neutralizing antiserum was obtained by serial injections of ADNF into mice. In cerebral cortical or spinal cord cultures, ADNF antiserum (1:10,000) decreased neuronal counts (50-65% of control) after five days treatment. Co-administration of purified ADNF prevented neuronal cell death associated with the antiserum. Control antiserum had no effect. These data support the conclusion that ADNF is important to the survival of a subset of cortical and spinal cord neurons.

545.15

VASOPRESSIN INDUCTION OF THE IMMEDIATE EARLY GENE, *zif/268*, IN CULTURED HIPPOCAMPAL GLIAL CELLS.

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The neural peptide, vasopressin (AVP), has been associated with growth responses in both neural (Brinton & Gruener, 1987; Brinton, 1990) and nonneural cell types (Rosengurt, 1979). The immediate early gene (IEG), zif/268, is one of several genes induced in response to growth factors (Bartel et al., 1989). Because of AVP effects on nerve cell growth, we investigated the influence of AVP on induction of zif/268 expression in cultures of hippocampal neurons and glial cells. Hippocampal cells from E18 rat pups were cultured onto polylysine coated chamber slides in serum containing medium. Following 3 days in culture, cells were exposed to 10, 100, 500 or 1000 nM AVP or other peptides for 15 or 30 min. Paraformaldehyde fixed cells were treated with TEA and $C_4H_6O_3$ prior to hybridization with a ^{35}S-labeled zif/268 cRNA probe. Slides were dipped in NTB-2 emulsion, exposed at 4°C for 3-4 wks and analyzed using a BioQuant image analysis system. Results of those studies showed AVP-induction of zif/268 in glial cells that was dose and time dependent. Greatest grain density was observed in cells treated with 10 or 100 nM AVP whereas grain densities in glial cells treated with 500 or 1000 nM AVP were not as dense. Maximal induction of zif/268 was observed in glial cells exposed to AVP for 15 min. AVP-induction of zif/268 was IEG and cell specific, in that cfos was not induced by AVP and AVP-induction of zif/268 was specific to glial cells and not observed in neurons. Experiments to determine receptor specificity indicated that AVP-induction of zif/268 is mediated via a V1 receptor since the specific V1 agonist Phe^2,Orn^{8}Vasotocin at a concentration of 10 nM induced zif/268 expression. *Supported by NIH grants MH46036 and SO3 RRO3011 to R.D.B. and NS01337 to S.S.S.*

545.16

VIP RECEPTOR DISTRIBUTION IS RELATED TO ONTOGENETIC EVENTS IN THE DEVELOPING CORTEX, HIPPOCAMPUS AND CEREBELLUM.

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VIP has trophic effects in the CNS through its action as an astroglial mitogen and by stimulating astrocytic secretion of neurotrophic agents (Brenneman *et al. PNAS*: 83:1159, 1986: *J. Neurosci. Res.* 25:386, 1990). *In vitro* autoradiography with ^{125}I-VIP was performed on E14, E16, E19, DO, D7, D14, D21 and adult CNS tissue to determine the patterns of VIP receptor distribution in the cortex, hippocampus and cerebellum throughout development. VIP binding was high from E14, and was localized to the intermediate layers in cortex and hippocampus. In the intermediate layer of the cortex, the presumptive astrocytes and pioneer neurons of the cortical plate are developing. During this period neurogenesis takes place in the germinative zones and VIP binding was moderate to low in these layers. By E19 the highest VIP binding occurred in the germinal layers which are the sites of gliogenesis at this time. Binding remained very high in these layers until the end of gliogenesis. From D0 to D14, a period characterized by glial and neuronal migration, differentiation, axogenesis, dendrogenesis, synaptogenesis and the beginnings of myelination, a relatively uniform blanket of moderate VIP binding was seen throughout the brain. By D21 the adult pattern of VIP binding to discrete neuroanatomical sites began to emerge. The changing patterns of VIP receptor distribution occurring in relation to ontogenetic events, especially those related to gliogenesis and glial maturation, suggest that VIP plays an important role in the developing nervous system.

OTHER FACTORS AND TROPHIC AGENTS: INJURY

546.1

NEUROTROPHIN-3 mRNA EXPRESSION IS STIMULATED IN DEGENERATING PYRAMIDAL CELL LAYERS OF CA1 AND CA4 HIPPOCAMPAL FIELDS OF THE RAT.

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Unilateral intrahippocampal injection of the endogenous excitotoxin quinolinic acid (120 nmols) induces seizures together with a delayed ipsilateral neurodegeneration particularly of the pyramidal cell layer of CA1 and CA4 hippocampal fields. In situ hybridization histochemistry was used to analyze the spatio-temporal pattern of hybridization of brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and neurotrophin-3 (NT3) mRNAs after this treatment. As in other excitatory paradigms, a rapid and transient increase of BDNF and NGF mRNAs together with a delayed and also transient decrease in the NT3 mRNA level, were observed in the contralateral hippocampus. Analysis of mRNA levels in the ipsilateral hippocampus showed a different situation. BDNF and NGF mRNA expression was only stimulated in the areas not-directly in contact with the excitotoxin. Around twelve hours after the insult, and in parallel with the begining of the neurodegenerative process there is an increased expression of NT3 mRNA in the specifically degenerated CA1 and CA4 pyramidal cell layers.

The main findings from this work are: (i) the increased expression of NT3 mRNA in the adult brain, associated with neurodegeneration, (ii) the differential regulation for the different neurotrophic factor genes in response to seizures, (iii) the stimulatiom of BDNF and NGF mRNA expression not direct but transynaptically mediated by NMDA receptors.

546.2

BDNF AND *trk*B mRNA REGULATION FOLLOWING SELECTIVE DEAFFERENTATION OF ADULT RAT HIPPOCAMPAL NEURONS

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We have previously suggested that BDNF may participate in the synaptic remodelling responses observed in the adult hippocampus following the administration of systemic kainic acid (Dugich-Djordjevic et al., Neurosci., 47, 303-315, 1992). In order to further investigate the possible role of BDNF in neuronal plasticity in the adult, we used in situ hybridization histochemistry to examine the alterations in BDNF and *trk*B mRNA expression following selective deafferentation of the rat hippocampus. After selective destruction of CA3 pyramidal neurons by intrahippocampal kainic acid (IHKA) injections, BDNF mRNA was no longer detectable in the ipsilateral CA3 pyramidal population but was increased in the ipsilateral DG and medial CA1 region at 3 d post lesion. At 10 d following lesion, BDNF mRNA was elevated only in the ipsilateral DG and control levels were observed at 21 d. *Trk*B mRNA expression was elevated in apparent non-neuronal populations of the septal aspect of the ipsilateral dentate molecular layer at 10-21 d following IHKA. Unilateral destruction of dentate granule cells by colchicine resulted in a loss of expression in the ipsilateral DG and a pronounced increase in BDNF mRNA in the ipsilateral CA3 pyramidal layer and hilar region on day 3, with slightly elevated expression in the contralateral DG and CA3 at 10 d post lesion. *Trk*B mRNA expression was increased along the entire septotemporal portion of the ipsilateral dentate molecular layer from 10-21 d post lesion, with elevated expression in areas adjacent to the CA1, CA3, and DG regions at 10 d following lesion.

In summary, the expression of BDNF mRNA was increased at neuronal sites of axonal projections of deafferentated areas, while altered expression of *trk*B was localized to apparent non-neuronal populations adjacent to denervated target regions. This finding suggests that BDNF and *trk*B may participate in neuronal plasticity in the adult rat brain following injury. Alternatively, the more delayed increases in *trk*B mRNA expression suggest a coordination with neurodegenerative processes.

546.3

EXPRESSION OF NEUROTROPHIN RECEPTOR mRNAS IN AXOTOMIZED FACIAL AND RUBROSPINAL NEURONS.

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Neuronal atrophy and/or cell death after axonal injury is a major component of the failure of CNS neurons to regenerate. This problem is compounded by the lack of information as to the trophic dependencies of CNS neurons. We have examined the expression of the *trk* family of high affinity neurotrophin receptors, on facial motoneurons or rubrospinal neurons after axotomy, using *in situ* hybridization with oligonucleotide probes. **Facial** motoneurons contralateral to axotomy express significant levels of mRNA for *trk*B and *trk*C, but not *trk*A nor p75^{NGFR}. Seven days after axotomy *trk*B expression increased two-fold while *trk*C expression decreased. As reported by others, the expression of p75^{NGFR} was dramatically enhanced following axotomy. **Rubrospinal** neurons express significant levels of *trk*B mRNA, and to a lesser extent *trk*C, but as for facial motoneurons no detectable levels of *trk*A nor p75^{NGFR}. Seven days after transection of the rubrospinal tract, at cervical level C3, there were no significant changes in *trk* mRNA expression. Nevertheless, at this time point p75^{NGFR} expression was significantly increased - this message was not detectable in the contralateral red nucleus. These data suggest that after axotomy, facial motoneurons and rubrospinal neurons may become responsive to BDNF and NT3. (Supported by MRC)

546.4

IMMUNOREACTIVITY TO MOTONEURONOTROPHIC FACTOR 1 (MNTF1) IN TONGUES OF ADULT RATS FOLLOWING DENERVATION AND REINNERVATION.

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Monoclonal antibody raised against MNTF1, a 35 kD muscle-derived motoneuronotrophic factor shown to promote survival of motoneurons in vitro and in vivo after axotomy, was used to study its cellular localization in the tongue following denervation and reinnervation. The right hypoglossal nerve of female Sprague-Dawley rats 2-3 months old was transected under anesthesia. Rats were perfused with 4% paraformaldehyde in PBS at 4 d, 1,2,3,5 wk, and 2,5 month postaxotomy (PO). Tongues were embedded in Paraplast and cut transversely for immunocytochemical procedures. Positive immunoreactivity was observed in the sarcoplasm of skeletal muscle fibers and in the axoplasm of many large, myelinated nerve fibers on both intact and denervated sides 4 d PO. At 1 wk PO, the intensity of immunostaining and number of immunopositive muscle fibers were reduced on the denervated side, and immunopositive nerve fibers were virtually absent. By 2 wk PO, most muscle fibers were stained immunonegative on the denervated side. By 5 wk PO, the immunostaining of muscle fibers on the denervated side had returned to levels close to that of the contralateral intact side, at a time when reinnervation of muscles had occurred and the contractile force of muscles had reached plateau. However, immunopositive nerve fibers reappeared later. These results suggest that MNTF1 is present in the lingual muscles of adult rats, probably taken up into axoplasm from motor end-plate regions, and production of MNTF1 is neurally regulated.

546.5

WITHDRAWN

546.6

SPINAL INJURY ALTERS CIRCULATING NEUROTROPHIC ACTIVITY. F. Roisen*, C.L. Lu, J.R. Johnson, R.D. Linden, G. Niznik, L. Ray, C.B. Shields, L-J. Wang, G. Yorke, and Y.P. Zhang. Departments of Anatomical Sciences & Neurobiology, Orthopedic Surgery, and Surgery (Division of Neurosurgery), University of Louisville School of Medicine, Louisville, KY 40292.

It is likely that trophic agents play a key role in regulating neuroplasticity and regeneration. To determine if circulating neurotrophic activity is altered by spinal cord injury, we used T_2 weight drop to produce known levels of injury and *in vitro* neuronal models to determine the resultant trophic response. Anesthetized adult Wistar rats (250 g) underwent surgery to produce a mild or medium spinal lesion. Blood was collected every 2 days, separated and stored in aliquots at -70°C. Serum taken at day 0 prior to trauma served as a control. Control or trauma-derived sera were incorporated into the medium at 5% and applied to cultures of 6 day embryonic chick spinal cord (AHC) or 9 day sensory ganglia (DRG) which were maintained for 4 days. The degree of resultant neuronal development was evaluated microscopically on coded cultures. Cell viability was determined by measuring mitochondrial dehydrogenases via MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide, Sigma cell growth determination kit). Sera obtained from normal rats established that minimal intra- and inter-animal variation occurred during the 28 day testing period. Our preliminary studies demonstrate that sera from lesioned animals contain factor(s) which increased the viability of the heterogeneous cell population. The degree of enhancement was dependent on the severity and time after injury. Maximum DRG and AHC viability was obtained with sera collected around day 15, both returned to pre-injury levels by day 28. The viability level was related to extent of injury; sera obtained from animals with mild lesions were more supportive than those obtained from animals given a medium lesion. The effects of spinal-lesioned-sera on neuronal survival are under evaluation with immunohistochemistry and quantitative microscopy. At present the nature and role of the factor(s) are unknown. Supported by Alliant Community Trust Fund, Louisville, KY.

546.7

TROPHIC INTERACTIONS OF AFFERENT NERVES AND EPIDERMIS: NORMALIZATION OF CUTANEOUS ABNORMALITIES FOLLOWING NEURAL LESIONS IN MONODELPHIS DOMESTICUS. S. A. Bogush and B. L. Munger.* Dept. Neurosci. Anat., Penn. State Univ., Hershey, PA 17033 and Dept. Anat., Univ. of Tasmania, Hobart, Tasmania 7001.

The present study evaluates the long-term effects of neural lesions on the differentiation of the skin in post-natal opossum pups. Lesions of dorsal root ganglia &/or spinal cord produce acute effects in the skin including hyperplasia of the epidermis, hyperinnervation of the dermis and epidermis, and suppression of hair formation in the first week following neural lesions. By the second week precocious hairs (more mature than expected) are present often with significantly abnormal shape and direction of the shaft relative to the skin surface. By three weeks the skin appears grossly normal and no abnormalities are visible to the eye. However on studying serial sections the residual lesions of neural tube, DRG, and neural arches confirm the prior neural lesion. The only microscopic abnormality in the skin is the presence of isolated abnormal hairs in terms of shape and direction. These findings indicate the extent of trophic interaction of afferent nerves and the skin, and that afferent nerves control the differentiation of their targets in skin as well as muscle (Zelena, 1957). Supported in part by USPHS Research Grant NS 19406

546.8

COMPARATIVE EFFECTS OF BASIC FGF (bFGF) AND NGF ON HIPPOCAMPAL CHOLINERGIC FUNCTION IN FIMBRIECTOMIZED RATS. D. M. Araujo*, P. A. Lapchak and F. Hefti, Andrus Ger. Ctr., USC, Los Angeles, CA, 90089-0191.

Although bFGF, like NGF, appears to be trophic for cholinergic neurons of the basal forebrain, its effects on cholinergic function have not been demonstrated. In rats with unilateral partial fimbrial transections, chronic administration (icv, qod for 21 days) of bFGF (10 ng) or NGF (1 μg) resulted in increases of ChAT activity in the lesioned hippocampi of similar magnitude (39 and 46%, respectively), compared to that of a control group of rats treated with cytochrome c (cc). In the control cc-treated group, ACh content, basal and evoked ACh release, were significantly reduced by the lesion by 56, 40 and 53%, respectively. However, whereas only modest increases in these measures (10-18%) in the bFGF-treated group compared to the control cc-treated group were observed, marked enhancements in the NGF-treated rats were apparent. Thus, in the NGF-treated group, ACh content, basal and evoked ACh release in the lesioned hippocampi were augmented by 67, 46, and 51%, respectively. In addition, no synergism between bFGF and NGF was observed, since in rats receiving combined administration of bFGF/NGF, the increases in hippocampal cholinergic parameters were not markedly different from those measured in rats treated with NGF alone. Moreover, the binding of [^{3}H]vesamicol, a presynaptic cholinergic marker, was similar in the lesioned hippocampi of the NGF- and the bFGF/NGF-treated groups, and modestly enhanced compared to either the cc- or the bFGF-treated group. In conclusion, the limited effectiveness of bFGF in counteracting lesion-induced reductions of cholinergic function, indicates that it may not be the treatment of choice in reversing the cholinergic deficit associated with certain neurodegenerative diseases.

546.9

CHRONIC NEUROTROPHIN ADMINISTRATION TO FIMBRIECTOMIZED RATS: EFFECTS ON PRESYNAPTIC HIPPOCAMPAL CHOLINERGIC FUNCTION AND WEIGHT GAIN. P.A. Lapchak*, D.M. Araujo and F. Hefti, USC, Andrus Ger. Ctr., Los Angeles, CA, 90089-0191

Recent pharmacological studies have shown that BDNF increases ChAT activity in cultured septal cells suggesting that BDNF may be effective in stimulating the expression of the cholinergic phenotype in the adult brain. The present study determined the effects of chronic administration (icv, 1.4 μg qod for 21 days) of recombinant human (rh) NGF or rhBDNF on hippocampal cholinergic function measured in vitro in adult rats with partial fimbrial transections. Partial fimbrial transections reduced hippocampal high affinity choline uptake (by 54%), ChAT activity (by 41%), and [^{3}H]ACh synthesis (by 63%). Chronic rhBDNF treatment failed to enhance the level of these cholinergic parameters. In contrast, chronic treatment with rhNGF increased high affinity choline uptake (by 112%) compared to lesioned control values. rhNGF treatment also increased hippocampal ChAT activity by 54%. Moreover, rhNGF treatment increased [^{3}H]ACh synthesis by hippocampal synaptosomes by 65% compared to lesioned control values. Thus, chronic rhNGF treatment, but not rhBDNF treatment effectively enhances markers of presynaptic hippocampal cholinergic function following partial fimbrial transections. Lastly, chronic treatment with either rhNGF or rhBDNF produced significant reductions in body weight (23-26 grams) over the 21 day treatment schedule. Thus, although rhBDNF does not affect central cholinergic function it does seem to interact with CNS neurons to regulate weight gain. The results of the present study suggests that NGF will be more effective than BDNF in counteracting the cholinergic deficit associated with the cognitive decline observed in Alzheimer's disease.

546.10

Brain Derived Neurotrophic Factor (BDNF) Increases the Survival of Basal Forebrain Cholinergic Neurons Following a Fimbria-Fornix Transection. J.K. Morse,* R.F. Alderson, Y.You, N.Cai, C.A. Altar, S.J. Wiegand, R.M. Lindsay, Regeneron Pharmaceuticals, 777 Old Saw Mill River Rd., Tarrytown, NY 10591.

We have demonstrated an increase in septal cholinergic neuron survival and phenotypic differentiation in response to BDNF *in vitro* (Alderson, et. al., 1990). We show here the efficacy of BDNF *in vivo* in rescuing this neuronal population from the cell death associated with transection of the fimbria-fornix pathway.

The fimbria-fornix was transected unilaterally by knife cut in adult (175-225 gm) Sprague-Dawley female rats. A cannula was implanted for delivery of BDNF into the caudal pole of the septum. BDNF was delivered continuously at rate of 12 μg/day for two weeks, via an osmotic pump.The ability of BDNF to increase cell survival was evaluated on the basis of cell counts in 30 μm coronal sections stained for choline acetyltransferase (ChAT) or low affinity NGF receptor (LNGFR). Cells were counted in sections taken at 180 μm intervals through the rostral-caudal extent of the medial septal nucleus. In control animals a 64% decrease in the number of ChAT- and a 45% decrease in LNGFR- positive cells was observed on the side of the lesion compared to the intact side. In contrast only 37% of ChAT- and 21% of LNGFR- positive neurons were lost when BDNF was delivered into the septum.

These *in vivo* results substantiate our previous *in vitro* findings and suggest a role for BDNF in rescuing cholinergic neurons following deafferentation injury or disease.

546.11

EFFECT OF INTRAVENTRICULAR BDNF ADMINISTRATION ON HIPPOCAMPAL BDNF AND *trk*B mRNA EXPRESSION IN ADULT RATS WITH PARTIAL SEPTO-HIPPOCAMPAL LESIONS. J.L. Venero*, B. Knüsel, K.D. Beck and F. Hefti. Andrus Gerontology Center, University of Southern California. Los Angeles, CA 90089-0191.

In situ hybridization was used to evaluate changes of mRNA expression of BDNF and its *trk*B receptor after daily intraventricular administration of BDNF and after unilateral partial fimbrial transections. 24 and 48 h after the lesion, expression of BDNF was decreased in the pyramidal cell layer of Ammon's horn, this effect being most prominent in CA2, CA3 and CA4. In contrast, expression of BDNF mRNA in the granule cell layer of the dentate gyrus was enhanced at these time points. BDNF administration not only completely abolished the lesion-induced increase of BDNF mRNA in the dentate gyrus but reduced levels on the lesioned side below control values in this area. No effect of BDNF administration was seen on the lesion-induced decrease in the different fields of Ammon's horn. Neither fimbrial transections nor BDNF administration affected *trk*B mRNA levels after 24 or 48 h. Emulsion in situ hybridization analysis is in progress to assess whether, at the level of individual cells, BDNF and *trk*B share common patterns of mRNA expression. Our data suggest that injury of septal inputs result in sub-region specific changes of hippocampal BDNF mRNA expression, whereas *trk*B expression does not seem to be affected. The experiments with exogenously administered BDNF suggest that BDNF can specifically alter its own expression in the dentate gyrus.

546.12

NEUROTROPHIN-3 INCREASES THE REGENERATIVE SPROUTING OF THE LESIONED RAT CORTICOSPINAL TRACT. M.E. Schwab*(1), R. Kolbeck(2), Y.-A. Barde(2) and L. Schnell(1). (1) Brain Research Institute, University of Zurich, 8029 Zurich, Switzerland and (2) Max-Planck-Institute for Psychiatry, Dept. of Neurobiochemistry, 8033 Planegg-Martinsried, Germany.

Sprouting occurs as a spontaneous reaction to axotomy in many CNS and PNS tracts. In case of the adult rat corticospinal tract (CST) spontaneous sprouting is very limited. Transplants of E14 rat spinal cord tissue greatly increased this sprouting in a zone of 1 - 4 mm proximal to a thoracic CST lesion in young adult rats. The frequently occurring retraction of lesioned axons was also prevented. Very similar results were obtained following a single injection of the neurotrophic factor NT-3 (0.2 - 0.5 µg into the lesioned spinal cord). No effect was seen after injection of BDNF. The maximal distance of regenerative growth was restricted to 0.5 - 1.5 mm in all these cases. - Applications of mABs IN-1 or IN-2 directed against the neurite growth inhibitory constituents NI-35/250 of CNS myelin resulted in 1.) a further increase in local sprouting, and 2.) a subsequent elongation of CST fibers over distances of 2.5 - 20 mm. Regenerating fibers were often found in anatomically aberrant positions, largely related to their way across the lesion site. Branching and terminal arborizations could be seen in the gray matter. Our results confirm that regenerative sprouting and elongation are two distinct processes; CST sprouting can be enhanced specifically by NT-3, wherease elongation strictly depends on the neutralization of the powerful myelin-associated neurite growth inhibitory proteins.

546.13

TGF-β1 REGULATION OF HIPPOCAMPAL mRNAs FOLLOWING ENTORHINAL CORTEX LESION. T.E. Morgan, N.J. Laping, N.R. Nichols, C.S. Young-Chan, B.W. Bernstein*, and C.E. Finch. Andrus Gerontology Center, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191.

This study examines the role of transforming growth factor-β1 (TGF-β1) in regulating glial fibrillary acidic protein (GFAP) and sulfated glycoprotein-2 (SGP-2) mRNAs during lesion-induced synaptic reorganization. GFAP, a marker for reactive astrocytes, and SGP-2, with a putative role in membrane transport, increase in the deafferented hippocampus after unilateral entorhinal cortex lesion (ECL). TGF-β1 is a multifunctional peptide that plays an important role in peripheral wound healing. Results of RNA titration indicate that TGF-β1 mRNA increases 3-fold in the deafferented hippocampus 4 days after ECL. *In situ* hybridization indicates that TGF-β1 mRNA is localized to brain regions that show elevated microglial reactivity (OX-42 immunoreactivity) after ECL. To assess TGF-β1's contribution to lesion-induced synaptic reorganization, a cannula was implanted into the lateral ventricle of adult male rats on the same day as ECL. TGF-β1 (100 ng) or vehicle was infused 24 hours before sacrifice; rats were sacrificed 2 or 4 days after ECL. Two days after ECL, the GFAP and SGP-2 levels were unaffected by TGF-β1 infusion. However, preliminary results show the expected elevation in GFAP and SGP-2 mRNAs at 4 days after ECL was prevented by TGF-β1 infusion. Thus, TGF-β1 may regulate astrocytic reactivity and other events involving membrane transport during synaptic reorganization. Supported by NRSA AG-05589 (TEM), NRSA AG-05528 (NJL) and AG-07909 (CEF).

546.14

MDF INCREASES TYROSINE HYDROXYLASE ACTIVITY AND CATECHOL LEVELS IN INJURED BUT NOT INTACT DOPAMINERGIC NEURONS. B.K. Jin*, J.S. Schneider, X.Y. Du and L. Iacovitti. Dept. of Neurology, Hahnemann University, Philadelphia, Pa. 19102.

We have previously demonstrated that a 10,000-fold purified muscle-derived factor termed MDF will increase tyrosine hydroxylase (TH) activity and catechol levels in lesion-spared dopamine neurons following a 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal system. In the present study, we examined whether prior damage to dopaminergic neurons that resulted from 6-OHDA treatment was essential for MDF's effect. To test this hypothesis, either partially-purified MDF or PBS+BSA (control) was infused unilaterally into the intact rat striatum. In order to minimize mechanical damage and maintain the intact state, we used a 28 gauge cannula connected to a mini-osmotic pump (ALZET 2002: infusion rate of 0.5 µl/hr). After 12-13 days of infusion, rats were sacrificed and postmortem tissue on both the ipsilateral and contralateral sides was rapidly dissected for TH immunocytochemistry/in situ hybridization and biochemical measurement of TH enzyme activity, dopamine (DA) and its metabolite, dihydroxyphenylacetic acid (DOPAC). Evidence of a mechanical lesion was not observed anatomically or biochemically in control (PBS-treated) rats. Thus, there was no disruption of TH immunoreactive fibers except in the area immediately adjacent to the cannula tip, and no differences in TH enzyme activity, DOPA or DOPAC levels when compared to untreated or contralateral striata. Similar to PBS, but in marked contrast with the changes previously observed in 6-OHDA-lesioned rats, MDF infusion failed to increase biochemical indices of DA function in the intact striatum. Thus, TH activity in MDF-treated striata (371±59) remained at PBS-treated (366±45) levels. Similarly, there was no change in postmortem levels of DA (97%) and DOPAC (106%) (expressed as a % of contralateral striata). In situ hybridization analysis of TH mRNA also showed no detectable change in TH mRNA/cell in the parent cell bodies of the substantia nigra. These findings indicate that MDF treatment has no effect on the intact dopaminergic neuron. Taken together with our previous studies on 6-OHDA lesioned rats, these data suggest that prior damage is essential for MDF's effect on adult nigral dopaminergic neurons in vivo.

546.15

MUSCLE-DERIVED MOTONEURONOTROPIC FACTORS PROMOTE SURVIVAL OF AXOTOMIZED MOTONEURONS OF THE FACIAL NERVE. **W.H.A. Yu*[1], R.M.W. Chau[2] and F. Ren[2]. [1]Dept. of Cell Biol. and Anat. Sci., City Univ. of New York Med. Sch., New York, NY 10031 and [2]Dept. of Anat., Univ. of Hong Kong, Hong Kong.**

Two motoneuronotrophic factors (MNTF1 & MNTF2), isolated from the peroneal muscle of 3-week old rats and shown to promote the survival of anterior horn motoneurons in vitro and in vivo after axotomy, were tested for their efficacy on axotomized motoneurons of the cranial region. The facial nerve of 10-day old rats was transected unilaterally distal to its exit from the stylomastoid foramen under hypothermia. MNTF1 and MNTF2, separated by Phast gel electrophoresis and migrated at 35 and 22 kD, respectively, were cut out from the Phastgel, minced into small pieces and placed between the two nerve stumps. For controls, either blank or Phastgel devoid of neuronotrophic activity was used. At 1 and 2 wk postaxotomy, rats were perfused with 4% paraformaldehyde in PBS, and brain stems were embedded in Paraplast. Neuronal cell numbers in the motor nuclei of the facial nerve were counted on serial transverse sections stained with cresyl violet. The total number of motoneurons on the side of axotomy was divided by the respective contralateral number to determine the percentage survival. Results indicated that MNTF1 and MNTF2 individually reduced the severity of chromatolysis and increased the survival of axotomized facial motoneurons. However, mean percentage survival with each factor was less than that observed in motoneurons of the sciatic nerve, and MNTF1 and MNTF2 in combination did not show synergistic action. It is concluded that responsiveness to neuronotrophic activity of MNTF1 and MNTF2, isolated from hindlimb muscles of young rats, extends not only to somatic motoneurons of the spinal cord, but to cranial motoneurons as well.

546.16

SYNERGETIC EFFECT OF MOTONEURONOTROPHIC FACTORS (MNTF) 1 AND 2 ON SURVIVAL OF AXOTOMIZED MOTONEURONS OF SCIATIC NERVE. R.M.W. Chau, W.H.A. Yu, L.S. Jen* and F. Ren. Dept. of Anatomy, Univ. of Hong Kong, Hong Kong; Dept. of Cell Biology & Anatomical Sciences, CUNY Medical School, New York, USA and Dept. of Anatomy, Charing Cross & Westminister Medical School, London, UK.

MNTF1 (35kD) & MNTF2 (22kD) were isolated from the peroneal muscles of 3-week-old rats by the "protein band-fishing by cells" method. In vitro, both factors could support the survival of anterior horn motoneurons. In vivo survival of axotomized motoneurons of sciatic nerve of 10-day-old rats were studied. The factors in minced gel were placed near the site of the lesion for rescue experiments for 2 weeks. The results revealed that the percentages of axotomized motoneuron survival compared to those of the respective contralateral side were 44.6±7.5 for the control without factor, 76.5±10.8 for the rescue experiment with MNTF1, 71.3±8.7 for those with MNTF2, and 86.8±5.8 for those with both MNTF1 and MNTF2. These results indicated that MNTF1 and MNTF2 can individually promote the survival of axotomized motoneurons of the sciatic nerve, in vivo, both with statistical significance $P<0.05$, $n=7$; however, when both MNTF1 and 2 are used concurrently they can provide a very significant synergetic effect on the survival of axotomized motoneurons, in vivo, with $P<0.01$, $n=7$. These results are consistent with previous in vitro evidence.

546.17

Low molecular weight brain-derived proteins and neurotrophins can prevent motoneuron cell death induced by spinal deafferentation. Y. Qin-Wei*, R.W. Oppenheim, J.E. Johnson and D. Prevette. Dept. of Neurobiology and Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, N.C. 27157-1010

Our previous studies have shown that extracts prepared from embryonic (E16) chick brains can enhance the survival of developing spinal motoneurons during normal cell death or death induced by spinal deafferentation. The current experiments were designed to partially separate and characterize brain extract (BEX) survival activity, and to determine the effects of members of the NGF-like neurotrophin family on deafferentation-induced motoneuron cell death.

Thoracic spinal cord segments were removed from chick embryos (E2) creating a spinal gap and lumbar segments were assayed for motoneuron survival on E16. Daily treatment (E9-E15) with protein from a 25%-75% ammonium sulfate fraction of BEX prevented motoneuron cell death induced by spinal deafferentation. BEX activity was partially separated and characterized with tandem gel exclusion chromatography (Sephadex G-50 and BIO-GEL P-60), preparative isoelectric focusing electrophoresis and PAGE. Preliminary results suggest that activity is confined to low molecular weight proteins (<20 Kd) with a pI of 8 (high activity) or 5 (low activity). Interestingly, while all members of the neurotrophin family tested (NGF, BDNF and NT-3) promote motoneuron survival following spinal deafferentation, only BDNF appears to support survival during normal cell death.

546.18

ACTH/MSH PEPTIDES PROMOTE NEUROMUSCULAR EFFICIENCY FOLLOWING TRAUMA OF THE DEVELOPING NERVOUS SYSTEM. L.A. Zuccarelli* and F.L. Strand, Dept of Biology, Center for Neural Science, New York University, New York, NY 10003.

ACTH 4-10 and α-MSH positively affect the development and regeneration of peripheral nerve[1]. Here we investigate their effects on integration at the developing neuromuscular junction and on the contractile properties of muscle following denervation. 2 d old Sprague-Dawley rats are subjected to sciatic nerve crush and peptide or saline vehicle treatment (10μg/kg/48h/8d). At 18, 21 and 35 d of age, the extensor digitorum longus muscle is attached to a force transducer and the sciatic nerve exposed. A stimulating electrode, placed proximally to the crush site, delivers graded stimuli (0.1 V) to determine motor unit recruitment and maximal twitch contraction amplitude. Super-maximal stimuli elicit isometric twitches, tetani at fusion frequency and at 120 Hz, twitches after fatigue at 400Hz for 10s and 10Hz for 10 min.

Results at 18 d indicate that α-MSH prevents fatigue in that twitch amplitude and rate are not different from sham controls. At 21 d, peptide treatment significantly ($p<.05$, ANOVA) promotes the recovery of numerous contractile properties of both twitch and tetanus: amplitude, rate, post-tetanic twitch amplitude and post-tetanic twitch rate as compared to saline treatment. After 10 min of stimulation at 10 Hz, no differences exist among sham and peptide groups in 1/2 relaxation time. Preliminary data at 35 d indicate peptide efficacy on twitch rate and tetanic fusion frequency, tetanic rate and post-tetanic twitch amplitude, and post-tetanic twitch rate. These results continue to support the efficacious role of ACTH/MSH peptides in PNS regeneration during development. [1]Strand et al., Prog. in Neurobio. 33, 1989.

546.19

SEX STEROID AND CASTRATION EFFECTS ON THE REGENERATING EDL MUSCLE AFTER PERONEAL NERVE CRUSH. **J. Kume*, R. Baradarian, and F.L. Strand. New York University Depart. of Biology and Center for Neural Science. NY, NY 10003.** Sex steroids have been shown to affect peripheral nerve regeneration in a muscle that has been typically characterized as essentially non-androgen sensitive, the rat extensor digitorum longus muscle (EDL). (Kume, J. and Strand,F.L. Soc. Neuroscience, Nov. 1991). We investigated the influence of sex steroids on regeneration of the crushed peroneal nerve from the perspective of muscle reinnervation and motor functional recovery. Sprague-Dawley male and female rats were divided into 3 groups: sham-nerve crushed; nerve-crushed; and castrated, nerve crushed. Another group of castrated male rats was nerve-crushed, implanted with testosterone propionate (TP)(20mm, id.058",od.077"), and given ORG 2766, an ACTH 4-9 analog (40ug/kg/48 hrs). To assess motor functional recovery, changes in toespread and print length were measured from footprints. 1-5 digit distances, peroneal functional indices, and % toespread recovery were calculated. At 9 days post-crush, rats were anesthestized with a xylazine/ketamine combination (5mg/kg; 75mg/kg respectively) and EDLs were indirectly stimulated via the peroneal nerve. Nerve crushing, regardless of sex, results in significant ($p<.05$, ANOVA) decreases in twitch and tetanic peak responses, a lower fusion frequency, and an altered peak rate. PTP responses were similarly smaller in response peak and altered peak rate. Twitch/tetanic peak response ratios are significantly greater in castrated, nerve crushed rats but are restored in males to normal values with TP. It can be concluded that removal of sex steroids for either gender seriously hinders recovery from peroneal nerve crush. This deleterious effect is apparent when corresponding to motor functional recovery tests.

OTHER FACTORS AND TROPHIC AGENTS: CYTOKINES

547.1

TEMPORAL EXPRESSION OF BRAIN CYTOKINES INDUCED BY LPS. S. De, R.M. Klein, G. Wood and N.E.J. Berman*. Depts. of Anatomy and Cell Biology and Pathology and Oncology, University of Kansas Medical Center, Kansas City, KS 66160

Extensive tissue remodeling occurs in the brain during development and following injury. Many of the cellular events of tissue remodeling, including phagocytosis of cellular debris, are accomplished by brain macrophages or microglial cells. In culture, these cells produce cytokines in response to stimulation by bacterial lipopolysaccharides (LPS). To determine how these cells respond to LPS stimulation *in vivo*, we injected LPS intraperitoneally or directly into the brain of adult CD-1 mice and studied the mRNA levels for various cytokines (IL-1α, IL-1β and TNF-α) in the brain and liver using Northern blot analysis. Following intraperitoneal injection of 10 μg LPS, tumor necrosis factor alpha (TNF-α) message was increased in the liver at 1 and 2 hr, and decreased by 4 hr. No change was observed in the brain at these times. Following injection of 10 μg LPS into cerebral cortex, TNF-α message levels in the brain increased at 2 hr, decreased by 4 hr, but increased again significantly 4 days later, stayed high for up to 7 days, and declined to the basal level by day 8. TNF-α expression in the liver was stimulated at 2 hr, declined at 4 hr, and stayed low through day 8. Following LPS injection into the brain, changes in interleukin-1-alpha and beta (IL-1α and IL-1β) expression followed the same time course in brain and liver. Both were increased at 4 hr but declined by 1 day and stayed low until 8 days, showing no late second peak in expression levels. These results demonstrate a biphasic temporal response of TNF-α mRNA in brain following LPS stimulation. The differences in expression between liver and brain suggest tissue-specific temporal expression of certain cytokines. Supported by MH38399.

547.2

EXPRESSION OF INTERLEUKIN-6 AND INTERLEUKIN-6 RECEPTOR mRNA IN RAT HYPOTHALAMUS. R. A. Gadient, R. G. Bernasconi* and U. H. Otten. Dept. of Physiology, Univ. of Basel, CH-4051 Basel, Switzerland.

The cytokine interleukin-6 (IL-6) mediates a number of biological activi ties, including hepatic acute phase response, maturation of activated B-lymphocytes, and inflammation. In addition, IL-6 exerts specific effects in the central nervous system, such as activation of the hypothalamic-pituitary-adrenal (HPA)-axis, promotion of mesencephalic neuron survival, and modulation of neurotrophin production. Using reverse transcription followed by polymerase chain reaction we investigated the expression of IL-6 and its receptor mRNAs in rat brain during postnatal development. IL-6 and IL-6 receptor signals were compared to the constitutively-expressed ribosomal protein S12, to check RNA integrity and efficiency of reverse transcription. Of the various brain regions examined the hypothalamus showed highest levels of IL-6 and IL-6 receptor mRNA. Hypothalamic IL-6 and IL-6 receptor mRNAs were developmentally regulated. Both mRNAs gradually increased reaching maximal levels at postnatal day 70. At all developmental stages studied the IL-6 mRNA content was about 10-fold lower than that of its receptor mRNA. The results of our work are in close agreement with in situ histochemical studies and suggest that IL-6 is involved in brain function, including in particular the control of HPA-axis.

547.3

INTERFERON-γ (IFN-γ) INCREASES CHOLINE ACETYLTRANSFERASE IN CULTURED BASAL FOREBRAIN. G.M. Jonakait* and L. Ni. Dept. of Biol. Sci., Rutgers Univ., Newark, NJ 07102

In order to study the effects of immune cytokines on cholinergic neurons of the basal forebrain, we have grown dissociated cell cultures derived from fetal rats. We measured choline acetyltransferase (ChAT) activity as a marker for cholinergic neurons. Cells plated at 2 X 10^6 cells/ml showed a 12-fold increase in ChAT activity over cells plated at 1/4 that density. In subsequent studies, therefore, density was carefully maintained at 1.5-2 X 10^6 cells/ml. ChAT activity increased steadily over the first week in culture; then it slowly declined.

Recombinant murine IFN-γ (Schering-Plough) produced a dose-dependent increase in ChAT activity in one-week-old cultures taken from embryonic day 16 (E16). At 50 U/ml this activity peaked, showing a 10-fold increase over control. Striatal ChAT activity measured in sister cultures was unaffected by IFN-γ. The effect of IFN-γ was dependent upon fetal age. Cultures taken from E18 rats showed a significant but truncated (1.7-fold) increase in ChAT. E19 cultures did not respond to IFN-γ even at 500 U/ml.

The effect of IFN-γ is partially reversed in the presence of an antibody to NGF (5%; Mike Coughlin, McMaster Univ.), suggesting that NGF or another neurotrophin mediates at least some part of IFN's action.

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547.4

LEUKEMIA INHIBITORY FACTOR MEDIATES THE INTERLEUKIN-1 (IL-1) INDUCTION OF SUBSTANCE P IN SYMPATHETIC GANGLIA. A.M. Shadiack*, R.P. Hart, and G.M. Jonakait, Dept. Biol. Sci., Rutgers Univ., Newark NJ 07102

Immune cytokines act as growth and differentiation factors in the nervous system. Superior cervical ganglia (SCG), cultured either as explants or as dissociated cells, respond to IL-1 with increased levels of substance P (SP). Since IL-1 does not alter SP expression in pure neuronal cultures, a molecular intermediate derived from non-neuronal cells must exist. Therefore, we collected medium from IL-1-treated ganglia (IL-1CM). IL-1CM elevated SP in naive explanted ganglia even in the presence of an IL-1 receptor antagonist (Synergen). Importantly, IL-1CM raised SP in pure neuronal cultures. Preliminary characterization of the active factor in IL-1CM showed it to be a protein with a molecular weight >10Kd.

Since IL-1CM, leukemia-inhibitory factor (LIF) and ciliary neuronotrophic factor (CNTF) raises both SP and choline acetyltransferase activity in explanted ganglia, we tested LIF and CNTF as possible intermediates. Immunoprecipitation of IL-1CM with an antibody against CNTF (Regeneron) did not affect IL-1CM activity. However, immunoprecipitation with an antibody specific for LIF (Dr. P.H. Patterson, Cal. Tech.) eliminated the SP-inducing activity of IL-1CM on pure neurons. These data suggest that the elevation of SP in response to IL-1 may be secondary to an induction of LIF in sympathetic ganglia.

Sponsored by ONR and NIMH.

547.5

INTERLEUKIN-1 RECEPTOR mRNA IS INDUCED IN CULTURED RAT SYMPATHETIC GANGLIA. R.P. Hart*, C.-L. Liu, A.M. Shadiack, C.D. Carlson, R. McCormack and G.M. Jonakait. Dept. Biol. Sci., Rutgers Univ., Newark NJ 07102.

Interleukin-1 (IL-1) increases substance P (SP) gene expression in cultured rat superior cervical ganglion (SCG) explants. This increase is blocked by the depolarizing agents KCl or veratrine. Furthermore, IL-1 induces SP in dissociates but not in "pure" neuronal cultures, suggesting that IL-1 acts at a non-neuronal cell. In order to study the molecular mechanism of IL-1 action, we have cloned IL-1 receptor (IL1R) cDNA from rat brain and SCG. The cDNA sequence is strongly homologous with mouse and human IL1R cDNA of the fibroblast type (type I). mRNA specific for IL1R can be readily detected in intact SCG by S1 hybridization or RT-PCR reactions. However, the level of IL1R mRNA increases 5-fold by 2 days in culture. This increase is independent of the presence of IL-1, IL-1 receptor antagonist protein (Synergen), veratrine or tetrodotoxin. 40 mM KCl reduces the level of IL1R mRNA slightly. Identification of the IL1R mRNA-containing cell type is in progress. The induction of IL1R following explantation, a model of nerve injury, may provide a mechanism linking inflammatory signalling to neuronal phenotypic changes. (Supported by ONR and NIMH).

547.6

EFFECTS OF MURINE AND HUMAN CYTOKINES ON PC12 CELLS IN VITRO. W.J. Bell, S. Rudnick, M. Palmatier, L. Christenson* and T.R. Flanagan, CytoTherapeutics, Inc., 2 Richmond Square, Providence, RI 02906.

Neurons are known to be responsive to a broad range of biologically active peptides. Considerable information has been accumulated describing peptide neurotrophic factors. At the same time, a tremendous body of information has been assembled describing immunologically active trophic factors (cytokines). Neurons might reasonably be exposed to a number of these cytokines during acute inflammatory episodes. We have examined the effects of 4 recombinant cytokines (IL-1, IL-2, IFN, TNF) over a 100 fold concentration range on the metabolic activity of a neuronal pheochromocytoma (PC12) cell line during 2 days of culture. Metabolic activity was measured by mitochondrial oxidation of MTT, and, as such, reflects either a proportional loss of mitochondrial oxidative potential or fractional cell death.

In PC12 cells grown in attached cultures in serum-free media, a 10 to 40% depression of net metabolic activity was seen after exposure to the highest doses of all the tested cytokines. However, PC12 cells grown in serum supplemented media displayed a depressed metabolic activity only in response to the highest doses of murine gamma interferon (1000 units per ml). This metabolic depression progressed with increased culture time. Combinations of all four cytokines at half maximal doses did not detectably depress metabolic activity more than interferon alone, and at lower concentrations a 10% metabolic stimulation was suggested in cells cultured with incomplete media. The inhibitory effect of high levels of murine gamma interferon was less pronounced in PC12 cells grown as spinner cultures.

These studies indicate that the metabolic activity of PC12 cell cultures can be depressed by elevated levels of cytokines and that the magnitude of this effect is influenced by the conditions used to culture these cells.

547.7

CELL CONTACT REGULATES NEUROPEPTIDE Y EXPRESSION IN CULTURED SYMPATHETIC NEURONS. M. Freidin, C. Kalberg*, and J.A. Kessler. Depts. Neurology and Neuroscience Albert Einstein College of Medicine, Bronx, NY 10461.

Neurotransmitters and neuropeptides coexpressed by the same neuron may be regulated independently. The peptide transmitter, neuropeptide Y (NPY), has been identified in approximately 60% of rat sympathetic superior cervical ganglion (SCG) neurons, virtually all of which are catecholaminergic. However, we find that environmental signals which effect catecholaminergic traits in cultured rat SCG, regulate NPY expression differently. Relatively large increases in neuronal density only slightly alter catecholaminergic properties in sympathetic neurons. However, levels of NPY were highly depependent on cell density. In pure neuronal cultures, increasing cell numbers from 6500 to 12,000 neurons per culture resulted in a 14-fold decrease in NPY. Coculture of SCG neurons with ganglion nonneuronal cells further reduced NPY levels at all neuronal densities examined. Treatment with the neuropoietic cytokine leukemia inhibitory factor, which increases cholinergic traits and SP and decreases catecholaminergic expression, decreased NPY by 50% in cocultures and only slightly in pure neuronal cultures. Finally, neither glucocorticoids nor the immunoregulatory cytokine interleukin-1β, which regulate other transmitter systems, altered NPY expression. These observations indicate that although norepinephrine and NPY are often coexpressed, they are regulated independently in cultured SCG neurons. Further, expression of NPY is highly influence by cell-cell contact.

547.8

MULTIPLE CYTOKINES/GROWTH FACTORS REGULATE TRANSMITTER EXPRESSION IN CULTURED SYMPATHETIC NEURONS. J.A. Kessler, D. Javitz*, J. Kim and M. Freidin Depts. Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.

Coculture of sympathetic neurons with ganglion nonneuronal cells stimulate neuronal expresssion of cholinergic traits and and of the peptide transmitter, substance P (SP). The molecular mechanism mediating these interactions are not fully understood. Treatment of sympathetic neurons with the cytokine leukemia inhibitory factor (LIF), which is found in conditioned medium from nonneuronal cells, likewise stimulates cholinergic and SP expression (Yamamori, T., et al, 1989). However, treatment of cocultures with an anti-LIF antibody (which completely blocks the effects of exogenous LIF) only reduces choline acetyltransferase activity (ChAT) and SP by 30-50%, suggesting that LIF mediates some but not all of the effects of coculture with nonneuronal cells. Cocultures also produce a molecule similar to or identical with CNTF, suggesting that the factor also mediates some of the effects of culture. Finally, another cytokine, interleukin-1β (IL-1β) is endogenously produced by these cultures. Treatment of cocultures with an antagonist to the interleukin 1 receptor significantly reduced levels of SP. These observations suggest that the effects of nonneuronal cells on sympathetic neurotransmitter and peptide expression are mediated by a complex array of growth factors/cytokines including, but not restricted to, LIF, CNTF, and IL-1β.

547.9

INDIRECT DEVELOPMENTAL MODULATION OF CHOLINERGIC AND DOPAMINERGIC PROPERTIES OF RAT CNS NEURONS IN CULTURE BY D-FACTOR (CDF/LIF). C.H. Schmelzer, L.E. Burton, F. Hefti and B. Knüsel*. Andrus Gerontology Center, Univ. of Southern California, Los Angeles, CA 90089 and Genentech Inc., South San Francisco, CA 94080.

Cholinergic differentiation factor (CDF), identical to leukemia inhibitory factor (LIF), induces cholinergic phenotype in normally adrenergic sympathetic neurons. LIF mRNA is also found in rat CNS. We tested whether LIF might play a role in the transmitter-specific development of rat mesencephalic neurons. Chronic treatment of primary cultures of embryonic (E15) ventral mesencephalon with LIF reduced the uptake activity for radiolabeled dopamine (DA) to approx. 50% of control after 1 week *in vitro*. In identical cultures the activity of the cholinergic marker enzyme choline acetyltransferase (ChAT) was increased two-fold. Immunocytochemical staining of the cultures for neuron-specific enolase, tyrosine hydroxylase, ChAT and glial fibrillary acidic protein revealed little effect of LIF on numbers of neurons, including the dopaminergic and cholinergic ones, but showed a significant effect on prevalence and morphology of astrocytes. Dose requirements for effects on DA uptake and ChAT activity were identical, with ED_{50}s of 1 ng/ml. However, the reciprocal effects on cholinergic and dopaminergic properties were not due to transdifferentiation of DA neurons. Selective elimination of DA cells by pretreatment with 10 μM MPP^+ for 24 hrs abolished DA uptake of both, control and LIF treated, cultures but did not affect the LIF mediated ChAT activity increase. Our data suggest that LIF affects cholinergic and dopaminergic mesencephalic neurons by the same, albeit indirect mechanism, possibly reflecting a specific, receptor mediated effect of LIF on development and differentiation of astrocytes.

547.10

CNTF AND LIF ACT ON NEURONAL CELLS VIA SHARED SIGNALLING PATHWAYS THAT INVOLVE THE IL-6 SIGNAL TRANSDUCING RECEPTOR COMPONENT GP130. N. Y. Ip*, S. H. Nye, T. G. Boulton, Y. Li, S. Davis, S. J. Birren, T. Taga, T. Kishimoto, D. J. Anderson, N. Stahl and G. D. Yancopoulos. Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591.

Recent analyses suggest that ciliary neurotrophic factor (CNTF) and a number of hemopoietic cytokines including interleukin-6 (IL-6) and leukemia inhibitory factor (LIF) appear to be distantly related, as are some of their receptor components. While LIF shares several common actions with IL-6 outside of the nervous system, it also elicits responses in some neuronal cells similar to those of CNTF. We have identified biologically relevant responses to CNTF in a sympathoadrenal progenitor cell line (MAH), which also responds identically to LIF. Comparison of the tyrosine phosphorylations and gene activations induced by CNTF and LIF in MAH cells, as well as in other neuronal cell lines, reveals that they are indistinguishable and are also very similar to signalling events which characterize LIF and IL-6 responses in hemopoietic cells. We provide a basis for the overlapping actions of these related cytokines by demonstrating that the shared CNTF and LIF signalling pathways involve the IL-6 signal transducing receptor component gp130. Thus the receptor system for CNTF is unlike the trk receptor kinases used by the nerve growth factor family of neurotrophic factors, but instead shares components with the receptor complexes for a subclass of hemopoietic cytokines. Interestingly, we find that the receptors for both the CNTF and the nerve growth factor family are not only expressed in discrete populations of adult neurons, but are also present during early neuronal development. We provide evidence that these two distinct classes of neurotrophic factors, utilizing distinct signalling pathways, can interact to effect the growth and differentiation of neuronal progenitors.

547.11

GROWTH FACTOR REGULATION OF mRNA'S ENCODING RECEPTORS FOR NEUROTRANSMITTERS IN CULTURED SYMPATHETIC NEURONS. W.H. Ludlam*, Z. Zang§, and J.A. Kessler. Depts. of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx NY 10461, and §Center for Molecular & Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ 07102.

Neuronal receptor and neurotransmitter phenotypes are both regulated by similar factors in cultured sympathetic neurons of the neonatal rat superior cervical ganglion (SCG). Leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF), factors previously shown to stimulate levels of choline acetyltransferase activity (CAT) and of substance P, have recently been shown by our laboratory to also block the normal developmental increase in muscarinic receptor expression in cultured SCG neurons. In this study we have characterized the muscarinic receptor genes expressed by sympathetic neurons, and defined the effects of LIF and CNTF treatment on muscarinic receptor mRNA (subtypes m1, m3, and m4). In untreated SCG cultures, significant levels of the m1 subtype were detected using solution hybridization/RNase protection analysis. By contrast, m3 and m4 subtypes mRNA's were much less abundant. After 12 days of treatment with LIF or CNTF (both at 5 ng/ml), the level of m1 muscarinic receptor mRNA was approximately 50% lower than control. We are currently investigating the regulation of the m2 and m5 subtypes. Further, we are examining the regulation of other receptor mRNA's which may be elevated by LIF and CNTF treatment. These findings indicate that LIF and CNTF regulate differential receptor expression at the mRNA level, and that these factors may influence both transmitter development and receptor phenotypic expression of the neuron.

NUTRITIONAL AND PRENATAL FACTORS

548.1

EFFECT OF PRE- AND/OR POSTNATAL CHOLINE SUPPLEMENTATION ON WORKING AND REFERENCE MEMORY (SPATIAL AND NONSPATIAL) AND OPEN FIELD BEHAVIOR IN THE RAT.
R.C. Tees* and J.L. Johnston. Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1Z4.

Research has shown that choline supplementation during development enhances spatial working and reference memory in adult rats (Meck et al., 1989). Cholinergic blockade studies report spatial memory impairments (Ellen et al., 1986) and diminished responses to novel cues as well as a disruption of normal search and exploratory behaviors (Whishaw & Tomie, 1987).

The present experiment was designed to assess the effect of dietary choline supplementation on spatial and non-spatial memory and exploratory behavior. Subjects received either no supplementation, supplementation pre-natally to postnatal day 30, or supplementation from post-natal days 16 to 30. Test battery consisted of an open field task, visual discrimination task, 17-arm radial arm maze and delayed-nonmatching-to-sample.

Significant group differences were found, as well as a significant group by sex interaction effect in the post-natal group. These findings suggest that dietary choline supplementation enhances several aspects of memory perfor-mance and that postnatal supplementation alone has a differential effect depending on sex.

548.2

SEX DIFFERENCES IN THE EFFECT OF PRENATAL CHOLINE TREATMENT ON SEPTAL CELL SIZE AND HIPPOCAMPAL NGF.
R. Loy,*D. Heyer, J. Miller and M.D. Lindner. Canandaigua VAMC and Department of Neurology, University of Rochester, 435 E. Henrietta Road, Rochester, NY 14620.

Choline treatment restricted to embryonic days 12-17 (E12-17) is sufficient to produce long-lasting enhancement of spatial memory in male and female rats, although the effect in females is not as robust. We tested rats treated *in utero* from ED12-17 with choline chloride (300 mg/kg/day, *p.o.* to the dam) for changes in the size of NGF receptor-positive neurons. Basal forebrain NGF receptor-positive neurons increased in size from 70 sq microns on PD0 to more than 200 sq microns on PD30. Males tended to have larger NGF receptor-positive neurons than females, but choline increased cell size in both males and females. Cells were no longer increasing in size after PD30, and by PD90 only the males still exhibited a significant increase in cell size due to choline treatment. Choline treatment also increased levels of NGF protein in the hippocampi of both males and females at PD20. In males at PD90, NGF levels were higher in the right hippocampus than in the left, and at this age choline treatment increased NGF protein levels equally on both sides. Future studies remain to determine whether the laterality of NGF protein levels is different for females than for males, whether choline effects on hippocampal NGF protein levels persist in females, and what the role of NGF may be in either the increased cell size or enhanced memory function with choline treatment.

Supported by AG09525.

548.3

EFFECT OF PRENATAL EXPOSURE TO METHADONE (M) ON ACETYLCHOLINE (ACH) TURNOVER IN ADULT FEMALE RATS. S.E.Robinson,* H. Guo, J.R. Pascua, K.P. McDowell, and H.M. Allen. Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298-0613.

Prenatal exposure to M affects ACh content and the turnover rate of ACh (TR_{ACh}) in specific brain regions of neonatal and weanling female rats (Dev. Brain Res. 57: 296, 1990; 64: 183, 1991). In order to determine whether abnormalities in cholinergic function persist into adulthood, the following experiment was performed. On gestational day 7, female Sprague-Dawley rats anesthetized with methoxyflurane were implanted with 14-day Alzet osmotic minipumps filled with water (W) or M (9 mg/kg/day). An untreated control (C) group of pregnant rats was also included. Within 24 h of parturition, the litter size was reduced to 10 (5 of each sex) and the pups fostered to untreated dams. On postnatal day 90 (P90) and beginning 11 min after i.p. injection of saline or morphine SO_4 (20 mg/kg), rats were infused via the tail vein with deuterium-labeled phosphorylcholine (15 µmol/kg/min) for 9 min and euthanized by microwave radiation focussed to the skull (10 kW, 1.3 sec). Using a mass fragmentographic technique, relative incorporation of deuterium into choline and ACh, and hence, TR_{ACh} were determined in brain regions. Unlike findings in the weanling rat, prenatal M exposure did not affect TR_{ACh} in the parietal cortex, hypothalamus, or striatum of female rats at P90. However, although morphine reduced TR_{ACh} in the hippocampus of C and W rats, it did not have this effect in M-exposed rats. Thus, although basal cholinergic activity returns to control values in adult females exposed to M prenatally, this treatment affects the cholinergic response to morphine. (Supported by NIDA grant DA-05274).

548.4

ACTIVATION OF PYRIDOXAL KINASE BY ZINC METALLOTHIONEIN IN TROUT BRAIN. R. Hao, R.F. Pfeiffer and M. Ebadi*. Div. of Neurology and Dept. of Pharmacology, Univ. Nebr. Coll. Med., 600 S. 42nd St., Omaha, NE 68198-6260.

With the exception of calcium and magnesium, zinc is the most abundant cation in the brain. Metallothionein (MT) are low-molecular weight metal binding proteins consisting of 25-30% cysteine, containing no aromatic amino acid or disulfide bonds, and binding between 5 and 7 g atoms of group IIB heavy metals per mole of protein. The precise physiological functions of these ubiquitously occurring proteins, which may vary in different organs and tissues, are not fully understood. Since the basal level of MT synthesis varies in the hippocampus, cerebellum, striatum, pineal gland, and retina, we have proposed that MTs may regulate the transport and homeostasis of essential trace metals in these brain regions. By using G75 gel filtration and DEAE Sephadex chromatographies, we have purified MT from trout brain, which exhibited only one band on SDS polyacrylamide gel. Furthermore, the trout brain MT possessed a MW of 6.720 ± 200 as judged by studies on SDS PAGE and G75 gel filtration chromatographies, and contained a zinc content of 9 µg/mg protein. The trout brain zinc metallothionein stimulated the activity of pyridoxal kinase (ATP:pyridoxal 5-phosphotransferase EC 2.7.1.35) at the concentrations of 6-30 µM, whereas the thioenin was devoid of any stimulating activity. The results of these studies are interpreted to suggest that metallothionein stimulates pyridoxal kinase by cross-linking and donating zinc to it (Supported in part from a grant from USPHS ES 03949).

548.5

EFFECTS OF CHRONICALLY ADMINISTERED FLUOXETINE HCl DURING GESTATION ON SPRAGUE-DAWLEY OFFSPRING. M. Stanford, M. Gilbert, L. Mills and J. H. Patton*. Department of Psychology, Baylor University, Waco, TX 76798.

Fluoxetine HCl (Prozac®, Dista) is an antidepressant chemically unrelated to the tricyclic antidepressants. Its antidepressant action appears to be linked to its selective inhibition of 5-HT reuptake in the CNS (Wong, Horng & Bymaster (1974), *Life Sciences*, 15, 471-479). Recent research has demonstrated that Fluoxetine HCl causes a down regulation of ^3H - Imipramine binding sites in the cerebral cortex of rats exposed in utero (Montero, de Ceballos & Del Rio (1990), *Life Sciences*, 46, 1619-1626). In the present study gravid Sprague-Dawley rats were administered 5.62 mg/kg Fluoxetine HCl by gavage beginning day 6 or 7 of pregnancy and ending the day of birth. A control group received distilled water by gavage during gestation. Both groups received distilled water during the lactating period (birth to day 21). At birth Fluoxetine exposed pups showed a statistically higher frequency of skin hematomas when compared to water controls (Fluoxetine: 63 / 189, Water: 2 / 113, $X^2 = 34.61$, $p < .001$). This result is consistent with an earlier report in which Fluoxetine HCl was shown to cause bleeding episodes in 8 patients treated for obsessive-compulsive disorder (Tobias, Kirschen, Ninan & Mosberg (1991), *Am J Psychiatry*, 148, 949).

548.6

EFFECTS OF PRENATAL PROTEIN MALNUTRITION AND AGE ON CENTRAL NEUROTRANSMISSION. J.-C. Chen, G. Turiak and L. Volicer*
Dept. Pharmacology, U.Mass,Med.Sch., Worcester,MA 01655 and Dept. Pharmacol., Boston U.Med.Sch., Boston,MA 02118.

The effects of prenatal protein malnutrition on central neurotransmitters were assessed in postnatal day 1,15,30, 45,90 and 220 days old rats. Malnourished rat (6/25 group) were males born to dams fed a 6% casein diet during gestation and fostered at birth to dams fed a control (25% casein) diet. We observed: (1) in 220 days old rats, there was an enhanced basal release of hippocampal serotonin(5HT) dopamine (DA), norepinephrine (NE) and their acidic metabolites from incubated slices of 6/25 as compared with control (25/25 group). The basal release of these neurochemicals in 90 days old animals showed a similar pattern, however, was less significant. When challenged with 60mM KCl, there was no difference in KCl-induced neurochemical release between the two groups at both ages. (2) Tissue concentrations of 5HT, DA, NE, their precursors and metabolites from hippocampus, striatum, cortex and brain stem were similar in the two groups at each age. However, individual chemicals displayed ontogenic difference during postnatal development and varied in different brain regions. (3) by SDS-PAGE and Western immunoblotting, we found there was a difference in contents of striatal and hippocampal synaptophysin between 6/25 and 25/25 groups in 90 days old rats. We hypothesize that some molecular species that regulate neurotransmission are vulnerable to prenatal protein malnutrition. (HD-22539).

548.7

EFFECTS OF PRENATAL MALNUTRITION AND POSTNATAL NUTRITIONAL REHABILITATION ON THE DENTATE GYRUS OF THE RAT. L. Cintra,* L. Granados, S. Díaz-Cintra, T. Kemper and P.J. Morgane. Inst. Invest. Bioméd., UNAM 04510, and U. de Invest. Salud Infantil INP-SSA 14410, México D.F. Neurology Unit, Boston MA, 02118, and Worces. Found. Exp. Biol., Shrewsbury MA, 01545 USA.

We analyzed effects of prenatal malnutrition and postnatal rehabilitation on areas of granule cells (GC) in the dorsal leaf of the dentate gyrus (DG) and of mossy fibers (MF) stained with Timm's technique. 50 male Sprague-Dawley rats were divided in two groups, control and rehabilitated, consisting of 15, 30, 90 and 220-day-olds. 96 brain sections in the frontal plane at two levels (rostral and medial) of the dentate gyrus were analyzed with an imaging system. Results showed a significant decrease ($p<0.05$) in the area of GC at 220 days of age in the medial part of the DG in malnourished rats. 90-day-old rats showed a significant increase ($p<0.05$) in this parameter. MF area showed significant reductions ($p<0.05$) across two levels, rostral and medial, only in 220-day-old animals. These findings indicate a long-term effect of malnutrition on dentate granule cells and their efferent fibres. **(Supported by DGAPA IN202891, NIH HD-22539-04 and HD-23338-03).**

548.8

PRENATAL MALNUTRITION AND POSTNATAL NUTRITIONAL REHABILITATION EFFECTS ON CA3 HIPPOCAMPAL PYRAMIDAL CELLS IN RATS. M. García-Ruiz,* S. Díaz-Cintra and L. Cintra. Depto. de Fisiol. Inst. Invest. Biomédicas, UNAM, México, D.F. 04510.

Prenatal malnutrition (6% casein diet) and postnatal rehabilitation (25% casein diet), were studied for effects on CA3 pyramidal cells. We used 36 male Sprague-Dawley rats, divided in 2 groups: 1) control (25% prenatal/25% postnatal) and 2) experimental (6% prenatal/25% postnatal). Using the rapid-Golgi method, 216 CA3 cells were selected for morphometric analysis using an imaging system, and measurements of somal size, dendritic linear extent, area of dendritic thorny excrescences and the number of apical dendrites, as well as, the density, length and diameter of the synaptic spine bulbs were made. Results showed significant decreases ($p<0.05$) in somal size, number of spines, diameters of dendrites and spine bulbs, and significant increases ($p<0.05$) in the spine length and dendritic ramification. Rats fed with a 6/25% diet at 30-day-old showed a compensation in the number of spines (83%) and somal size (100%) in CA3 pyramidal cells. Prenatal malnutrition has a differential effect on hippocampal CA3 cells and this effect depends of the age of the rat. **Supported by DGAPA IN202891.**

548.9

EFFECT OF PRENATAL PROTEIN MALNUTRITION AND POSTNATAL NUTRITIONAL REHABILITATION ON CA1 HIPPOCAMPAL PYRAMIDAL CELLS IN RATS OF THREE AGE GROUPS. S. Díaz-Cintra*, A.Aguilar, A.Galván, L. Cintra, T. Kemper and P.J. Morgane. Inst. Invest. Bioméd., UNAM, México,D.F. 04510. Neurol. Unit, Boston City Hosp., Boston MA, 02118 and Worces. Found. Exp. Biol., Shrewsbury MA, 01545.

We have previously analyzed the effects of prenatal malnutrition and postnatal rehabilitation on pyramidal cells in CA1 in adult rats. In this study we used the same paradigm in 30, 90 and 220-day-old rats. 216 pyramidal cells were studied using an imaging system. We measured the somal size, linear dendritic extent in relation to *strata: radiatum and lacunosum moleculare* and the number of spines in three 50 micron segments corresponding to perforant path (PP), Schaffer collateral (SF) and commissural fiber input systems. We found significant reductions ($p<0.05$) in some cellular parameters in 30-day-old rats and most of these parameters in 220-day-old rats in malnourished animals. At 90 days, the spine length and apical dendritic diameters were significantly reduced ($p<0.05$). Prenatal malnutrition produces severe alterations in the apical dendrites where both PP and SF synapse in 30 and 220-days-old rats. **(Supported by DGAPA IN202891, NIH HD-22539-04, HD-23338-03).**

548.10

IMPACT OF PRENATAL PROTEIN MALNUTRITION ON LONG-TERM POTENTIATION IN JUVENILE RATS. P.J. Morgane*, R.J. Austin-LaFrance and J.D. Bronzino. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545 and Dept. of Engineering and Computer Science, Trinity College, Hartford, CT 06106.

We examined the ability of freely-moving 15- and 30-day old prenatally protein malnourished rats (designated 6/25) to support and maintain LTP of the perforant path/hippocampal dentate granule cell synapse. Tetanization in 6/25 animals resulted in a 37% enhancement of the EPSP slope measure at the 5 hr post-potentiation period. This level slowly decayed to 18% after 24 hrs. Enhanced synaptic activation, however, was not translated into enhanced cellular discharge. Population spike amplitudes from 6/25 animals declined continuously, dropping to 50% of baseline 24 hrs after potentiation. Thus, tetanization in the 6/25 group resulted in a net **decrease** in synaptic transmission efficacy. Although preliminary, 3 15-day old, 6/25 animals also failed to exhibit potentiation of the population spike component. Well-nourished controls did show enhancement in this measure beginning 3 hrs after potentiation, reaching levels of 50-70% above baseline 24 hrs after potentiation. Since no significant age-related differences were observed for baseline measures of population EPSP slope or population spike amplitude, the differential response to tetanization seen in 6/25 animals does not appear to result from immature granule cell physiology. We suggest that the dietary insult may impact intrinsic or extrinsic neuronal systems modulating dentate granule cell excitability. The dietary insult was found to alter both the establishment and maintenance of LTP, suggesting possible consequences for learning and memory function as a result of the animal's inability to properly receive, process or store information during the early post-weaning period. **Supported by NIH/CHD Grant # HD-22539**

GLIA AND OTHER NON-NEURONAL CELLS V

549.1

REGULATION OF GLIA-DERIVED NEXIN IN PRIMARY SCHWANN CELLS.
A. Bleuel and D. Monard*.
Friedrich Miescher Institute, P.O. Box 2543, CH-4002 Basel, Switzerland.

Glia-derived nexin (GDN) is a 43 kDA neurite-promoting glycoprotein with serine protease inhibitory activity. *In vivo* experiments have shown that expression of GDN is dramatically induced after injury of the rat sciatic nerve (Meier et al., Nature 343, 548-550 [1989]) and following hippocampal lesion caused by transient forebrain ischemia in the gerbil (Hoffmann et al., Neuroscience, in press).

In explant cultures of dorsal root ganglia, the GDN level is down-regulated and, as *in vivo*, can be induced following lesion of the processes emerging from the explants. The GDN increase detected by immunocytochemistry is restricted to the Schwann cells distal to the site of injury, where nerve degeneration has taken place. Neurotrophic factors, also known to be induced after lesion, have been tested for their ability to affect GDN expression in pure Schwann cell cultures. A significant increase of GDN mRNA and protein is seen after NGF treatment. NT-3 and BDNF remained inactive. Some neuropeptides also influence GDN synthesis. These results suggest that neuron-glia interaction, both by cell-cell contact and secretion of macromolecules, plays an important role in the regulation of GDN.

549.2

AGE-RELATED DIFFERENCES IN PROLIFERATIVE RESPONSES OF SCHWANN CELLS DURING WALLERIAN DEGENERATION.
A. Komiyama*, O. Hasegawa and K. Suzuki. Dept. of Pathology, Univ. of North Carolina, Chapel Hill, NC 27599 and Dept. of Neurology, Yokohama City Univ. Sch. of Medicine, Yokohama, Japan.

Proliferative responses of Schwann cells during Wallerian degeneration were investigated in the mouse sciatic nerves after nerve-transection at 3, 10 and 60 days, corresponding to the periods of early myelination, active myelination and postmyelination. As assessed by thymidine incorporation for the first 24 h in culture, Schwann cells from adult nerve proliferated rapidly within day 1 post-transection and reached a peak at day 3. In the nerves from neonatal or suckling mice, however, division rate of Schwann cells declined after transection, and was even less in the transected nerves than in the contralateral uninjured nerves. The reduction in thymidine uptake by Schwann cells was more pronounced in nerves sectioned at postnatal day 3 than those sectioned at day 10. By contrast, fibroblasts divided rapidly following transection regardless of age. These data suggest that in the degenerating nerves of young mice, their mitotic capacity of Schwann cells declined because of a loss of axonal mitogens and also the paucity of mitogens from myelin components, whereas proliferation of fibroblasts is stimulated by growth-promoting polypeptides common to any other tissues during wound repair.

549.3

IMPROVED YIELDS IN SCHWANN CELL CULTURES FROM ADULT PERIPHERAL NERVE UNDERGOING WALLERIAN DEGENERATION
A.D. Ansselin*, S.D. Corbeil and D.F. Davey, Microsurgery Research Centre, Sydney and Department of Physiology, University of Sydney, NSW 2006, Australia.

We have previously reported to the Society that a conditioning lesion to the sciatic nerve of adult rats facilitates the isolation of Schwann cells from the distal portion of the nerve several days later (Soc. Neurosci. Abstr. 17:932, 1991). It appears that the process of Wallerian degeneration causes both an increase in the number of Schwann cells *in situ* and makes it easier to extract the cells using conventional methods. We have exploited this observation further, and have found that the enzymatic treatment required to isolate cells from lesioned nerves can be much gentler than those required for intact nerve. This change in procedure produces a further marked increase in cell numbers. The left sciatic nerve in young adult Wistar rats was exposed, severed at the sciatic notch and deflected. After various survival times the severed (conditioned) and unoperated (control) sciatic nerves (20 mm each side) were excised using sterile techniques, and processed as previously described except for reduction of the digestion phase from 18h to 4h. The cells from each nerve segment were counted prior to plating approximately 3×10^6 cells per well in 6-multiwell plates (Linbro) coated with a laminin substratum. Schwann cell yield was assessed by counting the spindle shaped cells attached to the substratum 18h later. The identification of these cells as Schwann cells was confirmed with S-100 antibody staining. The optimal delay between lesion and cell harvest using this method is approximately 10 days.

549.4

PURIFICATION OF EMBRYONIC CHICKEN SCHWANN CELLS AND ANALYSIS OF P_0 EXPRESSION. A. Bhattacharyya*, R. Brackenbury, and N. Ratner. Dept. of Anatomy & Cell Biology, Univ. of Cincinnati Sch. of Med., Cincinnati OH 45267.

We have previously described an immunocytochemical marker, 1E8, that is specific for Schwann cells and their precursors (Bhattacharyya *et al.*, 1991, Neuron 7:831-844). We have now used the 1E8 monoclonal antibody to immunoselect Schwann cells from embryonic day 14 (E14) chicken sciatic nerve. When cultured, these immunoselected cells displayed many characteristics of mature Schwann cells, including S100-immunoreactivity and O4 antigen-immunoreactivity. As previously described for rodent Schwann cells, purified chicken Schwann cells divided slowly when cultured alone, but were responsive to mitogenic stimulation by neurons and by neuronal membranes.

The 1E8 monoclonal antibody recognizes the peripheral myelin protein, P_0. In rodent, P_0 expression increases at the time of myelination and is downregulated when Schwann cells are removed from axons. However, in chickens, P_0, as detected by 1E8, is expressed prior to myelin formation. We tested whether P_0 expression in the chicken is also regulated by axonal contact by culturing chicken Schwann cells in the presence and absence of axons. As in rodent, most Schwann cells purified using the 1E8 monoclonal antibody lost 1E8-immunoreactivity in 24-48 hours when cultured alone. When axonal contact was restored by adding purified Schwann cells to cultures of embryonic rat dorsal root ganglia neurons, most cells were 1E8-negative even after prolonged culture. Therefore, axonal contact *in vitro* is not sufficient to restore expression of the 1E8 antigen (P_0) in Schwann cells. These results suggest that additional signals may exist in developing nerve to maintain P_0 expression. Supported by NIH grant NS27227 and an Albert J. Ryan fellowship.

549.5

GOLDFISH SCHWANN CELLS AND OPTIC NERVE OLIGODENDROCYTE-LIKE CELLS ARE DISTINCT CELL TYPES M.Bastmeyer* and C.A.O.Stuermer; Faculty for Biology, University of Konstanz, Germany

Oligodendrocyte-like cells isolated from the goldfish optic nerve share many properties with mammalian Schwann cells. In particular like mammalian Schwann cells but unlike mammalian oligodendrocytes the fish oligodendrocyte-like cells support the growth of regenerating axons (Bastmeyer et al., J Neurosci 11, 626-640, 1991). Therefore, we isolated fish Schwann cells and compared them to the oligodendrocyte-like cells.

PNS glial cells were obtained by explanting small pieces from the IX. and X. cranial nerves of adult goldfish onto laminin coated coverslips. Two morphologically and immunocytochemically distinct cell types emigrated and multiplied in culture: long, bipolar Schwann cells and fibroblasts. Like goldfish oligodendrocyte-like cells fish Schwann cells expressed the O4-antigen and the fish myelin proteins IP1/2, which are related to mammalian P0. Both, Schwann cells and oligodendrocyte-like cells carried the HNK1-epitope, NCAM and the E587-antigen, which is related to L1 (Vielmetter et al., J Neurosci 11, 3581-3593, 1991). Oligodendrocyte-like cells differed from fish Schwann cells in that they transiently expressed fish-GFAP. Only the Schwann cells but not the oligodendrocyte-like cells were immunoreactive for anti-NGF-receptor.

Thus, despite substantial similarities, goldfish optic nerve oligodendrocyte-like cells and goldfish PNS Schwann cells are distinct cell types.

549.6

ROLE OF GLUCOCORTICOIDS IN SCHWANN CELL PROLIFERATION. T.J. Neuberger* and G.H. DeVries, Dept. of Biochemistry, Medical College of Virginia, Richmond Va. 23298.

The axolemma enriched fraction (**AEF**) contains a potent mitogen, which in the presence of serum induces Schwann cell proliferation. In serum free media, the AEF also induces Schwann cell proliferation but only if hydrocortisone is included in the media. Schwann cells grown in defined media containing hydrocortisone demonstrated a 5 to 10 fold greater response to AEF stimulation as compared to Schwann cells grown in defined media without hydrocortisone. The role of glucocorticoids in mediating the response of Schwann cells to the AEF associated mitogen was further investigated using two separate approaches, a) charcoal extraction of steroids from serum and b) inhibition of the glucocorticoid receptor. Addition of the AEF to Schwann cells grown in charcoal extracted serum containing media resulted in a 50% reduction in ^{3}H thymidine uptake as compared to Schwann cells grown in untreated serum containing media. By supplementing the charcoal extracted serum with hydrocortisone, a majority of the AEF associated mitogenic activity for Schwann cells was restored. Furthermore, addition of the glucocorticoid inhibitor, RU-486, to serum containing media and serum free media resulted in a dose dependent inhibition of the AEF induced ^{3}H thymidine uptake. This effect was completely reversed by the removal of RU-486 from serum media 24 hours prior to the addition of AEF. Since the rate of ^{3}H-thymidine by AEF stimulated Schwann cells was not effected by pretreatment with increasing concentrations of RU-486, RU-486 did not appear to adversely affect Schwann cell viability. Finally, increasing concentrations of RU-486 added to serum containing media resulted in morphological changes. Schwann cells grown in serum containing media had short, thick processes and formed large aggregates of cells. In contrast, in serum free media and serum containing media with 10μg/ml RU-486, Schwann cells had long, thin processes and were generally observed as isolated cells. This data suggests that the proliferative response of Schwann cells to the AEF in both serum containing and serum free media is dependent on the availability of glucocorticoids in the media. (This work was supported by NS 15408 and HL-07110-15)

549.7

THE EXPRESSION OF B-50/ GAP 43 IN SCHWANN CELLS IS UPREGULATED IN THE DEGENERATING DISTAL NERVE STUMP FOLLOWING NERVE INJURY. Plantinga,L.C., Verhaagen,J., Edwards,P.M., Jap Tjoen San,E.R.A.*, Bär,P.R., and Gispen,W.H. Rudolf Magnus Inst., Univ. of Utrecht, Utrecht, The Netherlands.

We have detected mRNA for B-50 (GAP 43, pp46, F1, neuromodulin), which was originally believed to be a neuron-specific protein, in non-neuronal cells in the rat sciatic nerve. In control rats, the level of B-50 mRNA in sciatic nerve tissue was much lower than in dorsal root ganglia. Following nerve crush or transection, the expression of B-50 mRNA in the distal nerve stump increased dramatically between 1 and 2 days postinjury. The B-50 mRNA levels in the distal stump of crushed nerves remained elevated for up to 4 weeks and subsequently returned to control levels after 7 weeks. In contrast, after nerve transection, B-50 mRNA levels in the distal nerve portion continued to increase up to 7 weeks postlesion. No changes in the levels of the B-50 transcript were observed in the proximal portion of either crush-lesioned or transected sciatic nerves. In situ hybridisation demonstrated B-50 mRNA associated with Schwann cells in the distal nerve stump. The observation that Schwann cells are capable of producing B-50 mRNA was confirmed by Northern blot analysis of total RNA isolated from primary Schwann cell cultures. Taken together these data suggest that B-50 mRNA is expressed by Schwann cells and that the expression of B-50 mRNA is regulated by contact between Schwann cells and intact axons.

549.8

EFFECTS OF NERVE SEGMENT SUPERNATANTS ON CULTURED SCHWANN CELL PROLIFERATION AND LAMININ PRODUCTION. Q. Zhang, G.L. Stoner* and H.deF. Webster. Lab. Exp. Neuropath., NIH, Bethesda, MD 20892.

Mouse sciatic nerves were transected and 3hr-16d later, proximal segments were removed and homogenized. Supernatants of these segments or of normal sciatic nerves were added to Schwann cells maintained in DMEM+15% FCS. After 6d, Schwann cells were solubilized and the protein content was measured using a Biorad protein assay. Samples containing the same amounts of protein were then applied to microtiter plates and the laminin content was determined by ELISA. Samples derived from cultures treated with 24hr supernatants contained significantly higher levels of laminin than controls or other intervals. Increased surface and cytoplasmic α-laminin immunoreactivity was found in Schwann cells treated with 24 hr supernatants. Schwann cell proliferation was compared in supernatant-treated cultures by using a BrdU ELISA. The 24hr and 2d supernatants increased Schwann cell proliferation significantly; 12hr, 4d and 8d supernatants produced smaller increases. The nature and origin of regenerating nerve constituents which increase Schwann cell proliferation and laminin production in our cultures is being investigated.

549.9

EXPRESSION OF C-JUN PROTEIN IN SCHWANN CELLS DEPENDS ON THEIR ENVIRONMENT. **E.Vaudano*[1,3], C.de Felipe[2], S.W.Davies[3], A.R.Lieberman[3] and S.P.Hunt[2]. [1]Dip. Anatomia e Fisiologia Umana, Univ. di Torino, Italy, [2]Molecular Neurobiology Unit, Cambridge, UK, and [3]Dept. of Anatomy and Dev. Biology, University College London, London, UK**

The Schwann cell, in contrast to glia derived from the adult CNS, is considered a permissive substrate for axonal regeneration. CNS neurons can regenerate an axon inside a peripheral nerve graft implanted into the CNS, although such regeneration is very limited in comparison with regeneration in the PNS. We show that the expression of the transcription factor c-jun in Schwann cells of adult rats is differentially regulated according to whether the cells are exposed to a PNS or CNS environment. Jun protein was studied in Schwann cells of adult rats in vivo and in vitro using a polyclonal antibody. c-jun mRNA was localized and quantified using in situ hybridization with radiolabelled oligonucleotides. In the intact tibial nerve, Schwann cells were Jun negative. However one week after transection of the nerve all Schwann nuclei in the distal stump are Jun positive. In contrast, if a segment of tibial nerve was cut and immediately autografted with one end (proximal tip) in the thalamus or the cerebellum, and the other end (distal tip) left free subcutaneously, only the Schwann cells in the distal tip had Jun positive nuclei, while Schwann cells in the portion of nerve deep within the CNS remained Jun negative. When Schwann cells were grown in vitro from dissociated adult dorsal root ganglia and peripheral nerve, c-jun mRNA and Jun protein were expressed at high levels within 24h of plating. These levels could be substantially reduced by treatment of cultures with dibutyryl cyclic AMP or forskolin. Thus it appears that the CNS environment also contains factors which can inhibit the upregulation of c-jun. The genes regulated by c-jun in Schwann cells are not known but they might well be involved in the ability of these cells to potentiate axonal regrowth, explaining at least in part, the relatively poor regenerative response of CNS neurons inside peripheral nerve autografts.

549.10

THE DEVELOPMENTAL REGULATION OF GROWTH FACTOR RECEPTOR EXPRESSION IN THE RAT SCIATIC NERVE: CORRELATION WITH THE PERIOD OF SCHWANN CELL PROLIFERATION. J. B. Davis*, The Salk Institute, PO Box 85800, San Diego, CA 92186.

During the development and myelination of peripheral axons, Schwann cells go through consecutive periods of proliferation, growth arrest, and differentiation. Schwann cell proliferation declines rapidly after birth (P0), inversely correlated with the onset of myelin gene expression.

The control of Schwann cell proliferation during the developmental process might be achieved by : (a) levels of growth factor, (b) control of Schwann cell receptor expression, or (c) a dominant inhibitory signal. In order to test (a & b), we have studied the expression levels, in sciatic nerves, of PDGF and FGF, and their receptors, over the P0-P14 period, by means of northern analysis.

There is a rapid decline in FGF and PDGF-B receptor mRNA expression over P0-P3, which correlates with the rapid decrease in Schwann cell proliferation rate over the same period. In contrast, the expression of the mRNAs for the growth factors remains constant, PDGF-B chain mRNA is absent, whilst FGF expression is retained in the adult. These data are consistent with the theory that regulation of growth factor receptor expression might control Schwann cell proliferation during development.

This work was supported by an EMBO fellowship.

550.1

DEVELOPMENT OF NEURONAL AND MESODERMAL COMPONENTS OF THE ELASMOBRANCH VIth NERVE AND LATERAL RECTUS MUSCLE. E. Gilland, R. Baker and R. Gould*. Marine Biological Laboratory, Woods Hole, MA 02543

Neuroepithelial and mesodermal components associated with the VIth nerve were studied by means of paraffin sections, SEM and the application of lipophilic fluorescent dyes in *Squalus acanthias* embryos. Motoneurons originated in rhombomere 6 and by Stage 23 axons exited into a mesenchymal matrix far caudal to the mesodermal precursors of the lateral rectus. Fiber outgrowth occurred later than any other cranial nerve except IV. By Stage 24 VIth nerve growth cones were observed to contact two separate epithelial-like rudiments of mesodermal origin. A distinct hyoid-derived component was located medial to the Vth ganglion and a mandibular component, Platt's muscle E, lay slightly more rostral. Each pre-migratory muscle rudiment received a separate VIth nerve branch. Confocal microscopy showed that some dye labelled abducens motoneurons in contact with the mesodermal targets retained ventricular attachment in the neuroepithelium. Between Stages 25-27 the two pre-muscle masses maintained their epithelial organization while extending forward onto the globe where, by Stage 29, they merged together. These observations demonstrate an autonomous development of two separate mesodermal derivatives that each receive a VIth nerve branch. Both moieties are displaced rostrally before fusing to form a common lateral rectus muscle. A caudally directed VIth nerve branch associated with the most caudal VIth nerve rootlet was present at Stages 26-29. Dye applied to the caudal branch labelled peripheral ganglion-like cells and showed entry of fibers into the neuroepithelium, but revealed no motoneurons. These data suggest that this nerve may originate from one of a series of ectopic neural crest-derived ganglia associated with the cranial nerves.

550.2

DEVELOPMENT OF NORADRENERGIC INNERVATION IN THE RAT HYPOGLOSSAL NUCLEUS. L.D. Aldes*. Dept. of Structural and Cellular Biology, Univ. S. Alabama, College of Medicine, Mobile, AL 36688

Although the functions of norepinephrine (NE) in the central nervous system remain speculative, recent studies have provided evidence that NE plays an important modulatory role in somatic motor reflexes. Studies from this laboratory have established a unique NE innervation pattern in the hypoglossal nucleus (XII) of the adult rat. Since tongue protrusor motoneurons are the principal targets of NE-nXII afferents, and this motoneuron group plays a crucial role in several oral behaviors, the sequence of NE pattern development in XII was investigated in the rat. This was accomplished immunocytochemically with antisera to tyrosine hydroxylase (TH). Experiments were conducted in Sprague-Dawley rats of both sexes. For prenatal studies, rat pups were removed surgically on gestational days (GD) 16-21 and euthanized by decapitation. Brains were fixed by immersion in aldehydes. For postnatal studies, pups ranging from 12 hrs to 40 days were perfused-fixed transcardially. Brainstem sections were cut serially through XII, incubated in anti-TH (1:20,000), biotinylated goat-anti-rabbit IgG (1:1,000), and steptavidin HRP (1:1,000) before reacting with DAB/H_2O_2. Results from this study include the following: 1) TH immuno-reactivity is initially found in XII on GD 16/17; 2) preferential targeting of protrusor motoneurons is first observed on GD 17/18, but is not consistently present until GD 19; and, 3) the density of NE innervation increases progressively from GD 19 to adulthood. These results establish the early prenatal presence of NE in XII of the rat, and suggest that the control of XII motoneurons and tongue reflexes by NE commences early in development. The temporal and spatial patterns of NE development in XII is interpreted as reflecting the importance of oral reflexes in both the newborn and adult.

550.3

MORPHOMETRIC CORRELATIONS BETWEEN MOTONEURONS AND MUSCLE FIBERS IN DEVELOPING RAT MUSCLES. W.Z. Zhan,* Y.S. Prakash and G.C. Sieck. Mayo Clinic and Foundation, Rochester, MN 55905.

It has been proposed that there is a relationship between motoneuronal size and the size of its peripheral field, i.e. motor unit (MU) size. In the rat, by postnatal day 21 (D21), synapse elimination and myogenesis is complete for the diaphragm (DIA) and medial gastrocnemius (MG) muscles. We hypothesize that subsequent growth of muscle fibers is paralleled by growth of phrenic and lumbar motoneurons (MNs). We obtained estimates of muscle fiber volume by measuring optimal fiber length and fiber cross-sectional area (CSA). Phrenic and lumbar MNs were retrogradely labelled with tetramethylrhodamine dextran via intramuscular injection. "Optical slices" of labelled MNs were obtained using a Bio-Rad Laser Confocal microscope. In both muscles at D21, type I and II muscle fibers were comparable in size, while type II fibers were larger in both adult muscles. Adult MG fibers were significantly larger than DIA fibers. Since innervation ratios are constant by D21, postnatal changes in fiber volume reflect MU growth. Phrenic MN somal volumes doubled between D21 and 10 weeks (adult), while lumbar MNs nearly tripled in size. At each age lumbar MNs were larger than phrenic MNs. This is consistent with the growth in MU size in the two muscles, although MU growth was not in direct proportion to changes in somal volume. A bi-modal distribution of somal volume was evident in adult MG MNs, but less so in the phrenic pool. We speculate that the bi-modal MN volume distributions reflect differences between slow and fast twitch MUs. Bi-modal distributions were less obvious at D21, which may reflect the absence of heterogeneity in fiber CSA between different muscle types at that age. Supported by NIH grants HL34817 and HL37680.

550.4

A motor neuron-specific epitope and the low-affinity nerve growth factor receptor display reciprocal patterns of expression during development, axotomy and regeneration. A.Y. Chiu,* E.W. Chen and S. Loera, Division of Neuroscience, City of Hope, Duarte, CA 91010.

Embryonic motor neurons express the low-affinity Nerve Growth Factor receptor (NGFr). However, in postnatal life, they lose NGFr-immunoreactivity, and acquire a motor neuron-specific epitope that is recognized by the monoclonal antibody, MO-1. We examined the effect of nerve injury on these two developmentally regulated markers in two populations of somatic motor neurons. Unilateral transection, ligation or crushing of the sciatic nerve resulted in loss of MO-1 binding and a concomitant rise in NGFr-immunoreactivity within motor neurons in lumbar levels of the spinal cord. Transection of the hypoglossal nerve, a pure motor nerve, resulted in a similar loss of MO-1 binding and a selective rise in NGFr immunoreactivity in neurons within the ipsilateral hypoglossal motor nucleus. These changes, detectable within 5 days following nerve injury, are reversed with reinnervation, but persist if reinnervation is prevented by chronic axotomy. Thus, regulation of the expression of NGFr and the MO-1 epitope appears to be dependent upon interactions between motor neurons and target muscles. These observations are also consistent with the idea that regenerating neurons may revert to a developmentally immature state; in the case of motor neurons, this state is characterized by the presence of NGFrs and the absence of the MO-1 epitope.

550.5

THE ROLE OF EXTRACELLULAR CALCIUM AND CALCIUM CHANNELS IN ACTIVITY DEPENDENT INTRACELLULAR CALCIUM CHANGES IN EMBRYONIC CHICK MOTONEURONS. Michael O'Donovan* and Stephen Ho. Lab. of Neural Control, NINDS, Bethesda, MD, 20982

Imaging studies have revealed that spinal neurons in the chick embryo accumulate intracellular calcium during spontaneous activity through an unknown mechanism. Here we examine the mechanisms responsible for activity dependent calcium accumulation in motoneurons retrogradely filled with fluorescent dextran-conjugated calcium sensitive dyes: fura-2 and calcium green. Fluorescent responses were monitored using real-time intensified video-microscopy during stimulation of spinal nerves or ventral roots. Fluorescent changes could be detected in motoneurons following a single stimulus and progressively increased in amplitude during stimulus trains (1-5 sec) at frequencies from 5-50Hz. The single stimulus transient peaked within 2-3 video frames after the stimulus, exhibited an approximately exponential decay with a 1/2 width of ~200 msec, which was briefer than transients recorded from dorsal root ganglion cells, but similar to transients in motoneurons loaded with fura-2AM. Fluorescent responses were almost abolished during perfusion with 0 Ca^{++}, and exhibited a dependence on the extracellular concentration of Ca^{++}. They were increased during perfusion with 4-aminopyridine (2.5 mM), and could be depressed by Ni^{++} (100-200 μM) and dihydropyridines (20-50 μM). A significant stimulus-induced fluorescent change remained in the presence of Ni^{++} and dihydropyridenes that could be blocked by Cd^{++}. These results suggest that the intracellular calcium changes accompanying action potentials in embryonic chick motoneurons are the result of calcium entry through low and high voltage activated calcium channels, although we cannot exclude a contribution from Ca-induced release from intracellular stores.

550.6

ONTOGENY OF MOTONEURONS, ACETYLCHOLINE AND MUSCLE FIBERS IN THE VOCAL MOTOR CIRCUIT OF A TELEOST FISH. M. Marchaterre*, M. Lindholm and A. Bass. Section of Neurobiology and Behavior, Cornell Univ., Ithaca, N.Y. 14853 and Bodega Marine Laboratory, Univ. California, Bodega Bay, CA. 94923.

Sexually mature males and females of the plainfin midshipman (*Porichthys notatus*) vocalize by the simultaneous contraction of sonic muscles attached to the lateral walls of the swimbladder (review: TINS 15:139). Previous studies have demonstrated that each muscle is innervated ipsilaterally by motoneurons located in caudal brainstem sonic motor nuclei. Biocytin was used as a retrograde tracer to identify paired, midline sonic motor nuclei in specimens ranging from 0.85-2.5 cm total length (~14-40 days post-fertilization). Following ipsilateral applications of biocytin to the sonic muscle, there is a Golgi-like filling of motoneurons; label is bilateral with increasing survival times. During these stages, myofibers differentiate and sonic motor axons form 'typical' neuromuscular junctions (cf. Brain Res. 251:312). Surprisingly, sonic motoneurons at these stages do not express choline acetyltransferase (ChAT), the rate limiting enzyme in the synthesis of acetylcholine. In contrast, other nearby motor nuclei are ChAT-positive, as are sonic motoneurons in sexually mature adults (AB8 monoclonal antibody; cf., J. Comp. Neurol. 275:87). The results suggest that: (1) acetylcholine synthesis is not requisite for early events in motoneuron and muscle fiber differentiation in this vocal pathway, and (2) there is late onset of ChAT expression, perhaps linked to sexual differentiation of the vocal motor phenotype. Supported by NSF, Cornell University Biotechnology Program and New York State Hatch Act.

550.7

THE ONTOGENESIS OF PROPRIOSPINAL CONNECTIONS IN THE OPOSSUM, MONODELPHIS DOMESTICA. G. Cassidy* and T. Cabana. Sciences biologiques, Université de Montréal, C.P. 6128, Succ. "A", Montréal, QC, Canada, H3C 3J7.

The small Brazilian opossum *Monodelphis domestica* is born more immature than placental mammals. At birth, the hindlimbs (HL) are little more developed than embryonic buds that do not move independent of the trunk, and HL muscles are not all individualized (Astrow & Thompson, Soc. Neurosci. Abst. 153.5, 1989). It is possible to observe postnatally the development of HL motility and the coordination between them and the forelimbs (Cassidy *et al.*, Soc. Neurosc. Abst. 377.6, 1991). Furthermore, supraspinal centers have not reached the lumbosacral enlargement (LS) of the cord at birth (Wang *et al.*, Soc. Neurosci. Abst. 377.4, 1991). In view of such immaturity of the newborn opossum's HL, we sought to verify if the connections between the two enlargements of the spinal cord, which contribute greatly to interlimb coordination and are more precocious than most projections descending from the brain, are established at birth. We have used DiI and WGA-HRP as retrograde markers to study those connections. At day 1, projections to LS originate mainly from the ventral horn of the brachial enlargement, presumably from presumptive laminae VII-VIII, and, to a lesser extent, from presumptive laminae V-VI. Projections from these laminae increase after birth and those from the more dorsal lamimae (Cabana & Parent, Soc.Neurosc. Abst. 282.2, 1989) are added. A proportion of propriospinal connections is thus established well before any behavioral evidence for them. Supported by NSERC.

550.8

EVIDENCE FOR SELECTIVE REINNERVATION IN ADULT CAT TIBIALIS ANTERIOR MUSCLE. G. A. Unguez*, S.C Bodine-Fowler, R.R. Roy, D.J. Pierotti and V.R. Edgerton. Dept. Physiological Science and Brain Research Inst., UCLA, LA., CA 90024 and Dept. Orthopaedics, UCSD, SD, CA 92039.

It is often assumed that following axotomy, reinnervation of adult mammalian muscle is non-specific for fiber type and that the consequential heterogeneity that results from the random reinnervation eventually becomes homogeneous as a result of fiber type conversion (Karpati & Engel, *Neurol* 18:447, 1968). We examined the fiber type composition of motor units (MUs) 6 months after the nerve branches innervating the anterior compartment of the tibialis anterior (TA) were cut and resutured in adult female cats. MUs were isolated via ventral root teasing, physiologically characterized and glycogen depleted (Bodine et al *J Neurophysiol* 57:1730, 1987). Fibers were typed using monoclonal antibodies specific to myosin heavy chains (MHCs) (provided by Dr. S. Schiaffino, Padova, Italy).

Based on MHC composition, at least 2 fiber types were observed in each of the 9 MUs (S, n=1; FR, n=6; FI, n=2) analyzed. Each unit, however, was markedly biased towards one fiber type. In one unit, 3% of the fibers coexpressed slow and fast MHCs within the same fiber. Chi-square analyses showed significant differences ($p<0.05$) between the observed frequencies of each fiber type within a MU and the frequency of occurrence of each fiber type within the territory of the unit. This suggests that the reinnervation process was not random. The mean percentages of slow (10%) and fast (90%) fibers in reinnervated muscles were similar to that of control TA muscles. Further, after self-reinnervation, the distribution of unit fibers expressing different MHCs within a unit displayed a normal gradient of fiber types across the muscle compartment, i.e. the proportions of slow, high oxidative fibers decreased, and fast, low oxidative fibers increased along the deep-superficial axis of the muscle. In spite of a greater than normal incidence of MU fibers to be adjacent (Bodine-Fowler et al *Muscle & Nerve* In Press), fiber type grouping was not apparent in any self-reinnervated muscles. Together, these data are consistent with the interpretation that TA motoneurons selectively reinnervated fibers and that this process was highly correlated with MHC expression. (Supported by NIH Grant NS16333).

550.9

MORPHOLOGIC AND FUNCTIONAL EFFECTS OF PHENOL ON PERIPHERAL NERVE AND MUSCLE S.C. Bodine-Fowler*, F.L. Dulbecco, L.R. Kitabayashi and M.J. Botte Dept. Orthopaedics, UCSD School of Medicine and VA Medical Center, San Diego, CA 92161-9151

Intraneural or perineural phenol injection is an accepted procedure used to produce a temporary but long lasting peripheral motor nerve block in spastic muscle. The purpose of this study was to evaluate the morphologic and functional effects of phenol application on peripheral nerves and skeletal muscles and to establish the degree to which nerve and muscle recover after the nerve block. A 5% aqueous solution of phenol was applied to the sciatic nerve of the rat by intraneural injection using a 30-gauge hamilton syringe or by bathing the the entire nerve with the solution. Phenol was injected into the nerve or applied extraneurally until a complete motor block was produced, i.e., no detectable nerve conduction or contraction of lower limb muscles. At 2 days, 1 wk and 2 wks the sciatic nerve and soleus (SOL) and tibialis anterior (TA) muscles were removed. At each time period, muscle contraction could not be elicited with stimulation of the sciatic nerve proximal to the site of phenol application. Phenol produced extensive degeneration of the axons and demyelination of the nerve fibers resulting in denervation of the SOL and TA muscles. At 2 days there was no significant atrophy in the SOL or TA. At 1 wk, the weights of the SOL and TA were 66 and 82% of control values, respectively. At 2 wks, the weights of the SOL and TA were 51 and 50% of control values, respectively. In general, the slow fibers atrophied more rapidly than the fast fibers. Phenol appears to produce a motor block by causing axonal degeneration without damaging the endoneurial tube. The degree to which axons reinnervate the appropriate muscle remains to be determined.

550.10

MILK PROMOTES COUPLING OF BEHAVIORAL SETS IN THE FETAL RAT. S. R. Robinson* and W. P. Smotherman. Laboratory of Perinatal Neuroethology, Center for Developmental Psychobiology, SUNY-Binghamton, Binghamton, NY 13902-6000.

During the late prenatal period, fetal rats exhibit spontaneous motor activity that is characterized by synchrony of movement, sequential organization, and temporal patterning (bout structure and cyclicity). The prenatal emergence of motor organization also is evident in the expression of behavioral sets: discrete periods of time comprising patterns of behavior that are similar based on their spatial or temporal distribution. In the present study, fetal rats were directly observed on day 20 of gestation during 60-min sessions to characterize behavioral sets defined by spatial (part of the body) or temporal (intervals between successive movements) criteria . Motor activity comprising rostral elements (head, forelimbs) tended to alternate with periods predominated by caudal elements (rearlimbs). Simulation studies employing Monte Carlo techniques indicated that the duration of spatially-defined sets was longer than expected if rostral and caudal movements were randomly ordered. Sets defined by temporal criteria (variability of inter-movement intervals) also persisted longer than expected by chance. In the absence of explicit stimulation, periods of high or low rearlimb activity and periods of high or low interval variability were poorly associated. However, a single 20 µl infusion of milk (bovine light cream) into the mouth of the fetus increased the duration of rostral-caudal sets and promoted synchronization of spatial and temporal variables. The independence and stimulus-evoked coupling of these behavioral variables in altricial rat fetuses suggests that behavioral state organization may result from self-organization and not from the maturation of central regulatory mechanisms during the prenatal period.

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550.11

ELECTRICAL ACTIVATION AND INHIBITION OF RESPIRATION IN VITRO. O. Hamada*, E. Garcia-Rill and R.D. Skinner, Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR

Previously we reported that the rib-attached brain stem spinal-cord preparation in vitro showed spontaneous rhythmic respiratory movements at a consistent rate around 0.1 Hz at a temperature of 25°C . Transection at the pontomedullary level caused an immediate increase in respiratory rate to 0.2 Hz, and exposure to high temperature (33°C) increased the rate as high as 0.3 Hz. We also were able to dictate the onset of respiratory activity with electrical stimulation of the cervical cord and drove it to 0.2 Hz (Hamada et al. 1991). Previous in vitro studies have shown that electrical stimulation of the caudal ventrolateral pons inhibited the respiratory rhythm generated by the medullary respiration generator (Hilaire et al, 1989).

The present study was undertaken to determine the effect of electrical stimulation delivered to the medulla, the pons and the combination of both so some of the functional interactions within the brain stem could be revealed. 25 S-D rats aged 0-2 days were used in this study. Stimulation required to activate respiration consisted of low frequency pulses (0.2-0.4 Hz) and the most effective area was the ventromedial medulla. In 15 of 19 cases, stimulation at 0.2-0.3 Hz activated respiration at the same frequency using low amplitude currents (23.3±13.5µA at 0.2 Hz, 24.7±13.6 µA at 0.3 Hz). In 7 out of these 15 cases, respiration followed 0.4 Hz stimulation. Stimulation of the pons at low (0.1-0.4 Hz) frequency did not activate respiration, whereas stimulation of the pons at high (3-10 Hz) frequency inhibited spontaneous respiration. The ventrolateral pons had a lower threshold for inhibition than the ventromedial pons (24.0±5.6 µA, 34.0±8.9 µA, respectively) and poststimulus inhibition was evident only when the ventrolateral pons was stimulated.

Our results suggest that the ventromedial medulla was most effective in driving, and the ventrolateral pons was more efffective than the ventromedial pons in inhibiting, respiratory activity. They also suggest that the upper limit of the respiratory rate generated in the medulla in this preparation is approximatley 0.4Hz.

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550.12

CENTRAL PATTERN GENERATORS AS REGIMES OF OPTIMAL MOTOR CONTROL SYSTEM FUNCTIONING. K.V. Baev*, Yu.P. Shimansky, Dept. of Physiol. of the Spinal Cord, A.A.Bogomoletz Inst. of Physiol., 252024 Kiev, Ukraine.

It was shown in experiments on scratching and locomotor reflexes in decerebrate cats that the most important peculiarities of their afferent control are common to the corresponding central pattern generators (CPGs). A concept of universal spinal motor control system, which is capable of controlling movements of both postural and rhythmic types by using the same functional mechanism, is put forward. From the mathematical view-point, this system should be considered as an optimal control system possessing, for its optimality, the intrinsic model of the object it controls. Thus, the problem of afferent control of CPGs is, actually, the problem of the mechanism of interaction between peripheral afferent inflow and the internal flow originated from the controlled object model. The key role of presynaptic inhibition in this interaction is revealed.

These ideas allow to ascribe functional meanings to all the main ascending spinal cord pathways. Ascending tracts convey informational signals of three types: i. about the current phase state of the controlled object obtained during the above interaction; ii. information about peripheral events not covered by the internal model; iii. information on mismatch between real peripheral events and those predicted by the internal model. A concept of interaction between the spinal motor control system and higher brain motor control sub-systems during control of complex motor automatisms and learning is suggested.

551.1

INFLUENCE OF THALAMIC PROJECTION PATTERNS ON DENDRITIC MORPHOLOGIES OF CELLS IN LAYER 4 IN CAT VISUAL CORTEX. A.Kossel* and J.Bolz, Friedrich-Miescher Labor der Max-Planck Gesellschaft, 7400 Tübingen, Germany

Thalamocortical afferents in layer 4 of the primary visual cortex are segregated according to eye of origin into alternating patches that represent the system of ocular dominance (OD) columns. The activity-dependent formation of OD columns during development and its perturbation by visual deprivation during the critical period has been thoroughly studied at the level of the thalamic afferents. However, it is not known whether this reorganization of the presynaptic terminals is accompanied by a remodeling of their postsynaptic target cells in layer 4. In the present study we made *in vivo* injections of the fluorescent tracer DiI into layer A of cat LGN to label anterogradely thalamic afferents of one eye only. By intracellular injections of Lucifer Yellow in slice preparations we were able to visualize simultaneously the morphology of cells in layer 4 and the termination pattern of the thalamic afferents. A quantitative analysis of the dendritic branching revealed that spiny stellate cells with their somata in the middle of OD columns as well as in the middle of layer 4 had nearly radially symmetrical dendritic fields. Cells near the upper or the lower borders of the thalamic afferents showed strong asymmetries in their dendritic fields; their dendrites were for the most part confined to the afferent recipient zone. Finally, the dendrites of cells near the border between two OD columns showed a tendency to avoid crossing the OD borders. We also examined monocularly deprived animals and found that cells in deprived visual cortex also respected the upper and lower borders of the thalamic input zone. Likewise, cells in the columns of the open eye respected the OD borders. In contrast, cells in the deprived columns extended their dendrites into neighboring columns. These results indicate that the dendritic fields of cortical neurons are shaped by patterns of afferent inputs. Thus, the segregation of thalamic afferents into OD columns during normal development and its perturbation by visual deprivation plays a significant role in defining the structure of cortical neurons.

551.2

THE DEVELOPMENT OF GENICULOCORTICAL PROJECTIONS IN ORGANOTYPIC CULTURES. R.B.Lotto*, S.Rennie and D.J.Price. Dept. of Physiology, Univ. Med. Sch., Teviot Place, Edinburgh EH8 9AG, U.K.

Our aim is to investigate factors that influence the development of the projection from the lateral geniculate nucleus (LGN) to the visual cortex using an in vitro organotypic co-culture system. Explants of occipital cortex and LGN from prenatal and neonatal mice were cultured on collagen-treated filters suspended in a chemically defined serum-free medium. Axons growing from geniculate neurones were labelled with the carbocyanine dye, diI.

When the LGN from embryonic day 16 (E16) mice was cultured alone, large fiber tracts developed within it but appeared unable to emerge onto the collagen filter. However, the presence of the occipital cortex, or the use of medium conditioned by the occipital cortex, promoted the outgrowth of axons onto the filter.

These results suggest that the occipital cortex releases a diffusable factor that induces outgrowth from the LGN, although it is not necessary for growth within the nucleus. It is possible that this substance plays a role in directing geniculocortical axons towards the developing cortex in vivo.

551.3

EARLY DEVELOPMENT OF CORTICOCORTICAL CONNECTIONS IN THE CAT'S VISUAL CORTEX. D.Caric* and D.J.Price. Dept. of Physiology, Univ. Med. Sch., Edinburgh EH8 9AG, U.K.

Our aim was to investigate the early postnatal changes in the organization of the area 17-to-18 association projection.

We injected neuroanatomical tracers in the visual cortex of 1-30-day-old kittens. In some, the retrograde tracer diamidino yellow (DY) was injected in area 18 or the white matter below it, to label cell bodies in area 17. In others, the axons of association cells in area 17 were visualized with anterogradely transported carbocyanine dyes, DiI and DiA.

Our results with DiI and DiA reveal that axons from area 17 have reached area 18 at birth. The majority of these fibres project highly divergently and terminate in deep layers; a few penetrate the superficial layers at regions topographically related to the position of the injection. As the pathway develops, the topographic projection to the superficial layers becomes denser, while the exuberant projection to the deep layers is reduced. Results with DY confirm these observations: injections involving the deep layers of area 18 and the underlying white matter produce more widespread labelling of area 17 than those restricted to superficial layers.

551.4

TRANSITORY CORPUS CALLOSUM AXON TERMINALS IN RAT SHOW SYNAPSE FORMATION IN VISUAL CORTEX. A.J. Elberger*, M.M. Hester and S.-L. Ding. Dept. of Anatomy and Neurobiology, The University of Tennessee Memphis, Memphis TN 38163.

A previous study using DiI to label corpus callosum (CC) connections in rat revealed a substantial number of transitory CC axons extending throughout visual cortex that gradually disappear during the first postnatal month (Elberger, Soc. Neurosci. Abstr. 1991). These axons had large *en passant* and terminal swellings in all cortical layers; the swellings were hypothesized to be synapses. To evaluate this hypothesis in neonatal rats, biotinylated dextran amine was injected into the visual cortex of one hemisphere to anterogradely label CC axons. The rats were sacrificed during postnatal week 2 and coronal sections were histochemically processed (Veenman et al., J. Neurosci. Meth. 41:239, 1992). Slices were processed for electron microscopy and regions within area 17 were selected for further analysis.

Labeled CC axons were detected by their shape and homogeneous dark appearance. Some regions of enlarged axonal diameters appeared to form synapses because within the labeled axon there were numerous vesicles, many clustered near the axonal membrane. In addition, there was a second parallel membrane forming a cleft; in some cases, a densification was coincident with the second membrane. These features support the conclusion that transitory rat CC axons form synapses in neonatal cortex; these presumed synapses were located as superficially as layer I. The formation of synapses by the abundant transitory CC axons extending throughout visual cortex provides an extensive opportunity for the CC to influence the development of visual cortex. Supported by NIH grant EY08466 (AJE) and State of Tenn. Neuroscience Center of Excellence (SLD).

551.5

INTERHEMISPHERIC CONNECTIONS IN NEWBORN GALAGOS (*Galago crassicaudatus*) AND OWL MONKEYS (*Aotus trivirgatus*). P.D. Beck* and J.H. Kaas. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

Interhemispheric connections of newborn primates were studied by injecting HRP and WGA-HRP at multiple sites in dorsolateral cortex of one cerebral hemisphere of three galagos and one owl monkey within the first postnatal week. Brains were cut in the coronal plane, or cortex was removed from the rest of the brain, flattened, and cut parallel to the surface to aid reconstructing surface-view patterns. In both primates, the total pattern of callosal connections was remarkably adult-like. In all regions of cortex, labeled neurons and processes were unevenly distributed, and there was no clear evidence of excessive exuberance. Callosal connections were concentrated along the 17/18 border in both primates, and they extended only a short distance into area 17 in owl monkeys. In galagos, area 17 was more extensively connected with the other hemisphere, and connections tended to overlap the CO blobs. In galagos, callosal connections were sparse in CO dense portions of S-I and dense in CO light portions. Such compartmentalization has not yet been reported in adult galagos, but it may be a common feature of S-I in mammals. (Supported by NEI EYO2686).

551.6

MORPHOLOGICAL DIFFERENTIATION OF LAYER 5 PROJECTION NEURONS FROM THE IMMATURE RAT VISUAL CORTEX STUDIED *IN VITRO*. Ekkehard M. Kasper* Joachim Lübke and Colin Blakemore. University Laboratory of Physiology and Dept.Human Anatomy, Oxford University, Parks Road, Oxford, U.K.

Recent studies have demonstrated that projection neurons in layer 5 (L5) of the adult rat visual cortex differ in their intrinsic morphological and physiological characteristics which are correlated with their respective axonal projection target (Kasper et al.,1991). Corticotectal neurons have a prominent apical dendrite extending into layer 1 where it forms an extensive arborisation. Interhemispherically projecting neurons possess a slender apical dendrite which tapers below layer 1 and does not arborize in form of an extensive terminal tuft.

To find out how early the two characteristic morphological types can be distinguished in cortical development, we have now studied L5 neurons in a fixed brain-slice preparation of late prenatal and early postnatal cortex (E17 - E19; P1 - P5). Brains were perfusion-fixed at various postnatal stages and coronal slices prepared. Individual cortical neurons were intracellularly injected with Lucifer Yellow, subsequently photoconverted, and drawn according to Buhl and Lübke (1989).

At E17 and E19, no morphological differences were observed between L5 neurons. However, at P5 corticotectal and interhemispherical neurons had aquired their distinct morphology.

551.7

LOCALIZATION OF NEURONS RETROGRADELY LABELED AFTER INJECTIONS OF ^{125}I-NGF INTO THE OCCIPITAL CORTEX OF RAT DURING POSTNATAL DEVELOPMENT.

L.Domenici*,[1]G.Fontanesi,[2]G.Traina,[2] P.Bagnoli,[3,4]A.Cattaneo and [5]L.Maffei. *Inst.of Neurophysiol.CNR, [1]Dept.of Physiol.and Biochem., [5]Scuola Normale Superiore,Pisa 56127; [2]Dept.of Environmental Science,Viterbo; [3]Inst.of Neurobiol.,CNR,Roma; [4]Sissa,Trieste,Italy.

We studied the distribution of neurons trasporting labeled NGF, injected in the rat occipital cortex at various postanatal ages. ^{125}I-labeled NGF (0.5-1 ul) has been injected into the posterior occipital cortex of rats at the postnatal age P14, P20, 3 months. NGF was iodinated by the chloramine T method (protein concentration=5 ng/ul; specific activity= $5x10^7$ cpm/ug). The animals were perfused (under chloral hydrate anaesthesia) 24 hours later. After an exposure time of 3 weeks, the autoradiograms were developed and the slices counterstained with Cresyl Violet. The results showed that NGF was taken up and retrogradely transported by forebrain neurons at all the postnatal ages investigated. After injection of NGF at P14 and P20 labeled cells were found within the visual cortex ipsilateral to the injection site. These cells were not found in adult rats (3 months). In conclusion, the present results confirm earlier findings in forebrain neurons projecting to the visual cortex (M.Seiler and M.Schwab,Brain Res.300:33-39,1984) and further suggest that in cortical cells, the expression of receptors for NGF or for related compounds could be modulated during postnatal development.

551.8

THE TOPOGRAPHIC ORGANIZATION OF PRIMARY VISUAL CORTEX (AREA 17) IN THE ALBINO FERRET M.P. Graham, E.V. Joynes and I.D. Thompson University Laboratory of Physiology, Parks Road, Oxford, OX1 3PT, U.K. (SPON: Brain Research Association)

Electrophysiological mapping of visual cortical receptive fields was performed on albino and pigmented adult ferrets (*Mustela furo*), which were anaesthetised (70:30 N_2O:O_2, Sagatal 1 mg/kg/hr) and paralysed (Flaxedil 35 mg/kg/hr). Electrophysiologically, the location of the 17/18 border was defined by a reversal in the progression of receptive field positions, and by qualitative changes in receptive field properties. The visuotopic mapping was subsequently related to cortical cytoarchitecture. In pigmented animals, area 17 contained a single visuotopic map of the contralateral hemifield, oriented as previously reported by Law et al. (1988). In the albino ferret, however, area 17 also included a representation of the ipsilateral hemifield, which was topographically continuous with that of the contralateral hemifield. The organization of the map is such that a given cortical site is associated with a single receptive field and the most ipsilateral fields are located adjacent to the 17/18 border. Such an organization is analogous to the "Boston" pattern reported for the Siamese cat (Hubel & Wiesel, 1971). The maximum extent of this ipsilateral representation ranged from 10° to 32° for different albino animals, compared with 2° to 4° for the pigmented animals. For a given animal, the extent of the ipsilateral representation increased with receptive field elevation; in one albino, for example, from just 9° ipsilateral at 13° below the horizontal meridian, to 20° ipsilateral at 10° above the horizontal meridian. (Receptive field positions are corrected for a standard optic disc projection of +30° azimuth and +11° elevation). We are now using anatomical techniques to investigate the development of the geniculocortical projections which presumably underlie these topographical arrangements.

551.9

DEVELOPMENT OF BINOCULAR VISUAL FUNCTIONS IN MONKEYS. Rick J. Brown, James R. Wilson, Yvette P. Veira, Jamie L. Goss and Ronald G. Boothe*. Depts. of Psychology, Ophthalmology, Anatomy & Cell Biology, and Yerkes Regional Primate Research Center, Emory University, Atlanta, GA 30322.

A number of characteristics of binocular vision are immature at birth and develop concurrently during postnatal development. These functions are often disrupted by visual deprivation rearing, but few norms are available regarding the exact sequence of emergence during normal development. Thus, it is impossible to differentiate deprivation effects that involve failure to develop from effects that involve deterioration. We are addressing this issue by using a combination of cross-sectional and longitudinal methods to track normal development of several binocular functions in infant rhesus (Macaca mulatta) monkeys. Our methodologies used for these assessments include visually evoked potentials (VEP), optokinetic nystagmus (OKN), preferential looking (PL), and corneal reflex photography. During the first few weeks after birth, ocular alignment changes from intermittent exotropia to predominantly orthotropic; stereoscopic sensitivity to horizontal disparity emerges, and there is a conversion of an immature motion response from a nasal directional bias to symmetry. Thus, visual deprivation has to begin shortly after birth in order to have the potential to disrupt emergence of these functions. Deprivation that begins later is more likely to involve deterioration than failure to develop. This situation contrasts with spatial resolution which continues to develop for several months, and thus remains susceptible for a longer period to both disruption of development and deterioration. Supported by NIH Grants T32EY07092, EY-05975, RR-00165, and The Bryan W. Robinson Foundation, Inc.

551.10

DEVELOPMENT OF DISPARITY SENSITIVITY IN A CORRELATIONAL-BASED NETWORK MODEL OF THE VISUAL CORTEX REQUIRES TWO PHASES. GS Berns, P Dayan and TJ Sejnowski*, The Salk Institute, La Jolla, CA, 92037.

A correlational-based model of development of disparity sensitivity in the visual cortex was simulated. Two one-dimensional input layers, representing retinal and thalamic inputs from each eye, were fully connected to a single one-dimensional cortical layer with fixed intra-cortical connections. The weights were modified by a linear Hebb rule using correlations both within and between eyes and were subtractively normalized. Weights that reached zero were frozen. Three developmental paradigms were investigated: 1) retinal activity locally correlated within each eye but not between eyes, which might occur during prenatal development; 2) retinal activity locally correlated both within and between eyes, which might occur during postnatal development; and 3) two-phase development with the first phase corresponding to paradigm 1 and the second phase corresponding to paradigm 2, modelling both pre and postnatal development. The development of disparity and ocularity are intimately linked in our model. With no between-eye correlation, the cortex developed only monocular cells without any disparity sensitivity. Between-eye correlations throughout development led to a cortex of uniformly binocular cells with the receptive fields of both eyes aligned and thus tuned to zero disparity. The two-phase paradigm allowed the initial development of a monocular bias which was partially reversed by the addition of between-eye correlations. This resulted in a cortex populated by both monocular and binocular cells, the binocular cells tending to have zero disparity and the more monocular cells having nonzero disparity, thus matching the experimentally observed relationship of disparity and ocularity in the cat (LeVay & Voigt, *Vis. Neurosci.* 1988). (Supported by the Howard Hughes Medical Institute and SERC).

551.11

DEVELOPMENTAL ON/OFF COMPETITION CAN QUANTITATIVELY ACCOUNT FOR PREFERRED SPATIAL FREQUENCIES OF CORTICAL SIMPLE CELLS K.D. Miller*, Division of Biology, Caltech, Pasadena, CA 91125.

I have previously shown that a Hebbian or similar developmental competition between ON- and OFF-center inputs can lead to development of orientation-selective simple cells and their periodic organization into orientation columns (1989, 1990, this meeting; *NeuroReport* **3**:73-76 (1992)). The key assumption is the existence of a reversal in the correlation structure between ON- and OFF-center inputs, as follows: at small retinotopic separations, two inputs of the same center type (two ON-center inputs) are better correlated with one another than are two inputs of opposite center type (an ON- and an OFF-center input); but at larger retinotopic separations, where one input's receptive field (RF) center overlaps another's RF surround, two inputs of opposite type are best correlated.

Here I show that this hypothesis also can quantitatively account for the mean preferred spatial frequency (MPSF) of cortical simple cells. The model predicts that the MPSF of these cells is that which maximizes the Fourier transform of the correlation function $C^D(\alpha) \equiv C^{\mathrm{ON-ON}}(\alpha) - C^{\mathrm{ON-OFF}}(\alpha)$; this function tells the difference between like-type and opposite-type correlations, as a function of the retinotopic separation α. Precise comparison with experiment will require measurement of this function in the developing LGN. However, Mastronarde (1983) measured the separations at which like-type correlation and opposite-type anticorrelation falls to zero in adult cat retinal dark activity. Assuming that this corresponds to the distance at which reversal in ON/OFF correlations occurs in LGN, we obtain a prediction for MPSF in cat visual cortex that agrees well with measurements across eccentricities (Movshon, Thompson and Tolhurst, 1978).

552.1

SUPPRESSION OF THE RELEASE OF GABA BY NORADRENALINE INFUSION IN THE KITTEN VISUAL CORTEX. Shirokawa, T.* and Ogawa, T. Dept. of Physiology, Akita Univ., Sch. of Med., Akita 010, Japan.

Release of γ-aminobutyric acid (GABA) was measured by brain microdialysis and high-performance liquid chromatography in the visual cortex of anesthetized kitten. First, we confirmed that visual stimulation gave rise to a marked increase in GABA release which was completely suppressed by infusion of tetrodotoxin (TTX). However, GABA release induced by nipecotic acid, a GABA uptake inhibitor, was not affected by the TTX infusion. Next, we examined the effect of local infusion of noradrenaline (NA) on the release of GABA. Exogenous NA caused a complete suppression of visually-induced GABA release as well as basal release of GABA. These results suggest that cortical GABA release may play a role in the NA-induced ocular dominance plasticity that cortical infusion of NA caused an obvious shift of ocular dominance in favor of the eye exposed monocularly to moving visual stimuli in the kitten visual cortex (Imamura and Kasamatsu, Exp. Brain Res.,1991).

552.2

POSTNATAL DEVELOPMENT OF GABA IMMUNOREACTIVITY IN CAT VISUAL CORTEX. G.D. Mower*, J. Hoffpauir, I. Kaplan, N.G.F. Cooper. Department of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, Ky. 40292.

Immunohistochemistry (anti-GABA) was used to describe postnatal changes in the laminar distribution of GABAergic neurons and puncta in cat visual cortex. Cats at postnatal ages .5,1,5,10,15,20 weeks and adult were studied.

At one week of age and younger, a dense population of immunoreactive cells is present in two distinct bands, one superficial (layer I, future layer II) and another deep (Layer VI, subplate and white matter). There is a marked reduction in immunoreactivity in the middle of the cortical plate at these ages. At 5 weeks, there is an marked reduction in immunoreactivity in all layers, with only a few palely stained neurons present. From 10 to 20 weeks there in a gradual increase in the density and staining intensity of GABA neurons. There is a laminar gradient in staining across these ages, with cells in deeper layers (IV-VI) being densest at earlier ages and superficial layers (I-III) filling in by 15-20 weeks. A steady increase in the density of GABAergic puncta was evident from 5 to 20 weeks. By 20 weeks, staining was indistinguishable from that in the adult.

These results suggest a two stage process in postnatal development of GABA immunoreactivity in cat visual cortex. At very young ages (up to 1 week), there is dense staining of cells which could be primordial cells of the marginal zone and subplate. The subsequent decrease in GABA immunoreactivity leads us to suggest that these cells largely disappear. After formation of the mature laminar pattern, GABA immunoreactivity steadily increases in a manner suggesting an inside to outside pattern of neurochemical differentiation.

552.3

THE POSTNATAL DEVELOPMENT OF EXCITATORY AMINO ACID BINDING SITES IN FERRET VISUAL CORTEX. A.L. Smith and I.D. Thompson (SPON: Brain Research Association). University Laboratory of Physiology, Oxford University, Parks Road, Oxford OX1 3PT, U.K.

We have used a combination of transneuronal tracing techniques and quantitative *in vitro* autoradiography to investigate how the distribution and levels of EAA binding sites in cortex correlate with the establishment of geniculocortical connectivity in ferrets. Intraocular injections of wheatgerm agglutinin horseradish peroxidase showed that geniculate afferents were present in subplate at birth, had started to enter the cortical plate by PD16 and exhibited an adult-like laminar distribution in cortex at PD40. Radioligand binding studies, performed on cryostat sections, showed marked differences in the expression of kainate, AMPA and NMDA binding sites. Kainate binding sites (labelled with [^{3}H]kainic acid) are highly localized throughout development and are found mainly deep in the cortical plate or in the subplate. AMPA binding sites (labelled with [^{3}H]AMPA), however, are widely distributed in the developing ferret brain and before eye-opening exhibit a much more homogeneous distribution in the cortical plate than kainate binding sites. A variety of radioligands (L-[^{3}H]glutamic acid, [^{3}H]CGP-39653 and [^{3}H]MK-801) were employed to localize NMDA binding sites and these gave rise to different distributions implying a heterogeneity in the binding sites for these radioligands. NMDA binding sites are only present at very low levels on the day of birth and throughout early development and they exhibit a more homogeneous distribution in cortex than non-NMDA binding sites. Shortly before eye-opening NMDA binding site levels increase dramatically. Taken together these findings suggest that the subtypes of EAA binding site play distinct roles in regulating thalamocortical connectivity during development.

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552.4

AGE DEPENDENCE OF MK-801 BINDING SITES IN VISUAL CORTEX: TISSUE SPECIFICITY, BINDING SITE SPECIFICITY, AND CHANGE IN PROPERTIES. B. Gordon*, Y. Tseng, and K. Tovar. The number of MK-801 binding sites in cat visual cortex is maximal at about 42 days (d) of age. This fact, among others, suggests that these receptors may be involved in visual cortex plasticity. We asked whether this age dependence of MK-801 binding sites is specific both to visual cortex and to the MK-801 binding site. To examine tissue specificity we compared MK-801 binding in visual cortex, retina and hippocampus. We selected retina because it is never plastic and hippocampus because it is, presumably, plastic throughout life. Retinal binding was very low and did not vary consistently with age. Hippocampal binding increased significantly from 7d to 42d and decreased slightly, but not significantly, in adulthood. Thus, development of MK-801 binding parallels the critical period in both visual cortex and hippocampus, but statistically significant decreases with increasing age occur only in visual cortex. To examine binding site specificity we compared the development of MK-801 and AMPA binding in visual cortex. AMPA binding increased from 7 to 42d, but did not decrease significantly in adulthood. Therefore, the binding peak at 6 weeks may be specific to the NMDA receptor. To determine whether the properties of the MK-801 binding site varied with age, we examined the age dependence of the ability of glutamate, glycine, and spermidine to enhance MK-801 binding. Preliminary data suggest that addition of a second enhancer may be more effective in adults than in younger animals, suggesting that the properties of the binding sites vary with age.

552.5

PATCHY EXPRESSION OF *C-FOS* IN AREA 17 OF KITTENS C. Beaver, K.M. Murphy and D. E. Mitchell*. Dalhousie University, Halifax N.S. and McGill University, Montreal, CANADA.

Physiological and anatomical investigations of area 17 of cats have revealed a system of functional modules or columns based upon two salient visual response characteristics of cortical cells, namely ocular dominance and orientation preference. Both systems of columns exist in an immature state at birth, but develop rapidly afterwards in normal kittens to attain adult-like anatomical form by about 6 wks of age. The postnatal development of both systems of columns requires visual experience since their development is both arrested in kittens reared from birth in total darkness and their pattern is altered in animals that receive biased early visual experience. Recent results obtained from the use of other anatomical methods (such as cytochrome oxidase histochemistry) on cat visual cortex point to the possible existence of other principles of modular organization in addition to those uncovered earlier. In order to investigate whether any system of modular cortical organization arises by factors intrinsic to the cortex itself we have exploited the recent observation of rapid expression of the immediate-early gene, *c-fos*, in the visual cortex of dark-reared kittens following very brief exposure to light. We have examined the tangential distribution of *c-fos* in kittens reared from birth in total darkness until 30d and then allowed 1-2 hrs of either monocular or binocular visual exposure. The *c-fos* positive cells were labeled immunohistochemically and vizualized in tangential sections from unfolded and flattened visual cortex. Surprisingly, distinct patches of *c-fos* expression were evident in layers 2 and 3 of area 17 of binocularly, as well as monocularly exposed kittens. The arrangement of the patches of *c-fos* expression were compared to the modular structure revealed by other immunohistochemical methods. However, the fact that *c-fos* expression in these dark-reared kittens is so non-uniform suggests that at least some aspects of cortical modularity may be intrinsically determined.

552.6

DEVELOPMENT AND REGULATION OF ALPHA ADRENOCEPTORS IN KITTEN VISUAL CORTEX

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Vancouver, B.C. Canada V5Z 3N9

Alpha1 and alpha 2 adrenergic receptors were localized in developing cat visual cortex by using [^{3}H]prazosin, and [^{3}H]rauwolscine, respectively. Effects of neuronal activity on development of the two receptor subtypes were also studied in animals with lesions at various sites of central visual pathway. Binding densities for both ligands increased during the first few postnatal weeks and declined thereafter. For both receptor subtypes, the highest concentration of binding sites was found in the subplate zone of the neonatal cortex, then concentrated in cortical layer IV beginning at postnatal day 30 and finally in the superficial cortical layers in adulthood. However, the developmental redistribution of α1 receptors began earlier than that of the α2 sites. Quinolinic acid lesions within cortex, lesions of the lateral geniculate nucleus and of optic tract reduced binding of both [^{3}H]prazosin, [^{3}H]rauwolscine to various extents in the cortex. Our results suggest that the two α-adrenoceptor subtypes were mainly located on cortical cells and their lamination is age-dependent and the density of the receptors is regulated by neuronal activity.

552.7

Transient overexpression of IP3 receptors during the critical period for kitten visual cortex plasticity. Y.L. Liu* and M.S. Cynader. Department of Ophthalmology, University of British Columbia, Vancouver, B.C., Canada. V5Z 3N9

Inositol 1,4,5-trisphosphate (InsP-3), a second messenger generated via receptor-stimulated hydrolysis of phosphatidylinositol 4,5-bisphosphate, mediates calcium mobilization from intracellular stores. Previous studies in kitten visual cortex have shown that neurotransmitter/modulator receptors linked with PI turnover are transiently concentrated in cortical layer IV during the postnatal critical period for kitten visual cortex plasticity. In order to investigate the correlation of this second messenger molecule with the molecular basis of visual cortex plasticity, we used an antibody directed against a purified InsP-3 receptor glycoprotein of relative molecular mass 260,000 (Ross *et al*, Nature, 1989) to visualize InsP-3 receptors in kitten visual cortex. InsP-3 receptor immunoreactivity was found to be concentrated in the pyramidal cells of the deep cortical layers in the first two weeks after birth, and in pyramidal cells of layers III and V in the third and fourth weeks. Between postnatal days 40 to 60, InsP-3 receptor immunoreactivity was most highly expressed in the cortex. Immunoreactive cells were present in all cortical layers, and were most dense in layers VI, IVab and III. After postnatal day 90, the number and intensity of InsP-3 receptor immunoreactive cells decreased gradually until adulthood. In adult cat visual cortex, immunoreactive cells were most concentrated in the superficial and deep cortical layers. These results show that InsP-3 receptor immunoreactivity is transiently concentrated in different populations of cortical cells during postnatal development and that the overall expression peaks during the critical period. The relationship between the transient expression of the InsP-3 receptor and the developmental alteration in cell surface receptors that evoke PI turnover is under study.

552.8

DEVELOPMENTAL EXPRESSION OF bFGF IN THE CAT VISUAL CORTEX. I. Cokgor* and M.S. Cynader, Department of Ophthalmology, Univ. of British Columbia, Vancouver, B.C., Canada V5Z 3N9

bFGF is an important neurotrophic factor that modulates glial cell differentiation and function, neuronal maturation, survival and growth of neuritic processes. It affects glial cell motility and migration, regulates protein synthesis and is also a major trophic factor operating at all stages of embryogenesis. It is known that bFGF is widely distributed in CNS and PNS as well as other tissues.

In this study, we immunohistochemically localized bFGF in the cat visual cortex. We used rat anti-bFGF (1/1000 dilution) and examined its distribution in microtome sections of different age kitten cortices. We showed that bFGF was heavily concentrated in glial cells in the white matter, the subcortical plate and the deep cortical layers of the gray matter in young kittens. Overall immunoreactivity peaked in 10 days old kittens. In 0 and 10 day old kittens bFGF staining was also shown in growth cones and neural processes in the visual cortex. In 20 and 30 day old kittens bFGF was abundant in the white matter and layer 1 of the gray matter. Its concentration showed a dramatic decrease in older age animals in which it was homogenously distributed throughout the cortex.

We studied the cellular distribution of bFGF with double-labelling immunohistochemistry, using GFAP as an astrocyte marker. Although it showed co-localization with GFAP (proving that it is localized in the astrocytes), its distribution was not restricted solely to astrocytes. The binding of bFGF with microglia in this system is still under investigation.

These results show that bFGF is overexpressed in young kittens prior to the critical period, suggesting that it may modulate glial proliferation, cell migration and may help to set up the cortex for activity dependent plasticity.

552.9

SEQUENCE ANALYSIS OF cDNA CLONES SELECTIVELY EXPRESSED DURING THE CRITICAL PERIOD FOR VISUAL CORTEX DEVELOPMENT.

Shiv Prasad* and Max S. Cynader Department of Ophthalmology, University of British columbia, Vancouver, B.C., V5Z 3N9 CANADA.

During the first few months of a kitten's life, the structure and function of the cortex can be dramatically altered as a result of abnormal visual input. During this critical period, which peaks at about 30 days of age, studies at the protein level have documented transient elevation of several important molecules within cortical cell populations.

We have isolated cDNA clones of mRNAs whose levels are higher during the critical period of development in kittens than in adult cats. Among approximately 12000 cDNA clones from a 30 day old kitten visual cortex cDNA library, 200 were identified by screening with a subtractive probe. About 150 of these cDNA clones were found to be unique. We have now tested 50 of these single copy cDNA clones with northern blots and found that 49 of these represent mRNAs whose levels are much higher in the 30 day old kitten visual cortex than in adult visual cortex. Partial nucleotide sequences for about 100 of these cDNA clones have been determined in order to search the EMBL DNA database for identities of these clones. Twenty-two of these clones were identifiable. Their identities showed that these sequences are involved in cell-cell communication, cellular remodelling, neurotransmitter release and processing, neurofilament assembly, energy metabolism, and RNA and protein synthesis.

552.10

ISOLATION AND CHARACTERIZATION OF cDNA CLONES OF mRNAs SELECTIVELY EXPRESSED IN KITTEN LATERAL GENICULATE NUCLEUS.

J.S. Bhangav, S.S. Prasad, R.M. Douglas* and M.S. Cynader Dept. of Ophthalmology, University of British Columbia, Vancouver, B.C. Canada.

During the first few months of a kitten's postnatal development, monocular visual deprivation causes marked effects on the morphology, growth, and connectivity of cells in the lateral geniculate nucleus (LGN) connected to the deprived eye. The same manipulation performed in adult animals has no effect. To understand the molecular mechanisms underlying the age and use-dependent plasticity in this nucleus we have used the method of subtractive hybridization to isolate genes that are differentially expressed in the LGN of 30-day-old kittens and not in adult cats.

Subtractive hybridization is a powerful technique for isolating differentially expressed mRNAs. A potential limiting factor for proper subtraction, however, is the requirement of large amounts of driver mRNA. We have utilized a subtractive hybridization protocol with modifications designed to avoid this problem. This method relies on a) Directional cDNA cloning b) Large scale in vivo phagemid excision and c) Generation of biotinylated run-off transcripts.

Thus far we have isolated five cDNA clones from a 30-day-old kitten LGN cDNA library that appear unique to kitten LGN. Characterization of the clones by Northern and DNA sequence analysis is underway.

552.11

ENZYMES, IONS AND RECEPTORS DISTINGUISH NOVEL, COMPLEMENTARY COLUMNAR SYSTEMS IN DEVELOPING CAT VISUAL CORTEX. Richard Dyck* and Max Cynader. Dept. of Ophthalmology, University of British Columbia, Vancouver, British Columbia, Canada, V5Z 3N9.

Using *in vitro* autoradiographic methods we have established that serotonin (5-HT) 1C receptors, labelled with [^{3}H]-mesulergine, are transiently expressed in columns through layer 4 of area 17 between 30 and 90 days postnatally (Dyck & Cynader, J Cellular Biochem, 1990), and that the columnar expression of these receptors is input- and experience-dependent (Dyck et al, Soc Neurosci Abstr, 1991). These 5-HT1C-rich columns are on average 420 μm in diameter with a centre-to-centre spacing of ~900 μm. Here, we present evidence that other molecules associated with serotonergic, glutamatergic and cholinergic function, as well as several metabolic enzymes, exhibit a patchy distribution in kitten visual cortex and are differentially localized within or between 5-HT1C columns to form an interdigitated mosaic. In carefully aligned, transverse sections through the opened and flattened kitten visual cortex, zinc-containing, glutamatergic, terminals were preferentially distributed in layer 4 of area 17, in the same columns as 5-HT1C receptors while the distribution of acetylcholinesterase- (AChE), cytochrome oxidase- (CO), and succinate dehydrogenase- (SDH) positive patches were limited to the layer 3 / 4 border and avoided the Zn / 1C columns. The patchy distribution of a second 5-HT receptor, the 5-HT2 subtype labelled with [^{125}I]-DOI, was also limited to the layer 3 / 4 border in area 17, but these patches were vertically aligned with Zn / 1C columns. Unlike the Zn /1C columns, 5-HT2 receptors were also highly expressed in area 18 and lateral suprasylvian cortex. This distinct anatomical distribution of 5-HT receptors suggests that the 1C receptor subtype is associated with the geniculocortical X pathway while 5-HT2 receptors mark the Y pathway transiently in development. The role of these multiple markers in column formation within the cortex is under investigation.

553.1

SUBMICROMOLAR CONCENTRATIONS OF LEAD CAUSE ABERRENT GROWTH OF RETINAL AXONS IN THE OPTIC TECTUM OF RANA PIPIENS TADPOLES. H. T. Cline* and L. C. Chen. Dept of Physiology & Biophysics, Univ. of Iowa, Iowa City, IA. 52245.

The retinal projection to the optic tectum of the frog tadpole is a well characterized system for the study of factors regulating neuronal development. We examined the possible influence of low levels of lead on axon growth. Lead (10^{-6}M - 10^{-8}M) was applied to the optic tectum of Rana pipiens tadpoles using the slow release polymer Elvax. We estimate that concentrations of lead released by the Elvax were10^{-8}M - 10^{-10}M, respectively. Tadpoles were T&K stage V at the beginning of treatment and stages X - XIII when we assayed the arbors. We reconstructed a total of 13 lead-treated HRP-labeled RGC axon arbors at the following concentrations: 4 at 10^{-6}M; 3 at 10^{-7}M; 6 at 10^{-8}M. RGC axon arbors which were exposed to lead for 2 months are severely reduced in size to about 30% of stage-matched control arbors. Despite their small size, the treated arbors display as many branchtips as controls. Consequently, the arbors have a branch density (branchtips/μm^2) about 4 times the normal value. The treated arbors also appeared to have many more growth cones than untreated arbors. These data suggest that lead may increase the rate of branch initiation, but the branches are not stabilized as they are in controls. We have begun to examine the development of tectal cells to determine if similar treatments might influence the growth of both the pre and postsynaptic partners in the retinotectal system. Supported by the McKnight Foundation.

553.2

NMDA AND NON-NMDA MEDIATED SYNAPTIC TRANSMISSION IN THE OPTIC TECTUM IN VITRO: DIFFERENCES BETWEEN XENOPUS AND RANA. S.G. Brickley and S. Grant (SPON: Brain Research Association). Div. of Neurophysiology & Neuropharmacology, NIMR, London NW7 1AA, and Dept. of Anatomy, CXWMS, London W6 8RP, UK.

A vision-dependent plasticity of synaptic connections in a commissural pathway, which re-aligns binocular tectal maps after early eye rotation occurs in Xenopus but not in Rana. The NMDA-type glutamate receptor has recently been implicated in this plasticity. Here we investigate the role of both NMDA and non-NMDA receptors in synaptic transmission at the tectum of these species, using an in vitro preparation. Postsynaptic potentials recorded in the superficial neuropil following optic tract stimulation showed early and late components, corresponding to the U1 and U2 responses seen in vivo (Chung et al, 1974: Proc. R. Soc. Lond. B. **187**, 421-447). Bath application of 20μM CNQX reduced the peak amplitude of both components (by ~60%) in both species. Application of 50μM AP5 affected only the long-latency U2 component in Xenopus(~30% reduction in peak amplitude) and had no effect in Rana. The NMDA-receptor mediated component of the long-latency response may provide a substrate for the vision-dependent plasticity that is exhibited only in Xenopus.

553.3

NMDA RECEPTOR CONTRIBUTION TO EPSCs IN THE DEVELOPING FROG OPTIC TECTUM. S. Witte* and H. T. Cline, Dept. of Physiology and Biophysics, University of Iowa, Iowa City, IA 52242.

The NMDA receptor has been implicated in a variety of neural plasticity phenomena, including long term potentiation, spatial memory, and activity dependent sorting of sensory afferents. Refinement of retinotectal projections in the frog is blocked by chronic application of the NMDA receptor antagonist APV. However, the sorting of nasal from temporal retinotectal fibers, occurring earlier in development, has been shown to be activity independent. We hypothesize that NMDA receptor activation may contribute differentially to EPSCs recorded from optic tectum neurons of tadpoles at different developmental stages.

Whole cell patch clamp recordings were made from a region in the middle third of the optic tecta of isolated superfused brains from Rana pipiens tadpoles. The perfusate contained 1.5 mM Ca^{2+} and 2.5 mM Mg^{2+} to reduce polysynaptic activity. Recorded neurons were labelled with neurobiotin or biocytin for later examination of their morphologies to seek possible correlations with electrophysiological findings. Stimulation of the optic nerve was delivered through a suction electrode attatched to the nerve stump. Several neurons appeared to have only indirect retinotectal connections. The majority of neurons displayed monosynaptic responses to optic nerve stimulation. For these neurons, separate current voltage curves were constructed from the early and late components of recorded EPSCs. Preliminary data show that in younger animals (stages III and IV) the late, voltage dependent component was very small compared to that in older animals (stage VIII). It appears that NMDA receptors contribute increasingly to retinotectal responses as tadpole development procedes. (Supported by McKnight Fund for Neuroscience.)

553.4

POLYSIALYLATED NCAM AND PLASTICITY IN XENOPUS TECTUM. S.B. Udin*, G. Rougon, J. Bandarchi, L. Gannon-Murakami, K. Murakami, S. Chen. Depts. of Physiology and Biochemical Pharmacology, SUNY, Buffalo, NY 14214.

The polysialylated form of the neural cell adhesion molecule NCAM (PSA-NCAM) has been shown to reduce membrane-membrane adhesion and thus may be permissive for reorganization of axonal connections. We have therefore compared the amount of PSA-NCAM in the Xenopus optic tectum, as revealed by immunostaining, with the degree of capacity for reorganization of ipsilateral connections to the tectum. During normal development, the ipsilateral eye's map, relayed indirectly via the nucleus isthmi, comes into register with the contralateral eye's map, relayed directly via the optic nerve. Visual input is required for this process. Abnormal visual input resulting from surgical rotation of one eye induces a systematic reorganization of the ipsilateral map. This reorganization normally can occur only during the critical period of development, lasting until about three months after metamorphosis. Plasticity can be prolonged by dark-rearing and can be re-established by chronic treatments of adult tecta with NMDA.

Using a monoclonal antibody to PSA-NCAM, we have found that the amount of staining in the superficial layers of the tectum is low in normal adults, in which plasticity is low. In contrast, staining is high in normal tadpoles, dark-reared adults, and NMDA-treated adult tecta, all of which show high levels of plasticity. These results suggest that the activity-dependent mechanisms associated with plasticity may affect connectivity in part by altering expression of variants of NCAM.

Supported by US P. H. S. Grant EY-03470 to S. B. U.

553.5

CHARACTERIZATION OF PROTEIN KINASES IN THE DEVELOPING OPTIC TECTUM. A.J. Scheetz*, L.O. Doan and M. Constantine-Paton. Yale University, Department of Biology, New Haven, CT

In order to maintain retino-tectal topography the synapses between retinal ganglion cells and their tectal targets in the *Rana pipiens* tadpole are being broken and reformed continuously throughout tadpole life. The maintenance of this topography is dependent on N-methyl-D-aspartate (NMDA) receptor activation. Such synaptic flux makes the retino-tectal projection an excellent model system for examining molecular mechanisms associated with plasticity of synapse formation. Using an *in vitro* kinase assay we have examined properties of the cyclic AMP-dependent (cAMP), calcium and calmodulin-dependent (CAM-II) and calcium and phospholipid-dependent kinases (PKC) in this tissue. Tectal kinase activity was similar to values for the mammalian enzymes, indicating that the amphibian kinases are functionally related to their mammalian counterparts. Additionally, we have investigated the effects of glutamate receptor activation on protein phosphorylation in intact tectal tissue. Fifty μM glutamate stimulated the phosphorylation of 12 proteins that we could identify as specific kinase substrates from our *in vitro* assays. Five have molecular weights and isoelectric points similar to proteins identified as CAM-II substrates; 3 appear to be PKC substrates and 4 are cAMP kinase substrates. Phosphorylation of some of these 12 substrates is likely an indirect effect of increased metabolic rate due to continuous glutamate receptor activation. We are investigating currently the difference between these general effects and specific receptor mediated events by selectively activating or inhibiting glutamate receptor subtypes, ie. NMDA receptors, in the presence of low concentrations of glutamate. In addition, we are coupling this manipulation with chronic treatments which are known to alter the specificity of new synapse formation in order to add to our understanding of the correlation between synaptic plasticity and kinase second messenger systems in developing neuronal pathways. This research is supported by NIE grant EY06039 to MC-P and NRSA EY06289 to AJS

553.6

DEVELOPMENT OF SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN THE OPTIC TECTUM OF TADPOLES. E.A. Debski* School of Biological Sciences, Univ. of Kentucky, Lexington, KY 40506.

In adult *Rana pipiens*, somatostatin-like immunoreactive (SOM-IR) tectal cells are found mainly in the caudal third of the optic tecta (Debski, Neurosci. Abstr. 17:1134, 1991). This non-uniform distribution implies that different regions of the tectum may be composed of functionally non-equivalent cells. To begin to understand what function(s) these SOM-IR cells may have, I investigated their distribution in tadpoles.

In stage XIX tadpoles, where caudal regions of the tectum are largely developed, the number and location of SOM-IR cells are about the same as in the adult. Neurites from some of the stained cells in layers 4 and 6 can be followed radially into layer 7 where they turn and extend horizontally. This morphology indicates that at least some of the SOM-IR cells may have efferent projections. In stage XIII tadpoles much of the caudal tectum is immature. Nevertheless, SOM-IR cells, present in much greater numbers than in the adult, are largely restricted to these regions. In caudal tectum that has a thickened but as yet unlaminated cellular layer, the cells are found in radially oriented clumps throughout the layer. Within the thin caudal regions of tecta that are the least mature, SOM-IR cells are seen above a thickened ventricular zone. Staining of neurites has not been observed at this stage. These data suggest that SOM-IR tectal cells are a late developing population of cells that may be specialized to adult function. Supported by BRSG SO7 RRO7114-22 and FFS GA91079.

553.7

THE EFFECTS OF RETINAL INNERVATION ON TECTAL DEVELOPMENT IN THE EMBRYONIC ZEBRAFISH. L.S. Ross and J.M. Zook*. Dept. of Biological Sciences, Ohio University, Athens, OH 45701.

Previous studies have suggested that retinal afferents regulate both the embryonic and regenerative development of the optic tectum in teleost fishes (Schmatolla, 1972; Raymond et al, 1983). We have examined the effect of eye removal on tectal development in the embryonic zebrafish to verify these effects and provide a foundation for future longitudinal studies.

One eye was removed from zebrafish embryos prior to the initiation of optic axon outgrowth, at 24 hours postfertilization. The development of the tectum was examined in sectioned material, with the opposite, innervated tectum serving as a control. In embryos as young as 72 hours, the non-innervated tectum was smaller than the innervated tectum. Volumes of the marginal and subventricular zones of the tectum were measured in 7 day old embyros. The mean volume of the marginal zone was reduced by 37.3%, and the subventricular zone was reduced by 28.0%. Examination of the tectal cytoarchitecture suggested that in the absence of innervation fewer cells migrated from the subventricular zone to populate the marginal zone. At 7 days, laminae of differentiated cells had formed in the innervated tectum; the non-innervated tectum lacked these laminae and contained 59.6% fewer cells. We are examining these proposed effects on tectal development with time-lapse video microscopy. Supported by NSF BNS91-11813 and OURC grant #861 to L.S.R

553.8

ABNORMAL RECOVERY OF RESPONSES TO VISUALLY PRESENTED PREY AND LOOMING STIMULI AFTER TRANSECTION OF THE OPTIC CHIASM IN *RANA PIPIENS*. R.F. Waldeck and E.R. Gruberg*. Temple Univ., Philadelphia, PA 19122

After complete transection of the optic chiasm frogs do not respond to visually presented prey and looming stimuli. Two months later there is recovery of function. However, unlike recovery after optic nerve transection, animals respond as if the stimulus were not at its actual position but at the symmetric position in the contralateral field. For instance, if a prey stimulus is located at an eccentricity of 40^0 left the animal will respond as if the stimulus were at 40^0 right. These animals typically respond to looming stimuli either by hitting the stimulus or jumping to the same side as the approaching stimulus. These behaviors persisted throughout the testing period, up to 13 months.

Electrophysiological recordings revealed visual activity in the optic tectum that is retinotopically organized but driven primarily by stimuli to the ipsilateral eye. Using HRP histochemistry, some retinal fibers were found to cross the midline of the chiasm. Thus, the midline is not impenetrable to crossing retinal fibers.

Abnormal behavioral recovery also occurs when 3/4 of the chiasm is cut. Initially, post-lesion animals responded to prey stimuli in 80% of the visual field. Responses were generally accurate. After two months these animals behaved similarly to the animals that recovered after complete transection. Physiological and histological results were also similar.

These results suggest there are important guidance cues at the chiasm for retinal axons. Supported by NIH grant EY04366.

VISUAL DEVELOPMENT: MAMMALIAN THALAMUS AND MIDBRAIN

554.1

DENDRITIC DEVELOPMENT OF LGN CELLS DURING LAMINAR AND SUBLAMINAR RETINOGENICULATE SEGREGATION IN THE FERRET. M. Rocha* and M. Sur, Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139; and Biophysical Institute, Federal University of Rio de Janeiro, 21941, Brazil.

The ferret LGN undergoes profound morphological changes during the first postnatal month. Retinogeniculate afferents first segregate into eye-specific laminae and then into on/off sublaminae during the first and third postnatal weeks, respectively. Here, we have examined how LGN cells acquire their mature dendritic form during this period. Ferrets between postnatal day 1 (P1) and 28 were used. Rhodamine-labeled latex microspheres were injected into primary visual cortex to backfill LGN relay cells. The detailed morphology of these cells was then revealed by intracellular injections of Lucifer Yellow in 300- μm, horizontal thalamic slices kept alive in a tissue-slice chamber. At P1, LGN cells were morphologically immature, with small dendritic arbors and few branches (mean (±s.d.) dendritic area: 2.2±1 $x10^3$ μm^2; 7±4 branch points/cell). By P7, dendritic arbor area increased several times (17.4±6.4 $x10^3$ μm^2). Dendrites were more branched (32±6 branch points/cell), distributed in a stellate or oriented pattern, and were covered with dendritic appendages (30 spines/cell). At P14, a more elaborate pattern of dendritic branching was observed (dendritic arbor area: 25.6±11.3 μm^2; 50±17 branch points/cell), and spine density had increased significantly (45 spines/cell). By P21, cells appeared adult-like in structure (dendritic arbor area: 33.6±18.9 $x10^3$ μm^2; 40±12 branch points/cell). Cells at P28 were similar in morphology, (dendritic arbor area: 29.8±5.0 $x10^3$ μm^2; 38±9 branch points/cell). We conclude that developing LGN cells undergo considerable dendritic remodelling to reach their mature morphology, attaining most of their adult features by P21. This indicates that dendritic development is correlated with the formation of appropriate retinogeniculate connections. Supported by EY07023 and CNPq.

554.2

RAPID INCREASE IN ASTROCYTIC GFAP IMMUNOREACTIVITY IN LGN OF ENUCLEATED RATS K. S. Canady*, J. F. Olavarria & E. W Rubel, Virginia Merrill Bloedel Hearing Research Center and Dept. of Psychology, Univ. of Wash., Seattle, WA 98195

We have previously found that the cessation of action potentials in the cochlear nerve (by deafferentation or TTX treatment) results in increased immunoreactivity for glial fibrillary acidic protein (GFAP-IR) in the chick cochlear nucleus within 1-3 hours (J. Neurosci. 12:1001, 1992; J. Comp. Neurol. 318:415, 1992). To determine whether this rapid astrocytic response to neuronal inactivity occurs in other systems, we examined GFAP-IR in the rat LGN at short times after unilateral enucleation. Anesthetized rats were perfused 3, 6 or 12 hours following eye removal. Paraffin sections containing the LGN were processed immunohistochemically using a polyclonal antiserum to GFAP. Area density of GFAP-IR in the deafferented LGN relative to the contralateral LGN was measured using a BioQuant microdensitometry system. Unoperated control rats showed no significant difference in GFAP-IR between the two LGNs. No reliable differences were found in the 3-hour enucleated rats either. At 6 and 12 hours following enucleation, a 30% increase in area density of GFAP-IR was found in the deafferented LGN relative to the contralateral LGN. For comparison, rats studied 3 weeks after enucleation show a 150% increase in GFAP-IR in the deafferented LGN, presumably in response to neural degeneration. Thus, our results suggest that the rapid (≤ 6 hours) astrocytic response to deafferentation is common across mammals and birds as well as across different sensory systems. Whether blockade of neuronal action potentials is sufficient to reproduce this effect remains to be determined.

Supported by PHS grant DC00393.

554.3

DIFFERENTIAL EXPRESSION OF CALCIUM BINDING PROTEINS AND CYTOSKELETAL PROTEINS IN HUMAN FETAL THALAMUS. X.-B. Liu, S.A. Mednick, S. Hodgins and E.G. Jones, Dept. of Anatomy and Neurobiology, University of California, Irvine, University of Southern California and Institute Philippe Pinel de Montreal, Canada.

The localization of calcium binding proteins, growth associated proteins and cytoskeleton proteins was examined by immunocytochemisty in the thalamus of human fetal brains at 15-20 weeks gestation.

Calcium binding protein immunoreactivity is absent from pulvinar and lateral group nuclei and stains different subpopulations of neurons and fibers in others. Parvalbumin is localized in cells of the geniculate, ventral, intralaminar and reticular nuclei, while calbindin appears in ventral nuclei cells only. Parvalbumin immunostaining is also more prominent in the optic tract and medial lemniscus. GAP-43 immunostaining is homogeneous in the thalamus and adjacent fiber tracts. Fibers immunostained for neurofilament proteins, NF-160 and SMI-32, are distributed in certain parts of thalamus, mainly following the pattern of parvalbumin fiber staining.

These results indicate the early developmental pattern of calcium binding protein immunostaining which may correlate with differential expression of cytoskeleton proteins. Temporal changes in expression of those proteins are under investigation.

Supported by NIMH grant, MH 44188-03.

554.4

FACILITATION OF LGN UNIT ACTIVITY ASSOCIATED WITH THE NON-RETINAL PONTO-GENICULO-OCCIPITAL (PGO) WAVE LACKS LAMINA SPECIFICITY. J.P. Shaffery*, H.P. Roffwarg and G.A. Marks. Sleep Neurophysiology Unit, UT Southwestern Medical School. Dallas, Tx 75235.

Development of the visual system has been shown to be dependent upon a competitive mechanism utilizing information contained in the afference from each respective retina. PGO waves represent a non-retinal source of LGN activation during the sleep phase most represented in young developing animals: rapid eye-movement sleep (REMS). Both retinal and PGO-related activation may play a role in the maturation of the visual system. Inasmuch as the retinal input to LGN demonstrates eye specificity among laminae in the cat, we have investigated whether PGO activation is also lamina-specific.

We report here that phasic PGO waves are associated with a very similar facilitation of unit activity in the A and A1 laminae of LGN. Regular and frequent PGO-like (PGOro) waves in LGN are observed in acutely urethanized, RO4-1284 (a reserpine-like drug)-treated adult cats. A bipolar, stainless steel, macro-electrode was placed in the right LGN to record photically evoked and spontaneous PGOro field potentials. An etched tungsen micro-electrode recorded neurons extracellularly in the A- and A1-laminae identified by responses to monocularly-presented photic flash. Perievent histograms of unit activity, which was analyzed in relation to PGOro wave triggers, demonstrated in both laminae clear facilitation associated with occurrence of the wave. Unlike the activation of LGN derived from the eyes, PGOro-related activation lacks laminae-specificity. These results are consistent with our observation that elimination of PGO exaggerates alterations of interlaminar cell-size disparity in LGN due to monocular deprivation.

554.5

LOCALIZATION OF CALCIUM-BINDING PROTEIN IN THE DEVELOPING CAT PRETECTUM. **B.Hutchins* and A.N.Taylor. Dept. of Biomed. Sci., Baylor Coll. of Dentistry, Dallas, TX. 75246.**

Routine Immunocytochemical techniques were used to localize the calcium-binding protein, calbindin-D 28K (CaBP) within the developing cat pretectum. Brains fixed in Bouin's were embedded in paraffin and cut in one of three stereotaxic planes. Tissues were sampled from neonatates and postnatally at 7, 14, 28, and 56 days. CaBP positive cells appeared to be randomly distributed within the nucleus of the optic tract (NTO), posterior (NPP), and medial pretectal nuclei (NPM). Within the anterior pretectal nucleus (NPA), CaBP positive cells could not be easily separated into the two subdivisions, NPAc and NPAr, however, there was a distinct ring of CaBP stained tissue along the border of NPA. The percentage of cells staining for CaBP either remained level or generally increased postnatally for NTO, NPA, NPP, and NPM, with the maximum cellular immunoreactivity never exceeding 26 %. Of particular interest, however, was the pretectal olivary nucleus. Neurons staining positive for CaBP numbered approximately 50 % for the first two weeks of neonatal life, dropping to approximately 25 % after two months of development. These preliminary data would suggest that the olivary nucleus is under considerably different developmental influences than the other four pretectal nuclei. (Supported by NEI EYO6977)

554.6

PROGRESSIVE LOSS OF SEROTONINERGIC SYNAPSES FROM THE SUPERFICIAL LAYERS OF THE HAMSTER'S SUPERIOR COLLICULUS. R.S. Crissman*, D. Jiang, E.A. Arce, C.A. Bennett-Clarke and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699

A previous report from this laboratory (Arce, E.A., et al., Synapse, in press) demonstrated that only a few (4.4%) serotoninergic processes make conventional synaptic contacts in the superficial gray layer of the superior colliculus of adult hamsters. The present study used a combination of immunocytochemistry and electron microscopic techniques to describe the development of the serotoninergic projection to the colliculus and we now report a surprising loss of synapses during the development of this projection. Serotonin-immunoreactive (5-HTIR) profiles were examined from pups killed on P-0 (the day of birth), P-3, P-7, P-12, P-15, and P-20. Of the 256 profiles examined on P-0, 36.3% made synapses. On P-3 (N=311 profiles), 40.5% made synaptic contacts; on P-7 (N=310 profiles) 32.3% made synapses; on P-12 (N=335 profiles), 37.6% made synaptic contacts; on P-15 (N=306 profiles), 17.3% made synapses; and on P-20 (158 profiles), 9.5% made synaptic contacts. Virtually all of the serotoninergic synapses made at each of these ages were axodendritic. Thus, there is a gradual decline in the percentage of synaptic contacts made by 5-HTIR profiles during postnatal collicular development in the hamster. Importantly, this change occurs without any apparent reduction in the density of the serotoninergic input to the superficial collicular laminae as evaluated by either light or electron microscopy. At present, the function of these transient serotoninergic synapses remains unknown. EY 04170, EY 08015

554.7

DEVELOPMENTAL REGULATION OF NITRIC OXIDE SYNTHASE mRNA AND NEURONAL NADPH DIAPHORASE ACTIVITY IN THE RAT SUPERIOR COLLICULUS. G. Prusky*, M. Hofer and M. Constantine-Paton. Department of Biology, Yale University, New Haven, CT 06511.

In rats, there is a topographical refinement of the retinal projection to the superior colliculus (SC) during development which is dependent upon NMDA receptor activation (Prusky et al, 1991). Since NMDA receptor activation can lead to the production of nitric oxide (NO), a messenger molecule that has been proposed as a diffusible retrograde signal at Hebbian synapses (Galley et al, 1990), we are investigating a possible role for NO in retinocollicular plasticity. Using a cDNA probe that recognizes NO synthase (NOS) (Bredt et al, 1991), we have employed quantitative Northern blot analysis of the rat superficial SC during postnatal development. NOS mRNA levels were very low during the first postnatal week, and increased moderately by postnatal day (P)12. The highest NOS mRNA levels were observed at P19. By adulthood, NOS message was reduced by 50% from levels detected at P19. Since NOS appears to be identical with nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase (Dawson et al, 1991), we have also used histochemistry of this enzyme as a marker of age-related alterations in NOS activity. There was no detectable NADPH diaphorase activity during the first postnatal week, but faint staining of neurons in the superficial SC could be detected by P12. By P19, NADPH diaphorase positive neurons were more abundant and were more intensely labelled. This trend continued and reached its peak by P27 when many neurons of different cell classes were labelled in the superficial SC. In the adult, the overall level of NADPH diaphorase reaction product in the superficial SC was lower than at P27. NOS markers have similar developmental profiles to NMDA message (Hofer et al, this meeting), and may indicate a relationship between NO production and NMDA receptor expression in the control of collicular synaptic plasticity. Supported by NEI EY06039 to MCP, and MRCC and EMBO fellowships to GP and MH respectively.

554.8

REGULATION OF NMDA RECEPTOR mRNA IN THE RAT SUPERIOR COLLICULUS. M. Hofer, G.T. Prusky and M. Constantine-Paton* Department of Biology, Yale University, New Haven, CT 06511.

Glutamate receptors have been implicated in several forms of central nervous system plasticity. Recently, it was demonstrated that the developmental refinement of the initially diffuse rat retinocollicular projection requires normal function of the N-methyl-D-aspartate (NMDA) glutamate receptor subtype (Prusky et al., 1991). Based on this observation, we are investigating the developmental expression of NMDA receptor mRNA and are testing whether it has any relation to the time course of the map formation in the superior colliculus (SC). *In situ* hybridization with a probe encoding the NMDA receptor subunit NMDAR1 (Moriyoshi et al. 1991) showed highest expression in the superficial layers of the SC at all ages examined. Northern blot analysis quantifying the 4.2 and 4.4 Kb transcripts in the superficial layers revealed low levels during the first postnatal week. A sharp increase was observed between postnatal day (P) 6 and P12 and maximal expression with tenfold higher amounts of NMDAR1 mRNA than at birth occurred at P19. In adults, NMDAR1 message was 30% lower than at P19. The ratio of the two NMDAR1 transcripts changed during this period with the 4.4 Kb band being more abundant than the 4.2 Kb band at early developmental ages and an opposite ratio at later stages. Chronic treatment with the NMDA receptor antagonist APV for 12 or 19 days led to a decrease in mRNA levels. The results show that the NMDA receptor is regulated at the mRNA level during collicular development. Furthermore, they suggest that treatment with receptor antagonists, which prevents normal retinocollicular map formation, modulates NMDA receptor expression. The sharp rise of NMDAR1 mRNA levels in the second postnatal week appears to parallel the refinement of the topographic map in the SC and supports a role for the NMDA receptor in collicular synaptic plasticity. Supported by NEI EY06039 to MCP and EMBO and MRCC fellowships to MH and GP respectively.

554.9

EARLY DEVELOPMENT OF THE RETINOCOLLICULAR PROJECTION IN THE OPOSSUM, *Monodelphis domestica*. T. W. Axlund, D. S. Sakaguchi, and C. D. Jacobson*. Dept. Zool and Genetics, Vet. Anatomy and Neurosci. Prog., Iowa State Univ., Ames, IA 50011.

The development of the retinocollicular projection in the Brazilian short-tailed opossum, *Monodelphis domestica* was examined from birth (PN1) until PN10. *Monodelphis* is a small, pouchless marsupial which breeds well under laboratory conditions and whose young are born in an extremely immature state. Retinal ganglion cell (RGC) axons were anterogradely labeled by placing crystals of the axon tracer DiI into the eyes of aldehyde-fixed specimens.

The eyes of PN1 opossums were relatively immature and immunocytochemical localization of neurofilament protein revealed axons of differentiated RGCs in only the central 1/3 of the developing eye. In PN1 opossums the contralateral projection has grown across the chiasm and proceeded a short distance up the optic tract. By PN3, RGC axons have grown approximately 2/3's the distance up the side of the midbrain towards the superior colliculus (SC). Ipsilaterally, a smaller number of optic axons were observed. RGC axons have reached the SC by PN6/7 and have begun elaborating axonal arborizations by PN8.

Examination of the optic tract region revealed numerous varicosities as well as side branches emanating from many of the RGC axons. Growth cones were identified in a number of specimens at various ages, displaying a variety of morphologies. Axons pioneering the retinal projection usually terminated in morphologically complex growth cones. We have also observed DiI labeled RGCs in the contralateral eye, suggesting the presence of inter-retinal projections at these early stages of development.

Since a great deal of the development of the visual projection occurs postnatally in *Monodelphis*, this species is likely to provide an excellent system for *in vivo* experimental manipulations and analysis.

554.10

ABNORMAL LAMINAR SELECTION AND SEGREGATION OF RETINOTECTAL PATHWAYS IN DEVELOPING RATS. C.A. Serfaty*[1,2] & R. Linden[1] [1]I. Biofisica da UFRJ, Rio de Janeiro & [2]Dept. Neurobiol. da UFF, Niteroi, Brazil.

The uncrossed retinotectal pathway in normal rodents terminates deeply within the upper grey layers and there is no binocular segregation in the tectum. We studied the retinotectal projections in rats using anterogradely transported horseradish peroxidase, after changing the balance between the pathways originating from both eyes as a consequence of either contralateral optic tract lesions (OTL) or small temporal retinal lesions (TRL) made at birth (P0). After left OTL an aberrant uncrossed projection develops at the surface of the right tectum at P3. At P10 this projection is fully developed and binocular segregation starts to appear. At P14 46% of the animals had gaps in the crossed pathway from the left eye. This figure increased to 55% at P42 and to 69% in adults. Neither bilateral nor unilateral (right) eyelid suture prevented segregation, but removal of the right eye at P14-P21 resulted in the absence of gaps in the crossed pathway from the left eye. Left TRL made at birth also produced an aberrant uncrossed projection in the right tectum, which was most prominent at the surface. Similar, though progressively smaller, rearrangements were obtained after TRL at P5-P21. We conclude that both laminar selection and segregation of the rat retinotectal pathways depend on a critical balance between the inputs from both eyes, which can be modified during a protracted period of development. *(CNPq, FINEP)*

554.11

EXPANSION AND ALTERED STRUCTURE OF RETINOTECTAL AXON ARBORS IN MONOCULARLY ENUCLEATED HAMSTERS. M. Xiong*, S.S. Lim, and B.L. Finlay, Dept. of Psych., Cornell Univ., Ithaca, NY 14853.

Neonatal removal of one eye in the hamster results in a massive loss of the contralateral input to the tectum and an expanded ipsilateral projection from the remaining eye (Finlay et al., 1976). The retinal terminal density, however, is still significantly reduced compared to normal. It is not known how the size and conformation of retinal axons are adjusted to the greater available tectal volume. In this study, we used an in vitro bulk fill technique, in which a bead of HRP was placed into the brachium of the superior colliculus to visualize individual axon arbors in tecta of both normal (N) and monoenucleated (E) hamsters. Tecta were processed with DAB histochemistry, retinal axons were drawn and the area, length and bouton number were quantified.

The retinal axon arbors of the monoenucleated hamsters had long, sparsely distributed branches with concentrated areas of boutons which covered a much larger area than those of normal animals (N=16,250±3170μm^2, n=15; E=147,200±51,070μm^2, n=6; t=4.169, p≤.001). They also had more boutons (N=161±16, n=15; E=273±76, n=6; t=2.125, p≤.05) and branches (N=39±5, n=15; E=72±20; t=2.283, p≤.05). This indicates that the increased tectal area for each retinal axon allowed them to make more contacts in the tectum than they do in the tecta of normal hamsters. However, the bouton density (N=.013±.002, n=15; E=.003±.001, n=6; t=3.82, p≤.005) and branch density (N=.003±.000493, n=15; E =.001±.000276, n=6; t=3.037, p≤.01) of the retinal axon arbors in the enucleated animals were lower, suggesting that there is an intrinsic limit to the number of boutons and branches that these axons can form.

Our previous study showed that retinal ganglion cells can reduce their arbor size by almost half to compensate for a decrease in available tectal area and remain viable. This study shows retinal ganglion cells can support greatly increased arbors, but with markedly altered clustering of terminal areas along their increased length. Supported by NIH R01 NS 19245.

554.12

REORGANIZATION OF RETINOCOLLICULAR TOPOGRAPHY FOLLOWING FETAL ENUCLEATION IN THE CAT. T.C. Steineke[2], Q.S. Fischer[1], P.D. Wilson[1], and M.A. Kirby[2*]. [1]Dept. of Psychology, U.C. Riverside, Riverside, CA 92521, and [2]Dept. of Pediatrics, Loma Linda University, Loma Linda, CA 92350.

We have examined disruption of binocular interactions during development of the retinocollicular projection in the fetal cat. Single and multi-unit recordings of light evoked responses in the superior colliculus (SC) were obtained using tungsten electrodes (1-10 megaohms) from eight adult cats, 4 that were monocularly enucleated on embryonic day 42 (gestation is 65 days). Fields were mapped on a tangent screen with reference to the optic disk and area centralis. Following the recordings, unilateral injections of horseradish peroxidase (HRP) were made into the SC. The animals were perfused 24-36 hours later and the retinas and brain sections were reacted for HRP.

In contrast to the control animals, the retinocollicular projection in the enucleates displayed several aberrant characteristics: (1) a disorganized progression of receptive field positions, (2) double response fields (simultaneous representation of extreme nasal and temporal fields at a single SC site), and (3) reversals in the caudal SC to the inappropriate hemiretina. Corresponding to the observed reversals in receptive fields, HRP labeled ganglion cells were found in the extreme nasal hemiretina. Morphologically, labeled cells resembled members of the major classes described in the normal adult retina.

Our data indicates the fetal enucleation in the cat results in a disruption of the normal topographic map combined with the production of nontopographic loci.

554.13

INNERVATION OF THE SUPERIOR COLLICULUS BY NASAL OR TEMPORAL RETINAL GANGLION CELL AXONS FROM ECTOPIC HEMIRETINAE K.T. Yee* and R.D. Lund Department of Neurobiology, Anatomy and Cell Science, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261 and Department of Anatomy, University of Cambridge, Cambridge, CB2 3BY, U.K.

Ectopic retinae introduced into postnatal hosts maintain intrinsic dorsal/ventral polarity, but afferents from these retinae show no such topographic ordering in their projection to the primary target, the host superior colliculus (SC) (Yee and Lund, '91), although synapses able to mediate behavior through the host animal are established (Lund, et al., '91). We have postulated that markers for establishing topography may no longer be present when ectopic retinal afferents arrive in the SC. Walter et al. ('87) have shown that temporal, but not nasal axons are repelled, *in vitro*, by posterior tectal membranes; this mechanism may be important for map formation in the tectum. We have examined whether a similar mechanism is operative, *in vivo*, following retinal transplantation.

Retinae transplanted to the midbrain were used to examine whether innervation patterns from nasal and temporal retinal ganglion cell axons differ. Nasal or temporal hemiretinae from embryonic day 12 mouse donors were transplanted over one SC of postnatal day 1 rat hosts. Animals were sacrificed at 3-5 weeks of age and midbrain sections were stained with a mouse specific antibody (anti-M4) to identify projections from the transplanted mouse retina.

Afferents from both nasal and temporal hemiretinae innervate the caudal aspect of the SC more heavily than the rostral portion, and show no differential patterns of innervation which reflect the region of retina transplanted.

While the hemiretinae show regional specificity of innervation, innervating only visual target nuclei of the host, mechanisms governing the establishment of normal retinotopy do not appear to be operative.

NIH EY 05308 and Action Research

554.14

DEVELOPMENT OF CORTICOTECTAL ARBORS AND SYNAPSES IN CATS. L.L. Bruce* and T.J. Neary. Div. of Anatomy, Sch. of Med., Creighton Univ., Omaha, NE 68178.

Maturing corticotectal axons undergo morphological changes that parallel development of synapses in the superior colliculus. To correlate morphological changes with synaptic development, electron micrographs were used to serially reconstruct biocytin-labeled axons and their contacts. Biocytin was injected into the visual cortex of cats aged 0 to 35 days. The tissue was processed to visualize the biocytin.

At the earliest age the labeled corticotectal axons were thin and unbranched with periodic dilations. These dilations contacted multiple vesicle-filled profiles that were absent along the lengths of axons lacking dilations. Profiles contained either clear pleomorphic vesicles or both clear pleomorphic and dense-core vesicles. Maturing labeled axons developed short side branches with presynaptic vesicles and contacted vesicle-filled postsynaptic profiles with synaptic clefts. Later axons developed elaborate arborizations with greater numbers of presynaptic vesicles and synaptic clefts that contacted dendritic profiles with fewer postsynaptic vesicles. Thus, vesicle-filled dendrites appear to interact with axonal dilations prior to the initiation of synapse formation and may guide local axonal reorganizations, and synapse selection and formation.

VISUAL DEVELOPMENT: RETINAL GANGLION CELLS

555.1

MOLECULAR DIFFERENTIATION OF GANGLION CELLS IN NORMAL AND *OCULAR RETARDATION* MOUSE RETINA. M.H. Hankin*, E. Hoover§ and D. Goldman§. *Med. Coll. of Ohio, Toledo, OH, and §Univ. Michigan, Ann Arbor, MI.

We are interested in molecular mechanisms underlying retinal ganglion cell (RGC) differentiation in normal and *ocular retardation* (orJ) mice. Since orJ mice (which show arrested retinal development) do not form retinofugal projections, we used this animal to test whether gene induction in recently differentiated RGCs is correlated with RGC axon-target interactions, as suggested in goldfish (Hieber et al., 1992. *J Neurochem* 58:1009).

We have assayed the retina for genes which are induced during RGC differentiation: gene expression was detected by in situ hybridization with cRNAs for GAP-43, nAChR α-3 and β-3 subunit genes (markers of RGCs which have migrated to their final position); protein expression was detected immunohistochemically using antibodies against neuron-specific β-tubulin (TuJ1) and a neuronal cell adhesion molecule mediating interactions between RGC axons (L1), both of which are expressed in early differentiating RGCs. In mouse retina, embryonic day 11 (E11) is the first day at which RGCs become postmitotic. At E11 we detected expression of β-tubulin (in soma and axons) and L1 (on axons) in cells spanning from the central ventricular margin to the central vitreal surface. In contrast, β-tubulin was not detected in the orJ retina until E12, implying that RGC development may lag behind that in normal mouse retina. Nicotinic AChR and GAP-43 gene expression was induced in vitreal RGCs at E12 in normal retina; by E13 the boundaries of nAChR and GAP-43 gene expression had spread peripherally. In striking contrast, nAChR α-3 and β-3 subunit genes were not induced in orJ RGCs, while the GAP-43 gene was expressed in a manner consistent with the normal retina.

These results indicate that RGCs develop in the orJ mouse, as revealed by positive TuJ1, L1 and GAP-43 expression. The lack of nAChR gene expression in orJ ganglion cells is consistent with the hypothesis that an interaction between a RGC axon and a brain target is responsible for nAChR gene induction. In addition, these results suggest that the mechanisms regulating the expression of temporally correlated ganglion cell-specific genes proceeds along diverging pathways.

555.2

AXOTOMY-INDUCED EXPRESSION OF IMMEDIATE EARLY GENES IN RAT RETINAL GANGLION CELLS. G.A. Robinson* and A.R. Light. Department of Physiology, University of North Carolina, Chapel Hill, NC 27599.

To assess the effect of axotomy on retinal ganglion cell (RGC) expression of immediate early genes (IEGs), we retrogradely labelled RGCs with Flouro-Gold (FG) applied to their targets in the midbrain and diencephalon in adult female Long-Evans rats, transected the optic nerve <1 mm from the eye and immunocytochemically identified IEG proteins (FOS, JUN and KROX 24) using Texas Red-conjugated secondary antibodies in retinal whole-mounts at various time points after axotomy.

These double-labelling studies (FG/Texas Red) revealed that JUN-like and KROX 24-like immunoreactivity increased in a small percentage of RGCs beginning approximately 3 days after axotomy and lasting at least a week after injury compared to control retinae. Expression of both of these IEGs was largely limited to FG-containing RGCs. FOS-like immunoreactivity also increased in the retina after axotomy. It is known that after axotomy close to the eye, only a small number of RGCs will sucessfully regenerate an axon into an autologous peripheral nerve graft. One possibility is that the observed expression of JUN and KROX in only a small percentage of RGCs may be related to the ability of some RGCs to sustain a regenerative response. Supported by NIH NS 14899 and 16433 and ADAMHA DA04420.

555.3

DENDRITIC STRATIFICATION OF DEVELOPING RETINAL GANGLION CELLS IS A GLUTAMATE-MEDIATED PHENOMENON. Stefan R. Bodnarenko*, Gayathri Jeyarasasingam and Leo M. Chalupa. Dept. of Psychology and the Center for Neurobiology, University of California, Davis, CA 95616

A fundamental attribute of vertebrate retinal ganglion cells (RGCs) is the stratification of their dendrites within distinct sublaminae of the inner plexiform layer (IPL). Whereas in adults dendrites of ON-center RGCs stratify nearer to the soma than dendrites of OFF-center cells, early in development RGCs are multistratified. The factors underlying the segregation of dendrites within the IPL are unknown.

We now report that repeated intraocular injections of 2-amino-4-phosphonobutyrate (APB), a glutamate agonist which in adults increases spontaneous activity of OFF-center RGCs and decreases spontaneous and light evoked activity in ON-center RGCs (Boltz et al. 1984), disrupts RGC dendritic stratification in neonatal cats. Animals received daily injections of APB into one eye beginning at age P2/P3. RGCs were labeled with DiI following application of the tracer into the fixed optic nerve. Examination of retinal cross-sections revealed a significant increase in the incidence of multistratified beta cells in the treated eye (survival age: normal vs. treated) P8: 21% vs. 39%; P10: 16% vs 29%; P13: 12% vs. 34%. The incidence of OFF as well as ON beta cells was decreased in the treated eye, suggesting that APB disrupted the normal stratification of both cell types. These results indicate that glutamate plays a role in the remodeling of RGC dendrites into sublaminae of the IPL, and imply that normal retinal activity is essential for the establishment of RGC mosaics. (Supported by EY03991 from the NEI).

555.4

DEVELOPMENT OF Na CURRENTS IN DISSOCIATED CAT RETINAL GANGLION CELLS. I. Skaliora*, L.M. Chalupa and R.P. Scobey. Depts of Psychology, Neurology and Neurobiology Graduate Group, University of California, Davis CA 95616.

We have previously shown that electrical excitability of retinal ganglion cells (RGCs) increases dramatically during the period of axonal pathfinding and initial synaptogenesis (embryonic days 30 - 38; gestation in the cat is 65 days). We now report quantitative changes in the properties of the voltage gated Na currents (I_{Na}) during the same period.

Whole-cell patch-clamp recordings are obtained from RGCs acutely dissociated from fetal and postnatal animals of known gestational ages. Ganglion cells are identified by retrograde labeling with rhodamine beads previously injected into the subcortical targets. Sodium currents were isolated by pharmacological channel blockers and ion substitution. The identity of I_{Na} was verified by its reversible block with TTX and by the influence of external [Na+] on current amplitude. The following ontogenetic modifications were observed (mean ± S.D.): (i) an increase in Na current densities, from a mean of 66.4 ± 32.4 pA/pF (n=25) at E30 to 142.4 ± 54.5 pA/pF (n=23) at postnatal ages. (ii) a significant ($p<0.05$) shift in the voltage dependence of both activation and steady-state inactivation toward the resting potential: the midpoint of the I_{Na} activation curve changes from -17.9 ± 2.4 mV (n=25) at E30 to -24.5 ± 4.1mV (n=23) postnatally; the midpoint of the inactivation curve shifts from -60.8 ± 3.7 mV (n=25) to -50.2 ± 3.4 mV (n=26) over the same developmental period. (iii) a decrease in decay time constants of the Na current, at membrane potentials negative to -15mV. These changes are largely occuring during the early part of the developmental period we studied (E30-38) and are consistent with the increase in excitability observed during the same period. (Supported by EYO 3991 from the NEI)

555.5

REGENERATING RETINAL GANGLION CELL AXONS BRIDGED BY PERIPHERAL NERVE GRAFTS CAN EXTEND INTO THE BRAIN STEM THROUGH THE TRIGEMINAL NERVE ENTRY ZONE. R. Pallini, E. Fernandez *, L. Lauretti, V. Bozzini and A. Spriccoli (#). Institutes of Neurosurgery and Neurology (#), Catholic University, Rome, Italy.

Axotomized retinal ganglion cells (RGC) of adult mammals can regenerate their cut axons over long distances into peripheral nerve (PN) grafts (Vidal-Sanz et al, J Neurosci 7:2894-2909, 1987). The regenerating RGC axons can be directed through the PN graft to selected central targets. However, most of the regenerating axons are blocked by the glial scar at the interface between the PN graft and the CNS. In the present study, we developed a new model aimed specifically at increasing the penetration of the regenerating RGC axons into the central environment.
The optic nerve (ON) of adult rats was cut intraorbitally and an autologous sciatic nerve graft (40 mm) was sutured to the ON stump. After a complete homolateral retrogasserian rhizotomy, the other end of the PN graft was apposed to the central stump of the trigeminal nerve (TN). Three-10 months after grafting, the regenerated RGC axons were labeled by anterogradely transported horseradish peroxidase (HRP) (WGA-HRP, Sigma, 8% solution) injected into the graft (0.05-0.2 µl) or vitreous body (1-2 µl).
We found that a substantial number of the RGC axons that had regenerated into the PN graft succeeded in growing through the TN entry zone and extended into the brain stem for distances of up to 1200-1300 um. Although many of these axons followed various different positional paths, a preferential growth into the spinal trigeminal tract was seen.

555.6

AN IMMUNOCYTOCHEMICAL MARKER FOR HAMSTER RETINAL GANGLION CELLS. P. G. Bhide*[1], W. C. West[1], K. R. Fry [2] and D. O. Frost[1]. [1]Dept. of Neurology, Massachusetts General Hospital, Charlestown, MA & [2] Center for Biotechnology, Baylor College of Medicine, Houston, TX.

About 50% of the neurons in the ganglion cell layer of the rodent retina are amacrine cells. Therefore, the presence of a soma in the ganglion cell layer *per se* does not identify that cell as a retinal ganglion cell (RGC). At present, hamster RGCs can be unequivocally distinguished only by retrograde labeling with neuroanatomical tracers injected into the brain.

To overcome this difficulty, we determined if AB5, a monoclonal antibody that selectively labels RGCs in rabbits, cats and monkeys (Fry *et al.*, Brain Res. 1985, 338: 360) selectively labels RGCs in developing and adult hamsters and if AB5 labels all RGCs. Retinae from postnatal day 0 (P0=first 24 hours following birth), P7, P10, P12, P14, P20 and adult (>P30) hamsters were immunostained as wholemounts or as 20-40 µm thick frozen sections using AB5 and biotinylated or FITC conjugated secondary antibodies. AB5 selectively labels perikarya in the ganglion cell layer, their proximal dendrites and their axons in the nerve fiber layer, in P12 and older hamsters. AB5 does not label somata or processes in other retinal layers or RGC axons in the optic nerve, chiasm or the brain. All RGCs that are retrogradely labeled by DiI or Evans blue injections into the optic tract of adult hamsters are AB5 positive. We are now investigating whether any AB5 reactivity remains when RGCs are ablated by neonatal optic nerve section. We could not detect AB5 immunoreactivity prior to P12. AB5 labeling is compatible with bromodeoxyuridine (BrdU) immunocytochemistry and therefore useful for studies of RGC generation.

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555.7

MORPHOLOGY OF RETINAL GANGLION CELLS IN OPERATED HAMSTERS WITH NOVEL RETINAL PROJECTIONS. Edna N. Yamasaki* and Douglas O. Frost. Dept. Neurol., Mass. Gen. Hosp., Charlestown, MA.

Neonatal surgery in hamsters can produce novel, functional retinal projections to non-visual thalamic nuclei. The morphological types of retinal ganglion cells (RGCs) that survive the neonatal surgery are unknown. To address this issue, RGCs, identified by retrograde transport of fluorescent latex beads injected into the superior colliculus (normal hamsters) or stained with acridine orange (operated hamsters), were intracellularly filled with Lucifer yellow (LY) *in vitro* to reveal their soma-dendritic morphology. Retinae were then fixed and immunostained with an antibody against LY. RGCs in operated hamsters were identified by the presence of an axon. RGCs were drawn using a computer microscope.

The 3 RGC types previously described for rats could be recognized in both normal (32 RGCs) and operated (12 RGCs) hamsters. There were quantitative differences between RGCs in normal and operated hamsters. Mean dendritic field diameter was significantly larger in operated than in normal hamsters (378.8 µm vs 324.9 µm, respectively; p=0.038). Mean total dendritic length was also greater in operated vs normal hamsters (5560.9 µm vs 3945.3 µm, respectively; p=0.0037). The total number of dendritic spines was also significantly greater in operated than in normal hamsters (335.7 vs 176.7, respectively; p=.0001) but spine density was not significantly different (0.047 vs 0.066 μm^{-1} respectively; p>0.05). The mean number of dendritic branch points in operated and normal hamsters was not significantly different (66.7 vs 56.7, respectively; p>0.05). There was no significant difference (p>0.05) in mean soma diameter between normal (21.8 µm) and operated (19.1 µm) hamsters. These findings suggest that RGCs in the operated hamsters maintain their basic morphology but their dendritic tree dimensions increase in response to the reduced RGC density in operated hamsters (Irons, Métin & Frost, Neurosci Abs. 16: 336, 1991).

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555.8

ESTABLISHMENT OF AXON DIAMETER CLASSES IN THE RAT OPTIC NERVE IS INFLUENCED BY OLIGODENDROCYTES. R.J. Colello[1], R.W. Guillery[2]* and M.E. Schwab[3]. [1,3] Brain Research Institute, Univ. of Zürich, August Forel-Str. 1, CH-8029 Zürich, Switzerland and [2] Dept. of Human Anatomy, Univ. of Oxford, South Parks Road, Oxford, England.

Axons within the rat optic nerve can be classified into distinct groups according to their conduction velocities (Fukuda, 1977) and axon diameters (Hildebrand and Waxman, 1984). In the rat, axon diameters are uniform in size at the time of birth (Colello and Guillery, 1992) and increase from P6 on. This maturation occurs simultaneously with myelination and is complete by the first postnatal month. What is not clear, however, is to what extent myelination helps to establish the adult fiber diameters.

In the present development study, we examine the possible role of oligodendrocytes in establishing the distinct axon diameter classes by, a: measuring the cross-sectional area of fibers within the optic nerve before (P4) and during (P8 and P15) myelination and, b: measuring axonal area in optic nerves where oligodendrocyte development was suppressed by neonatal X-irradiation.

At P4, before initial myelination, there is a unimodal distribution of axon areas with a mean axon area of .12-.14um^2 in both normal and X-irradiated optic nerves. At the time of initial myelination (P8), the mean axon area in both normal and X-irradiated nerves increases to .15-.18um^2, but distinct axon diameter classes cannot be detected yet. This, however, becomes evident in the normal nerve at P15, where a trimodal distrubution of axon areas is established: the smallest populations, represented by 42% of total fiber numbers are .5um^2 or less, whereas the medium-sized (30%) and largest fiber (28%) groups have axon areas of .5-.8um^2 and .8-2.7um^2 respectively. Interestingly, optic nerves free of oligodendrocytes at this age contain a majority of axons (85%) with axonal areas of .5um^2 or less; the remaining axon (15%) have axonal areas of .5-1.7um^2.

These results indicate that the formation of adult axon diameter classes in the optic nerve is a result of both intrinsic cues present in the ganglion cell and extrinsic cues presented by oligodendrocytes.

555.9

EFFECTS OF PRENATAL IONIZING IRRADIATION ON THE DEVELOPMENT OF MOUSE VISUAL PATHWAYS.

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Prenatal ionizing irradiation of pregnant mice produces in the progeny extensive shrinkage of the posterior halves of the cerebral hemispheres and the dorsal lateral geniculate nuclei (dLGN). In this study we describe the retinal projections to subcortical nuclei in adult mice irradiated prenatally. Pregnant mice were exposed to a gamma source at 16 days of gestation receiving a total dose of 3Gy. Adult mice (n = 13, irradiated progeny; n = 13, nonirradiated controls) received an eye injection (5μl) of a 30% solution of horseradish peroxidase (Sigma type VI). Tha animals were allowed to survive for 2 days. Then the brains were reacted for peroxidase using tetramethyl-benzidine as a cromogen and adjacent sections were counterstained with neutral red. Analysis of the retinal projections indicated that the general pattern of connection of normal and irradiated animals was similar. Bilateral retinal projections were found in dLGN, ventral lateral geniculate nucleus, posterior pretectal nucleus, olivary pretectal nucleus and superior colliculus. Label was found in nucleus of the optic tract only on the side contralateral to the injected eye. In spite of the damage to the striate cortex and the reduction of the dLGN, no abnormal retinal projection fields were detected in irradiated animals. These data differ from those obtained by other authors after neonatal surgical removal of the rat striate cortex. In these cases, aberrant pathways were consistently found. Our data suggest that neonatal or prenatal cortical damage may lead to distinct consequences regarding the redistribution of the retinal axons.

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VISUAL DEVELOPMENT: ABNORMAL DEVELOPMENT OF CORTEX

556.1

RESTRICTED CALLOSAL CELL DISTRIBUTION IN THE STRIATE CORTEX OF THE RABBIT FOLLOWING SYSTEMIC YOHIMBINE ADMINISTRATION DURING DEVELOPMENT.

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In the mature rabbit, the distribution of visual callosal cells which project to the contralateral visual cortex is limited to the 17/18 border. In the newborn rabbit callosal cells are distributed throughout most of the mediolateral extent of area 17. This developmental process of fine tuning in the visual cortex has been shown to be regulated in part by the level of noradrenaline (NA). In the present study we examined the effects of administration of an alpha-2 receptor antagonist, yohimbine, during the critical period, on the development of the visual callosal cell distribution. Yohimbine increases the release of NA by blocking presynaptic alpha-2 adrenergic receptors. Rabbits received intraperitoneal injections of yohimbine HCl (2.5 mg/kg) every day from postnatal day 5 through day 12 (N=4). Rabbits were raised until adult, at which time multiple injections of HRP (Boehringer, 20% in H_2O) were made (total volume injected: 8 μl) throughout one entire visual cortex. Animals were perfused 24 hours later and the brains were cut and reacted with TMB. Adult animals which received yohimbine injections had a significantly reduced tangential extent of the callosal cell distribution in area 17, and also had a significantly reduced callosal cell density in lamina II and III compared to normal animals. Yohimbine administration during the critical period enhanced the process of retraction of exuberant callosal projections. The results provide evidence that NA plays a role in the normal elimination of early exuberant projections during development.

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556.2

NEONATAL ENUCLEATION INCREASES THE COMPLEXITY OF THE VISUAL CALLOSAL PATTERN IN THE HAMSTER.

B. J. O'Brien, J.F. Olavarria, J. W. Lewis, E.A. Brenowitz*. Psychology, and Physiology & Biophysics Depts. Univ. of Washington, Seattle, WA 98195.

Callosal cells are widely distributed at birth but become restricted to specific cortical zones during development. It has been suggested that disruption of visual input neonatally arrests this normal process of reorganization, leaving what have been described as abnormally broad patterns of callosal connections. However, enucleation in young rats produces entirely new callosal features which cannot be explained by this mechanism alone. For instance, the most prominent anomalous feature in monocularly enucleated rats is an extra callosal band that runs rostrocaudally through the center of area 17 ipsilateral to the remaining eye. Analysis of these extra features offer clues for identifying extracortical mechanisms capable of altering the callosal pattern, but similar anomalous features have not been described in other species studied to date.

We have, therefore, investigated whether neonatal enucleation induces extra callosal features in golden hamsters. The callosal pattern was revealed with the TMB-HRP technique in coronal and tangential sections of the flattened cortex after multiple injections of HRP contralaterally. Although features of the normal callosal pattern were recognizable at the lateral border of striate cortex and in lateral extrastriate cortex, the callosal pattern in both monocularly and binocularly enucleated hamsters presented distinct anomalies that were very similar to those reported in the rat. In monocular enucleates we observed an extra callosal band in roughly central portions of striate cortex that was beaded in appearance due to fluctuations in the labeling density. Binocular enucleation markedly increased the width of the lateral callosal band in striate cortex, produced discrete areas of reduced callosal labeling within this band and small finger-like extensions of this band into medial striate cortex. Since the distribution of visual callosal connections is closely tied to the underlying topography, the increased complexity of the callosal pattern suggests that disruption of visual input markedly changes the organization of visual cortex.

556.3

Development of Retinotopy in Rewired Ferret Auditory Cortex - A Model

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A topographic map of visual space forms in primary auditory cortex of ferrets with retinal input directed to the auditory thalamus (Roe et al., Science 250: 818, 1990). Interestingly, the visual map in rewired auditory cortex shows retinotopic order despite rather convergent and divergent projections from the thalamus (Pallas et al., JCN 298:50, 1990). We have used a self-organizing model based on Hebbian plasticity of synaptic weights to investigate the possible mechanisms underlying map formation. As expected, auditory input, modelled as a column of thalamic cells activated synchronously, does not cause enhancement of topography in the isofrequency dimension within cortex. Visual input, consisting of local activation of adjacent input cells, guides the development of cortical retinotopy. Thus, the specific nature of input activity appears to play a crucial role in the development of different maps given the same underlying anatomy.

We investigated the relative importance of thalamocortical and intracortical connections in the development of retinotopy when the model is driven by visual input. Retinotopy does not develop when initial thalamocortical and intracortical weights are random. Short-range intracortical excitation leads to enhancement of retinotopy whatever the thalamocortical weights. A higher degree of retinotopy requires some initial topography in the thalamocortical weights; this is sharpened during development by the interplay between the thalamocortical and intracortical weights. Simultaneous changes in both sets of weights leads to the development of a greater degree of retinotopy than is possible by changing either set alone.

More generally, these results indicate that activity-dependent mechanisms can significantly improve the functional topography of widespread anatomical projections in developing pathways. Connections within the target layer (intracortical connections) play an important role in this process. [Supported by EY07719]

556.4

INTERFERENCE WITH GENERATION OF LAYER 4 PREVENTS THE FORMATION OF OCULAR DOMINANCE COLUMNS.

Peter Kind*, Frank Sengpiel, Jürgen Engelage and Colin Blakemore University Laboratory of Physiology, Oxford OX1 3PT, U.K.

In the cat, the LGN fibers arrive under the visual cortex and 'wait' before entering, while their principal targets, the cells of layer 4, migrate to their final position. At birth, the terminal fields of fibers from the two eyes overlap, but they gradually segregate into ocular dominance (OD) columns over the first few postnatal weeks.

To investigate the relationship between the generation of layer 4 and subsequent synaptogenesis and formation of OD columns, we used methylazoxymethanol acetate (MAM), a cytotoxin that destroys dividing cells, partially to disrupt the generation of layer 4. Time-mated pregnant cats were given a single injection of MAM (15mg/kg i.p.) on embryonic day 40.5 (the peak of layer 4 cell generation). The pattern of termination and extent of geniculocortical segregation were assessed in the adult offspring using transneuronal tracing with WGA-HRP.

Cortical thickness in the MAM-treated animals appeared virtually normal and lamination roughly equivalent to layers 4 and 2/3 was discernible; however, the density of cells in layer 4 was modestly reduced. Moreover, transneuronal tracing revealed an abnormal pattern of geniculate termination in some parts of areas 17 and 18 with dense termination extending up to the lower limit of layer 1. Even years after birth there was no hint of segregation into OD columns. There was also a marked down-regulation of the Cat-301 antigen in the upper and lower layers of areas 17 and 18. These data suggest that the cells of layer 4 play an important role in determining the laminar pattern of axon termination and in regulating the competition that underlies OD column formation. - Supported by the MRC.

556.5

RECEPTIVE FIELDS IN ASSOCIATION AND PRIMARY VISUAL AREAS OF MONOCULARLY DEPRIVED AND NORMAL CATS. U. Yinon*, G. Dobin and I. Volkon, **Physiol. Lab., Goldschleger Eye Res. Inst., Tel-Aviv Univ. Fac. Med., Sheba Med. CTR, Tel-Hashomer, 52621, Israel.**

Receptive fields (RF's) properties have been compared in each animal for two association suprasylvian (PMSA, AMSA) and for two primary (17; PMLS [Clare-Bishop]) visual cortical areas. Unit recording was extracellularly performed in early monocularly deprived (MD) and normal cats. Preliminary data (5 cats; 80 RF's) showed that the ocular dominance distribution of the cells is biased for all these areas in the MD cats and nearly symmetric in the control cats. In all areas both movement and stationary sensitive cells were found; however, the tendency was for more ambiguity in the association areas. RF's of the association areas had extended into the ipsilateral hemifield, albeit their geometric centres were found in the contralateral field. In the MD cats extremely large RF's were found for the association (average: 67.5 deg.^{2}) and PMLS (87.8 deg.^{2}) areas, in comparison to area 17 (2.3 deg.^{2}). In analogy, the RF sizes for normal cats were 258.7 deg.^{2} for the association and 286.9 deg.^{2} for the PMLS areas and 4.7 deg.^{2} for area 17. It has been further concluded that in all four areas studied RF's were remarkably smaller for the deprived cats.

Supported by the National Inst. for Psychobiology in Israel.

556.6

CHOLINE ACETYLTRANSFERASE FIBERS IN THE STRIATE CORTEX OF VERTICAL AND HORIZONTAL STRIPE-REARED KITTENS PREFERENTIALLY DEVELOP ORTHOGONAL TO THE SELECTED VISUAL ORIENTATION. Nancy J. Woolf*, Laboratory of Chemical Neuroanatomy and Dept. Psychology, UCLA, Los Angeles, CA 90024-1563, U.S.A.

Limited visual experience in young, light-deprived kittens to vertical or horizontal stripes has been shown previously to produce morphological changes in the basilar dendrites of pyramidal cells in striate cortex (Tieman and Hirsch, *J Comp Neurol.*, 211: 353, 1982). The present study addressed the possibility of developmental plasticity in one of the presynaptic inputs to cortical pyramidal cells, namely the cholinergic afferent axons. Kittens were reared in the dark until 5 weeks of age, at which time they were 1) exposed to a vertical stripe pattern, 2) exposed to a horizontal stripe pattern, or 3) given no light exposure. After multiple exposures spaced over a 2-week period, kittens were euthanized. Striate cortex was sectioned horizontally and processed immunohistochemically for choline acetyltransferase (ChAT). The orientations of ChAT fibers in and around the basilar dendrites of layer 5 pyramidal cells were clearly distinguishable and in the vast majority of cases were oriented nearly parallel or orthogonal (± 15°) to the vertical median. In the dark reared-condition, total lengths of ChAT axonal segments oriented parallel or orthogonal to the vertical visual meridian were roughly equal, but in stripe-reared conditions, ChAT fibers running parallel were decreased 25 - 68% in length relative to those running orthogonal to the preferred visual orientation.

556.7

A GLIOTOXIN, FLUOROCITRATE, REDUCES EFFECTS OF MONOCULAR DEPRIVATION. K. Imamura*, N. Mataga, K. Muguruma, and Y. Watanabe. Dept. of Neurosci., Osaka Bioscience Institute, Suita, Osaka 565, Japan.

Acetate and citrate have been shown to be selectively taken up into the glial cell's compartment. The corresponding fluorinated analogues, fluoracetate and fluorocitrate competitively inhibit the enzyme aconitase, resulting in the reduction of energy supply in this compartment (Clarke et al., 1970). Using the fluorocitrate as a gliotoxin, we studied roles of immature astrocytes in the regulation of the ocular dominance plasticity. Around 20µl of 10µM fluorocitrate was infused diffusely by pressure application into the visual cortex (area 17, subcortical depth 0-5mm) of 7-9 week-old kittens. Following the infusion, the contralateral eye was closed monocularly for 3 days. Physiological recordings from the vicinity of the infusion site (~1 mm) revealed that an usual shift in ocular dominance toward the open eye was prevented. Whereas a normal shift was consistently observed in the region ~ 4 mm remote from the infusion site in the same hemishere. The proportion of binocular cells was significantly higher in the region affected by fluorocitrate (average binocularity; 0.42, control; 0.23, t-test; $p < 0.05$). These results suggest that some functions of astrocytes in the immature kitten visual cortex is a prerequisite for the full expression of the ocular dominance plasticity.

556.8

UP-REGULATION OF POLYSIALYLATED NCAM EXPRESSION BY ACTIVITY BLOCKADE IN KITTEN VISUAL CORTEX G.E. Baker*, E.S. Ruthazer, U. Rutishauser and M.P.Stryker Keck Center for Integrative Neuroscience & Neuroscience Program, Univ. of California, San Francisco, CA 94143; Dept. of Developmental Genetics, Case Western Reserve Univ, Cleveland, OH 44106

Polysialylated NCAM (psa-NCAM) on developing axons has been shown to be directly involved in chick limb bud innervation, and the degree of NCAM sialylation is regulated by the level of neural activity (Landmesser et al., *Neuron* 4:655, 1990). In mammals, the organization of retinogeniculate and geniculocortical axonal arbors is profoundly influenced by patterns of neural activity during development. We have therefore studied the expression of psa-NCAM in the visual pathways of cats.

In the normal visual cortex, psa-NCAM was immunohistochemically nearly undetectable at birth and remained low thereafter. Tetrodotoxin (TTX) was chronically infused at 0.5 ul/hr into one visual cortex of kittens beginning at postnatal day 24. One week later, blockade of neural activity in a region extending approximately 7mm rostral to the infusion site was confirmed using conventional extracellular microelectrode recording. The brains were subsequently fixed, sectioned, and processed for immunoreactivity to psa-NCAM and to total NCAMs. The expression of psa-NCAM was elevated manyfold above normal levels in the region of cortex in which activity was blocked. In the cortical plate, the region in which this elevation was found extended at least 5mm from the site of infusion of TTX in the lateral gyrus. The highest density of staining was found in cortical laminae IV and VI. In addition, staining in the white matter was greatly increased and extended laterally into the suprasylvian and ectosylvian gyri, and medially through the corpus callosum into the contralateral hemisphere.

The finding that TTX blockade of activity results in up-regulation of psa-NCAM on axonal connections of the kitten's visual cortex raises the possibility of a direct causal link between the expression of psa-NCAM and activity-dependent changes in organization of the geniculocortical projection.

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556.9

CALCIUM INCREASE IN RAT VISUAL CORTEX NEURONS DURING TETANIC SYNAPTIC INPUTS.

M. P. Takahashi, M. Sugiyama, Y. Hata* and T. Tsumoto, Dept. of Neurophysiology, Biomedical Research Center, Osaka University Medical School, Suita, 565 Japan

An entry of Ca^{2+} into postsynaptic sites through NMDA receptor-linked channels is suggested to be a trigger for induction of long-term potentiation following tetanic synaptic inputs in the developing visual cortex. To test this hypothesis, we measured changes in $[Ca^{2+}]_i$ following tetanic stimulation of the white matter with fluorometry using a Ca^{2+}-sensitive indicator, rhod-2, in slice preparations of the visual cortex of rats aged from 15 to 30 days. Slices were loaded with 10 µM rhod-2/AM and fluorescence image of slices was recorded with a SIT camera. Simultaneously, field potentials to test stimulation of the white matter were recorded from layer II/III of the cortex. During tetanic stimulation (5 Hz, 1 min) of the white matter, the fluorescence increased in a columnar manner from the stimulation site to the pial surface and the increase was most marked in layer II/III. An application of D,L-2-amino-5-phosphonovaleric acid, an NMDA receptor antagonist (100 µM), reduced the tetanus-induced fluorescence increase in layer II/III to 75 ± 15 % of the control. Effects of a non-NMDA receptor antagonist, CNQX (20 µM), was tested in a Mg^{2+}-free medium and turned out to be ineffective. Nifedipine (10 µM), an L type Ca^{2+} channel blocker and Ni^{2+} (100 µM), a relatively selective blocker for T type Ca^{2+} channel, also exerted suppressive effects, but with less extent. Thus, tetanic synaptic inputs may lead to an influx of Ca^{2+} into cortical neurons at least partly through NMDA receptor-linked channels.

556.10

DARK-REARING POSTPONES RATHER THAN ABOLISHES THE CHANGE IN THE NMDA CONTRIBUTION TO THE VISUAL RESPONSE DURING THE DEVELOPMENT OF THE CAT VISUAL CORTEX. N.W. Daw*, D. Czepita and S. Reid. Dept. Anatomy and Neurobiology, Washington Univ. Med. Sch., St. Louis, MO 63110

The contribution of NMDA receptors to the visual response in layers IV, V and VI of the cat visual cortex drops from an average of 60% to an average of 10-15% between 3 and 6 weeks of age (Fox, Sato & Daw, J. Neurosci. 9: 2443-2454, 1989). This drop can be halted by rearing the cats in the dark to 6 weeks of age (Fox, Daw, Sato and Czepita, Nature 350: 342-344, 1991): it then proceeds when the cats are brought into the light. In the current experiments we tested whether this drop can be halted indefinitely by rearing in the dark. We reared three cats in the dark to 5 months of age, then assayed the NMDA contribution to the visual response by iontophoresing the NMDA antagonist APV near the cell being recorded. The average contribution of the NMDA receptors to the visual response was 27% in layer IV, and 16% in layers V and VI. Our conclusion is that rearing in the dark can delay the drop in NMDA contribution, but will not halt it indefinitely.

556.11

NERVE GROWTH FACTOR INDUCES NEURONAL PLASTICITY IN ADULT CAT VISUAL CORTEX. Q. Gu*, Y.L. Liu and M.S. Cynader. Department of Ophthalmology, University of British Columbia, Vancouver, British Columbia, Canada, V5Z 3N9.

Nerve growth factor (NGF) has been shown to be involved in neuronal survival, growth and differentiation. We have been examining whether NGF also plays an important role in neural plasticity in the visual cortex. During early postnatal development, neuronal connections in the visual cortex are highly susceptible to activity-dependent modification. For example, a few days of monocular deprivation renders most neurons in the visual cortex, which normally respond to stimulation of both eyes, unresponsive to stimulation of the deprived eye. However, this activity-dependent modification occurs only within a "critical period" in development and not thereafter. In adult visual cortex, neurons are no longer susceptible to monocular deprivation. To test the hypothesis that NGF may enhance neural plasticity in adult visual cortex, varied doses of NGF were continuously infused, by means of osmotic minipumps, into visual cortex of adult cats who had one eyelid sutured closed at the time of minipump implantation. After various times (minimum 2 weeks), the ocular dominance distributions of neurons in the visual cortex were assessed with single unit recording. We found that there was a dramatic change in ratio of binocularly activated neurons to monocular neurons in the adult visual cortex after monocular deprivation coincident with NGF-treatment. Our results demonstrate that intracortical infusion of NGF can recreate ocular dominance plasticity in adult cat visual cortex. The mechanisms by which NGF induces neural plasticity in adult visual cortex are now under investigation.

556.12

Anti NGF monoclonal antibodies affect the postnatal development of the rat geniculocortical system N. Berardi*, A. Cattaneo+,§, A. Cellerino§, L. Domenici, M. Fagiolini, L. Maffei§ and T. Pizzorusso§. Istituto di Neurofisiologia del CNR, I-56127 Pisa, § SNS, I-56125, Pisa, +SISSA, Trieste and §Ist. Neurobiologia del CNR, I-00156 Roma (Italy)

We have recently found that exogenous supply of NGF in rats monocularly deprived during the critical period of cortical plasticity prevents the effects of monocular deprivation, i.e., deprived eye amblyopia, shift in ocular dominance (OD) distribution of visual cortical neurons and shrinkage of cell bodies in the deprived Lateral Geniculate Nucleus (LGN) laminae. To test the role of endogenous NGF in the normal postnatal development of the geniculocortical system we have studied if and how it is affected by inhibiting the physiological activity of NGF. Hybridoma cells secreting an anti NGF antibody, aD11, known to inhibit NGF binding to the target cell (Cattaneo A. et al. 1988 J. Neurochem. 50(4), 1003) have been transplanted in the right lateral ventricle of 10 rats (age 15-18 postnatal days, P15-P18), to provide continuous supply of antibody. 2µl of Hanks solution containing 10^6cells/µl were injected. The presence of aD11 in the visual areas was readily detected by Western blot. Parental myeloma cells were transplanted in ten P15-P18 rats as control. At P45 cell soma size in cresyl violet stained sections of the LGN, OD of visual cortical cells and visual acuity (recordings from the left visual cortex) were evaluated for both groups and compared with those of normal rats of the same age. We found that cell soma size in the LGN of Hybridoma transplanted rats was 30% smaller than in normal rats, visual acuity was reduced by 40 % and the percentage of binocular cortical neurones was nearly halved. By contrast, these parameters were within the normal range in Myeloma transplanted rats. We conclude that inhibition of NGF activity affected both the LGN and the visual cortex, suggesting a physiological role for NGF in the development of these structures.

556.13

Transplant of Schwann cells in the lateral ventricle prevents the effects of monocular deprivation in the rat T. Pizzorusso§, M. Fagiolini&, M. Fabris#, G. Ferrari# and L. Maffei*§. §Scuola Normale Superiore, Pisa, & Istituto Neurofisiologia CNR, via S.Zeno,51 Pisa, # Fidia Research Laboratories Abano Terme (PD) (Italy).

Monocular deprivation (MD) during the critical period of mammalian visual cortex shifts the ocular dominance distribution of visual cortical neurons towards the non deprived eye. Recent results showed that an exogenous supply of NGF prevents the effects of MD in the rat visual cortex (Domenici et al., '90, PNAS 88, 8811-8815; Berardi et al. J. Physiol. '91, 434 14P). Since Schwann cells are known to produce NGF,BDNF and CNTF, we tested the possibility of using Schwann cells (SC) as biological pumps for neurotrophic factors in the CNS. 21 rats were used. 19 rats were monocularly deprived before eye opening (P14). 3 of them were transplanted with 10^6 SC, 7 MD rats with 6 10^5 SC, 2 MD rats with 3 10^5 SC, 3 MD rats with 10^5 SC and 4 MD rats were left untreated. SC were transplanted by intraventricular injection at P15. In 2 rats of each group SC were labeled prior to transplantation with Fluorogold. After one month, the impulse activity of single cortical cells was recorded from the primary visual cortex. We found that MD shifts the ocular dominance away from the deprived eye both in untreated and in 10^5 SC transplanted. Increasing amounts (3 10^5, 6 10^5) of SC gradually attenuate the shift in ocular dominance distribution and in 10^6 SC transplanted rats the ocular dominance distribution was similar to that observed in normal adults. The transplant did not affect the selectivity for the stimulus parameters of cortical cells. After the recording session, the rats transplanted with Fluorogold labeled SC were perfused and their brains sectioned. We found that SC were located on the white matter nearby the injection site and on the blood vessel walls of the whole telencephalon. We conclude that the transplant of SC in the CNS prevents the effect of MD, its effectiveness increases with the number of transplanted SC.

556.14

"MAGNO-WEIGHTED" STIMULI DISCLOSE BIASES OF VERTICAL AND HORIZONTAL MOTION PERCEPTION IN HUMAN INFANTILE STRABISMUS. A. Rastelli, S. Steinman+, L. Tychsen, and A.Burkhalter*. Dept Ophthalmology, Washington Univ Sch Medicine, and +School Optometry, Univ Missouri St Louis.

Humans who have onset of strabismus in infancy have maldeveloped visual motion processing when viewing monocularly (Tychsen and Lisberger, 1986). To determine if the motion anomaly could be elicited by a simpler stimulus optimally "tuned" for magnocellular neurons, we tested 5 subjects (2 controls, 1 motor nystagmus, 1 late-onset exotrope, and 1 infantile-onset esotrope) using stationary, low spatial frequency, high temporal frequency, reversing, black-and-white bar gratings.

Motion misperception was found only in the subject with infantile-onset strabismus. Vertical gratings were perceived to be moving nasally at a low velocity. Nasally-directed pursuit eye movement was induced when the misperception became robust. Horizontal gratings were perceived to be moving upward, and upward pursuit eye movement was induced. Optimal properties for inducing misperception were gratings with spatial frequencies < 1.6 cycles/deg and temporal frequencies > 6.7 cycles/sec. Absence of the misperception in the subjects with motor nystagmus and exotropia show that ocular motor abnormalities cannot account for misperceptions.

We conclude that a simple grating stimulus, designed to emphasize the spatio-temporal tuning properties of the magno-system, is a useful tool for detecting the motion pathway anomalies of infantile strabismus.

556.15

BEHAVIORAL FUNCTION OF NOVEL NEURAL CIRCUITS. L.A. Rothblat* and D.O. Frost. Dept. Psychol., George Washington Univ., Washington, DC and Dept. Neurol., Mass. Gen. Hosp., Charlestown, MA.

In hamsters, novel, retinal projections to the primary auditory (medial geniculate, MG), primary somatosensory (VB) or visual association (lateral posterior, LP) thalamic nuclei can form after neonatal surgery. We have previously demonstrated that the projections to the somatosensory system are functional at the neurophysiological level. We now offer preliminary data on the behavioral function of novel projections.

The discrimination of 3 horizontal rows vs 3 vertical rows of black squares on a white background normally depends upon the integrity of the retino-geniculo-striate system: 4 normal adult hamsters learned this task to a 90% correct criterion in 8, 17, 17 and 21 days (30 trials/day) respectively. After reaching criterion, 2 animals (which learned in 8 and 17 days) received lesions that included all of area 17 plus adjacent parts of areas 18a and 18b. These animals failed to relearn the task postoperatively within 40 days. 4 newborn hamsters received bilateral lesions of the superior colliculus, the brachium of the inferior colliculus and the occipital cortex (areas 17, 18a and 18b); the cortical lesions caused retrograde degeneration of the dorsal lateral geniculate (LGd), LP and lateral dorsal nuclei. In all 4 animals, as adults, the losses of area 17 and LGd were complete, but there were variable-sized remnanats of area 18a and LP; all 4 also had variable losses of MG and other thalamic nuclei. 3 of the 4 hamsters had novel retino-LP projections; 2 of these 3 had retino-MG projections. When tested behaviorally, the 3 hamsters with retinal projections to LP or MG learned the task in 14, 15 and 31 days; the 4th hamster still failed after 40 days. The 3 successful hamsters then received lesions of the remaining occipital and temporal cortices. All 3 failed to relearn the task after 40 days. Thus, it appears that the primary auditory- or an associative thalamocortical pathway can take over a visual function normally mediated by the geniculo-striate system. Supported by NIH, March of Dimes, ONR.

556.16

SYNAPTIC PLASTICITY FOLLOWING EARLY AND LATE DECOMPRESSION FOR INFANTILE HYDROCEPHALUS. J.P. McAllister II*, R.A. Morano, R.A. Ireland, D.V. Shroff, P.M. Hale and R.M. Kriebel. Depts. of Anatomy, Temple Univ. School of Medicine and Phila. Coll. Osteopathic Medicine, Philadelphia, PA 19140.

Controversy persists regarding the clinical decision of when to surgically decompress hydrocephalic children. To address this issue systematically, the present study continues our quantitative analysis of the neuropil of hydrocephalic animals before and after ventriculo-peritoneal (VP) shunting at two different time points. Obstructive hydrocephalus was induced in 10 day old kittens by intracisternal injection of kaolin; VP shunts were placed in hydrocephalic animals at early (6-8 days post-kaolin) and late (11-14 days) stages of ventriculomegaly. Normal animals served as age-matched controls. Hydrocephalic animals were sacrificed at times corresponding to immediate pre-shunt periods, and about 7 days after shunt placement. Tissue from primary visual cortex was processed for electron microscopy and the number of synapses per unit area determined for deep laminae. As shown below, early and late shunts restored synaptic density to within 32% ($p = 0.08$) and 35% ($p = 0.005$) of controls, respectively. These data modify our previous findings and suggest that only early decompression is effective in promoting synaptic recovery.

	Early Shunts			Late Shunts		
	Control	Hydro	Shunt	Control	Hydro	Shunt
Synapses/ 100 µm²	17.6 ±3.1	9.1 ±3.1	12.0 ±4.1	21.0 ±1.7	14.2 ±2.6	13.8 ±.14

557.1

LOCALIZATION AND CHARACTERIZATION OF SPECIALIZED MEMBRANE CONTACTS BETWEEN IMMATURE CONES IN THE PHOTORECEPTOR MOSAIC OF THE FETAL MONKEY RETINA Kenneth C. Wikler*and Pasko Rakic. Sect. of Neurobiol., Yale Univ. School of Medicine, New Haven CT 06510.

Developmental examination of the maturing photoreceptor mosaic in the fetal monkey retina has revealed a regular array of precocious cones that are transiently in apposition with their nascent cone neighbors (Wikler and Rakic, '91, Nature, 351: 397). Previous studies of dissociated cell cultures suggest that the development of photoreceptor phenotypes may be specified in part through early cell: cell interactions that may be mediated by either diffusable agents or through specialized membrane contacts (Watanabe and Raff, '90, Neuron, 2: 461). To examine the existence of potential contacts between neighboring cones in the primate retina we initiated an ultrastructural and immunocytochemical study of the photoreceptor mosaic at the onset of differentiation for the red/green- and blue- sensitive cone subtypes.

The perifoveal region of retinae from rhesus monkeys sacrificed at embryonic (E) days ranging between E60 and E110 were prepared for electron microscopy or immunocytochemistry. Examination of the retina at E60 or E70, prior to the development of outer segments, revealed the presence of punctate specialized membrane contacts between the inner segments of immature cones. At early fetal ages these contacts are unlikely to be gap junctions since neither our electron microscopic or immunocytochemical analysis using antibodies specific to the connexin- 32 fragment of the gap junction protein have revealed the presence of gap junctions between cones. These specialized intercone membrane contacts do resemble puncta adherentia having symmetrical filamentous thickenings facing the cytoplasmic compartments of the apposing segments and a wide intermembrane cleft. In some instances, vesicular profiles were found associated with these membrane junctions. In contrast, in the E100 or E110 cases, specialized membrane contacts between cones were not observed after the development of cone outer segments. The transient appearance of specialized membrane contacts between immature cone inner segments suggests a role for these junctions in the emergence of the photoreceptor mosaic. Supported by EY02593

557.2

DEVELOPMENT OF cGMP-PHOSPHODIESTERASE IN RETINAL PHOTORECEPTORS OF THE RAT. E. Strettoi*, and L. Colombaioni Istituto di Neurofisiologia CNR, 56127 Pisa, Italy

In retinal photoreceptors, the process leading from light absorption to cell membrane hyperpolarization involves the activation of a specific cGMP-phosphodiesterase (cGMP-PDE); this enzyme hydrolyses 3'-5' cyclic guanosine monophosphate that regulates the permeability of the light-sensitive channels.

We have investigated by immunofluorescence and electron microscopy immunocytochemistry the appearance of cGMP-PDE during the postnatal development of the rat retina. The distribution of cGMP-PDE during development has been compared with that of the adult retina. A monoclonal antibody, ROS1, specific for photoreceptor cGMP-PDE, has been employed. We show that a sudden increase in immunoreactivity takes place during postnatal day 5, when rod outer segments begin to form; immunoreactivity develops rapidly in the following days. Labelling is restricted to the developing photoreceptor outer segments, sparing other cellular districts and other retinal cells, as confirmed by electron microscopy. cGMP-PDE immunoreactivity mirrors the centre-to-periphery gradient of photoreceptor differentiation.

cGMP-PDE appears in photoreceptor outer segments concomitantly with photoactive rhodopsin and rhodopsin kinase, suggesting a coordination in the appearance of phototransduction proteins during development. If photoreceptor outer segments are assembled fully provided with their enzymatic machinery, it is conceivable that light dependent electrical events could take place in photoreceptors before the retinal circuitry has completely developed.

557.3

REGULATION OF INTEGRIN ALPHA AND BETA mRNA'S IN DEVELOPING CHICK RETINA. D. B. Gervin, G. M. Cann, A. D. Bradshaw, R. C. Cadwell, C. J. Cummings, A. W. Hunter, R. M. Lebel, E. S-H. Choi, and D. O. Clegg*. Neuroscience Research Institute and Division of Molecular and Cellular Biology, Department of Biological Sciences, University of California, Santa Barbara, CA 93106.

Interactions between developing retinal cells and the extracellular matrix may play roles in retinoblast migration or translocation, determination of cell fate, axonal pathfinding, and possibly synapse formation. Matrix proteins such as laminin, S-laminin, vitronectin, and thrombospondin have provocative distributions that in some cases correlate with developmental events. Receptors that mediate cell responses to matrix include those in the integrin family, but little is known about which receptors are used and how they are regulated.

To address these questions, we have attempted to identify integrin mRNA's that are expressed in the developing chick retina, and determine how they are regulated. We have employed a polymerase chain reaction (PCR) strategy using degenerate primers that recognize related integrin alpha or beta subunits. We have detected 5 alpha subunit mRNA's and 3 beta subunit mRNA's that are expressed in retinal tissue: Alpha's 2, 4, 6, 8, and v; and beta's 1, 3, and 5. PCR was also used to quantify changes in the relative amounts of these messages during development. We have found that alpha 6 and 8 mRNA levels decrease between embryonic day 6 to embryonic day 9, which correlates with changes in matrix responses that have been observed in cultured embryonic neurons. We speculate that changes in integrin expression may be important for retinal development, particularly during axonal pathfinding.

557.4

IMMUNOHISTOCHEMICAL LOCALIZATION OF CALPAINS AND CALPASTATIN IN THE EYE OF THE RABBIT. H. Persson* and J.-O. Karlsson. University of Göteborg, Dept of Anatomy and Institute of Neurobiology, Box 33031, S-400 33 Göteborg, Sweden.

The tissue distribution of m-calpain (calpain I) and μ-calpain (calpain II) in the eye of the rabbit was examined using polyclonal and monoclonal antibodies against the corresponding rabbit antigens. Purification of antigens was made as described previously (e.g. Nilsson, E. *et.al.*, Neurobiology of aging, 11 (1990) pp. 425-431) and purified antigens were injected into chickens to produce IgY antibodies recovered from the egg yolk. Monoclonal antibodies were a generous gift of Dr. S. Kawashima. Adult New Zealand White rabbits were perfusion fixed with 4 % paraformaldehyde and the eyes postfixed overnight after enucleation. 10 μm thick cryostat sections were cut, blocked with normal rabbit serum and incubated overnight with appropriately diluted primary antibodies. Bound antibodies were visualized with FITC-conjugated rabbit anti-chicken IgG fraction antibodies and sections mounted in DABCO before viewing in a Nikon Microphot FX epifluorescense microscope equipped with FITC optics. Distinct immunoreactivity against the two forms of calpains and calpastatin was observed in the epithelial cells on the external and internal surface of the cornea as well as in the epithelial cells covering the iris and ciliary body. The sclera and choroid layers showed a relatively weak immunoreactivity with anti-calpain antibody, while anti-calpastatin immunoreactivity was more distinct. By contrast, in the retina, the pigment epithelium, as well as the outer and inner plexiform layers, the inner and outer borders of the Müller cells and the retinal ganglion cells and the nerve fiber layer were distinctly labelled by the anti-calpain antibodies while anti-calpastatin immunoreactivity was less pronounced in these areas. The outer segments of many receptor cells showed a distinct immunoreactivity with both anti-calpain- and -calpastatin antibodies. Thus, labelling by antibodies against these antigens appears to be associated with areas expected to have a high degree of metabolic activity and/or membrane turnover, in line with the notion that calpains are protein degrading enzymes representing part of a non-lysosomal catabolic pathway. This study supported by The Swedish Medical Research Council, project #03157.

557.5

TRK PROTEIN IS DISTRIBUTED IN A DORSOVENTRAL GRADIENT IN THE EMBRYONIC RAT RETINA. R.H. Fryer,* D.R. Kaplan, and L.F. Kromer. Dept. of Anat. & Cell Biol., Georgetown University Medical Center, Washington, DC 20007 and the National Cancer Institute, Frederick, MD 21702

During embryonic development, retinal ganglion cells form specific topographic projections in the LGN and tectum. Although it is uncertain what governs this specificity, temporal and axial gradients of specific molecules on retinal neurons and axons are thought to be important for this process. Since retinal neurons respond to trophic molecules, such as NGF and BDNF, the objective of the present study was to determine whether receptors for these neurotrophins, which belong to the *trk* family of tyrosine kinase receptors, might also be associated with the appearance of gradients within the embryonic retina. For these experiments immunofluorescence techniques with a pan-*trk* antibody that recognizes a highly conserved intracellular domain present in *trk*A, *trk*B and *trk*C, were used to examine the timecourse of expression of these neurotrophin receptors. By embryonic day 14 (E14), all cells in the dorsal most retina and their associated axons in the initial optic nerve segment express *trk* proteins. At E15 and 16, *trk*+ cells are still restricted to the dorsal retina but *trk*+ axons are now observed in the optic chiasm (E15) and track (E16). By E18, *trk* staining is mainly localized to retinal ganglion cells present in both the dorsal and ventral retina. These results indicate that during a critical period in retinal development there is a gradient in the expression of *trk* receptors on retinal cells and their axons which might be involved in the formation of the retinotopic map.

557.6

DISTINCT PATTERNS OF SODIUM CHANNEL-LIKE IMMUNOREACTIVITY EXPRESSION DURING PRENATAL DEVELOPMENT OF THE CAT AND MONKEY RETINA. J.J. Miguel-Hidalgo*, K.J. Angelides+, L.M. Chalupa. Ctr. Neurobiology, Univ. California, Davis, CA 95616; +Physiol. & Molec. Biophysics Dept., Baylor Univ., Houston, TX 77030.

Polyclonal and monoclonal antibodies to the α subunit of the voltage-gated sodium channel (αVGNaC) were used to examine the distribution of sodium channel-like immunoreactivity during the prenatal development of the cat and monkey retina. At all prenatal ages studied, beginning embryonic day 29 (E29) in the cat and E45 in the monkey, both antibodies labeled optic axons. With the polyclonal antibodies the appearance of positive cells largely mirrored the onset of their morphological maturation. For instance, immunoreactivity appeared first in the somata of ganglion cells. A few weeks later horizontal cells displayed immunolabeling. This was followed by immunoreactive cones, with the bipolar cells labeled only postnatally. By contrast, with the monoclonal antibody some cells were found to be immunoreactive while their somata were still in the ventricular layer (E33 in cat and E52 in monkey). Many of these cells appeared to migrate to the outer portion of the prospective inner nuclear layer where they gradually acquired the morphological appearance of bipolar cells. These results indicate that different types of αVGNaC-like proteins are expressed in the mammalian retina at distinct developmental periods. They also suggest that these proteins could be playing developmental roles unrelated to their function at maturity. Supported by NIH and NMSS.

557.7

LIGHT-INDUCTED EXPRESSION OF IMMEDIATE EARLY GENES IN RAT RETINA

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Induction of immediate-early genes expression occurs in response to a wide range of extracellular stimuli. We present evidence for the expression of *c-fos* and *NGFI-A* in rat retina after exposing them to room light. Male Sprague-Dawley rats were maintained in a 12-h light/dark cycle with lights on at 0800 h. Animals were decapitated either in the dark or after various time intervals in room light. Total RNA from retina was extracted and *c-fos*, *c-jun* and *NGFI-A* mRNA were assayed by Northern blot hybridization. A dramatic increase of *c-fos* expression was observed in retina of light exposed animals which reached a peak (682%) after 30 min. In contrast, *NGFI-A* reached a peak after 15 min (246%) of light. Both mRNAs returned to basal values after 2 h of continuous light. Under our lighting conditions, we were unable to observe enhanced expression of *c-jun* in the retina. Basal content of *c-fos*, *NGFI-A* and *c-jun* were assayed at 0700, 1 h before the onset of room lighting. Injection of MK-801 (NMDA receptor antagonist) or SCH 23390 (D1 dopamine receptor antagonist) 30 min before exposure to light did not prevent the induction of either *c-fos* or *NGFI-A* in retina. Our results show that light transiently induces the expression of some immediate early genes in rat retina and that the induction is probably not mediated by glutamate or dopamine receptors.

557.8

CLONES AND POLYCLONES IN THE RETINA OF ADULT INTRASPECIES MOUSE CHIMERAS: D.S. Rice*, R.W. Williams, and D. Goldowitz
University of Tennessee, College of Medicine, Memphis, TN 38163

We are interested in the clonal structure of the mammalian retina. *Mus musculus* intraspecies chimeras were generated by fusing embryos from ICR and globin transgenic (GT) strains. GT cells were labeled with DNA probes in 5-μm paraffin sections of retina or in wholemounts. GT cells contribute a variable percentage of cells to chimeric retinas (0.5% to 70%). Clones and polyclones of GT cells are aligned radially and uniformly across inner and outer nuclear layers. As in interspecies chimeras (Williams & Goldowitz, 1992, PNAS 89,1184), boundaries between regions of different genotypes are well defined in our intraspecies chimeras. We have not noted any marked differences in the organization of clones or polyclones between intra- and interspecies chimeras. However, the number of small cohorts (1-2 cell diameters) appears to be greater in intra- than interspecies chimeras.

To reveal the pattern of clones and polyclones across the entire retina, we developed a method to label GT cells in wholemounts using DNA *in situ* hybridization. This makes it feasible to directly examine the distribution of the two genotypes in the ganglion cell layer of chimeras, as well as, to determine the correspondence between genotypes in the ganglion cell layer and in the inner and outer nuclear layers. In many instances, GT ganglion cells are displaced horizontally by as much as 20 to 30 μm from GT-labeled regions in inner and outer nuclear layers. Conversely, unlabeled ganglion cells are often found directly beneath cohorts of GT cells in the nuclear layers. This demonstrates that ganglion cells are often separated from the other members of their clones and suggests that these laterally-displaced cells make functional contacts with cells that have different clonal origins, and even different genotypes. Supported by NEI R01-8868.

557.9

DYNAMICS OF MOUSE RETINOGENESIS: A POPULATION AND CLONAL ANALYSIS. Dan Goldowitz* and Robert W. Williams. University of Tennessee, College of Medicine, Memphis, TN 38163

We have studied the dynamics of the increase in total cell number and clone structure in mouse retina. Estimates of the total number of retinal neuroepithelial cells were made in *Mus musculus* mice (albino ICR strain) at embryonic days E11, E12, E13, E15, the day of birth, and in adults. The retina consists of about 10,000 neuroepithelial cells at E11. There are essentially no postmitotic cells in retina at this age. By E13 the total cell population is ~40,000. By E15, the total cell population has increased to ~100,000, and at maturity the retina contains 3 to 4 million cells. To examine how this 300-fold increase between E11 and maturity is reflected in the clonal and polyclonal architecture of the retina, intraspecies chimeras were made using the globin transgenic (GT) mouse strain and the ICR strain. Chimeric retinas were examined at embryonic and early postnatal stages using in situ hybridization with labeled DNA to mark GT cells.

Cohorts and clones in these chimeric embryos typically form small radial arrays even at the earliest stages of development. The pattern is qualitatively similar to that seen in adult chimeras (Williams and Goldowitz, 1992, PNAS 89, 1184), except that cohorts contain far fewer cells—often less than 4 cells. In one E11 chimera, the right eye contained less than 1% GT cells, whereas the left eye contained no GT cells whatsoever. In this case, GT cells in the right retina were widely, but unevenly distributed. It is possible that the rudiment of this animal's right eye included a single GT cell, whereas the rudiment of the left eye included no GT cells. Our analysis indicates that a relatively small pool of progenitors—possibly less than 100—contributes to the murine retina and that even a single progenitor can give rise to a widely dispersed family of clones.

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557.10

DEVELOPMENTAL EXPRESSION OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) mRNA IN THE RAT RETINA. **G. Casini*, M. Molnar and N. Brecha, Depts. of Anatomy & Cell Biology and Medicine, BRI and CURE, UCLA and VAMC-West Los Angeles, Los Angeles, CA 90073.**

Using *in situ* hybridization histochemistry, we examined the developmental regulation of VIP mRNA in the rat retina. Retinas collected from birth to postnatal day (PND) 30 were hybridized with a rat VIP RNA probe (from Dr. Goodman) as whole mounts, and then cut either perpendicularly or parallel to the vitreal surface. In adult retinas, VIP mRNA is localized to somata positioned in the proximal inner nuclear layer (INL) and rarely in the inner plexiform or ganglion cell layer. These cells are amacrine and displaced amacrine cells. Neurons expressing VIP mRNA are sparsely distributed, with a non-random distribution and densities typical of wide-field amacrine cells. VIP mRNA is first detected in the retina during the first postnatal week. The hybridization signal is weak, suggesting low levels of VIP mRNA in these cells. As in the adult retina, labeled neurons are located in the proximal INL, suggesting that these cells express their transmitter phenotype when they are in their final laminar position in the INL. Stronger hybridization and more numerous VIP mRNA-containing neurons are present in the retina at PND 10, which is near eye opening. By PND 15 to 20, the maturation of this cell population is virtually complete. These results show that 1) VIP is expressed in a specific population of amacrine cells in the rat retina; and 2) the developmental expression of VIP is similar to that of other amacrine cell neurotransmitters.

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557.11

IMMUNOCYTOCHEMICAL CHARACTERIZATION OF RPE-CELL TRANSPLANTED RETINAS OF RCS DYSTROPHIC RATS. M.J. Sheedlo[1], L. Li[1], C.J. Barnstable[2] and J.E. Turner[1]*, Department of Neurobiology and Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC[1] and Department of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, CT[2].

This study was undertaken to further characterize the photoreceptor cell (PRC) rescue effect of RPE-cell transplants in retinas of Royal College of Surgeons (RCS) dystrophic rats (Li and Turner, 1988). Retinas of control and RCS dystrophic rats were immunostained with the monoclonal antibodies RET-P1 (N-terminus of opsin), SVP-38 (synaptophysin) and HNK-1 (a carbohydrate of N-CAM) using a paraffin technique (Sheedlo et al., 1989). RET-P1 in neonatal normal retinas immunostained the neuroblast layer, while in adult retinas outer and inner segments and PRC bodies were immunostained. In retinas of RCS rats, beginning by day 30, in addition to PRC constituents, a subretinal debris zone stained. By one year in RCS retinas, RET-P1 immunostained debris material was sparse and confined to the periphery. SVP-38 densely stained the outer plexiform layer (OPL), with lighter staining found in the inner plexiform layer (IPL) of normal retinas. In RCS retinas, a similar pattern was observed up to 2 months; however, at about 4 months, only the IPL stained. The HNK-1 antibody densely immunostained the OPL, IPL, a few cell bodies in the inner nuclear layer (INL) and ganglion cell bodies and their axons in control retinas. In RCS retinas, this staining pattern persisted until 2 months. Most importantly, in retinas of 4 month-old RCS rats transplanted with normal RPE cells at 26 days, the pattern and density of immunostaining for each of the above monoclonal antibodies was identical to that observed in control adult, healthy retinas, especially directly beneath the transplant. This result suggests that RPE transplants not only promote PRC survival, but also maintain the structural and, possibly, the functional integrity of dystrophic retinas. Two monoclonal antibodies (RET-PE2 and RET-PE10) which are specific for RPE cells are also being studied in RPE-transplanted RCS retinas.

Support provided by NIH EY 04337.

557.12

PRENATAL DEVELOPMENT OF HORIZONTAL CELLS IN THE RETINA OF THE RHESUS MONKEY. S.J. Kim and M.A. Kirby*, Department of Pediatrics, Loma Linda University, Loma Linda, CA 92350.

Recent studies have shown that the formation of the primate fovea is characterized by the lateral migration of cell classes. At present nothing is known of the effects of this movement on the morphological development of horizontal cells. Using retinal explants and horseradish peroxidase (HRP) we investigated the maturation of this cell class from embryonic day (E) 68 to E150 (term is 165 days). Animals were delivered by C-section, deeply anesthetized, and the retinas rapidly removed and placed into medium. HRP was inserted into the retina at different locations. After three hours, the retinas were fixed and processed for HRP.

At E68 horizontal cells at all locations had surprisingly complex arbors and could be classified as HI or HII cells described in the adult (Kolb et al., **JCN**, 1980, 189:31). Between E90 and E140 the soma and dendritic areas increased as a function of both age and eccentricity. The exception were cells in the central retina which were similar to cells observed in younger animals. At all ages and eccentricities the number of dendritic terminal clusters ranged between 6-12 per cell with the exception of peripheral HI cells in late term animals which had 14-19 per cell.

In summary, as early as E68 horizontal cells have surprisingly complex morphologies in both branching order and dendritic clusters. The mature appearance and lack of substantial growth in these cells during the majority of fetal development is surprising given the large migration of different cell classes to form the fovea.

557.13

DISPLACED RETINAL GANGLION CELL TOPOGRAPHY SUGGESTS A PROGRESSIVE DEGRADATION OF RETINAL HISTOGENESIS WITH CONTINUED RETINAL GROWTH. A. D. Springer*, Department of Cell Biology and Anatomy, New York Medical College, Valhalla, NY 10595.

Displaced retinal ganglion cells (dRGCs) in goldfish retina were backfilled by applying cobaltous-lysine to the severed optic nerve. dRGCs in flatmounts were defined as labeled cells that were not in focus with the orthotopic RGCs. In sectioned material, dRGCs were defined as cells touching the inner nuclear layer. The x-y coordinates of dRGCs were determined in retina having areas of 11mm² - 49mm².

dRGCs represented a nonhomogeneous RGC population since, in large retina, they varied in soma area from 20 - 300μm^2. However, the dendritic arbors of these cells were homogeneous in that they were consistently located in the outermost part of the inner plexiform layer. dRGCs represented 0.05% of the total RGCs in small retina and 0.2% of the total RGCs in large retina. This four-fold increase in the percentage of cells that were dRGCs was related to a progressive increase in dRGC **density** with retinal eccentricity and retinal area. dRGCs were not observed within a 250μm radius of the optic disc, and their density progressively increased to about 4 cells/mm² at a distance of 3.5 - 4mm from the optic disc. dRGC density did not differ as a function of retinal quadrant. Sectioned material showed a similar dRGC distribution. Furthermore, **doubly-displaced** RGCs were found near the **outer** plexiform layer in the retinal periphery.

The non uniform distribution of dRGCs suggests that repeated retinal germinal cell division at the retinal margin may be associated with a general, progressive degradation in retinal topography, as well as an increased incidence of ectopic retinal cells. Supported by grant EY-03552 from the NEI.

557.14

DISPROPORTIONATE INCREASE IN THE DENSITY OF LARGE DISPLACED CHOLINERGIC AMACRINE CELLS WITH RETINAL GROWTH IN GOLDFISH. E. Garcia-Valenzuela, A. D. Springer, A. B. Drakontides*, Department of Cell Biology and Anatomy, New York Medical College, Valhalla, NY 10595.

An antibody tc choline acetyltransferase (AB8) was applied to small (20mm²) and large (49mm²) flatmounted retinae. Two different sizes of cholinergic amacrine cells were found in the inner nuclear (INL) and ganglion cell layers (GCL). These cells had a mean soma area of 15μm^2 and 50μm^2 in the INL and 20μm^2 and 60μm^2 in the GCL.

Density of large displaced amacrine cells (dAC) decreased from 3.6/mm² in small, to 2.5/mm² in large retina. For small dACs, the density decreased from 1135.4/mm² in small, to 610.2/mm² in large retina. Similarly, small and large orthotopic cholinergic amacrine cell densities in the INL decreased as a function of retinal expansion. Cell density and retinal area were used to estimate the total number of cells per retina.

Concomitant with 2.5 fold increase in retinal area, the total number of orthotopic cholinergic amacrine cells and small dACs per retina increased by 25%. However, the number of large dACs increased by 71%. The nondisplaced cells in the INL, and the small dACs in the GCL were uniformly distributed across the retina. However, the density of large displaced amacrine cells progressively increased with eccentricity. This increased cell density was unrelated to that normally seen close to the germinal zone.

The paradoxical increase in the occurrence of large dACs as the retina grows may reflect a small degradation in histogenesis with continued production of new retinal cells. Furthermore, large dACs, because of their low incidence (0.3% of all cholinergic dACs), may be ectopic cells. Supported by grant EY-03552 from the NEI.

557.15

REGENERATION OF THE DOPAMINE-CELL MOSAIC IN THE RETINA OF THE GOLDFISH. P.F. Hitchcock* and J.T. VanDeRyt. Departments of Ophthalmology and Anatomy and Cell Biology, The University of Michigan, Ann Arbor, MI 48105.

A fundamental feature of the retina is the planar mosaic of each neuronal type. We have characterized the mosaic of regenerated dopaminergic interplexiform cells, and determined if the absence of the extant dopaminergic cells during regeneration modulated its density (see Reh and Tully, (1986) Dev. Biol., 114:463). Regeneration was induced locally by removing a 0.2-2.0mm^2 piece of retina, which was replaced during the subsequent 6-8wks. Two groups of eyes were studied: Group 1 (n=14) received surgical lesions only; Group 2 (n=18) received intraocular injections of 6-OHDA, to destroy all extant dopaminergic cells, 3 wks. prior to the surgical lesions. All retinas were immunostained with antibodies against tyrosine hydroxylase, and regenerated and normal cells were analyzed in wholemounts. The data from both groups were statistically identical: the somata of the regenerated TH-cells were more randomly arrayed than normal, and their planimetric density was significantly greater than control values. These data show that regeneration creates a less orderly inner retina than does *de novo* development, and suggest that, unlike retinal development, extant neurons do not modulate cell proliferation during retinal regeneration. Supported by NIH (NEI) grants EY07060 and EY07003 (CORE).

557.16

VISUAL FUNCTION IN REGENERATING TELEOST RETINA FOLLOWING RETINECTOMY AND CYTOTOXIC LESIONING. A. F. Mensinger* and M. K. Powers Vanderbilt Univ., Nashville, TN.

Components of the electroretinogram (ERG) were recorded in intact fish during regeneration of the neural retina. Portions of goldfish retina were removed by trans-scleral surgery or aspiration. Additional retinas were destroyed by intraocular injections of ouabain. Contralateral eyes served as controls.

Following trans-scleral removal of small retinal patches (10-25% retinal area, n=12), b-wave amplitude of experimental eyes increased from 70% (day 14) to 91% (day 75) of control eyes. Experimental eyes with large percentages (75-90%; n=8) of retina aspirated increased b-wave amplitude from 11% (day 14) to 30% (day 75). Complete aspiration (n=9) and ouabain injection (n=37) temporarily eliminated ERGs. Following complete aspiration, experimental eyes attained 3% of control b-wave amplitude by day 55. Ouabain treated eyes were 5-10% of controls at day 55 and increased steadily through day 210, when they were 50% of controls.

The experiments show that regenerating goldfish retina is responsive to photic stimulation. Partial retinectomy leads to swifter recovery presumably due to a larger population of undamaged retinal cells that can initiate repair. Histological examination of ouabain eyes revealed an occluded lens and a disorganized inner nuclear layer; this probably accounts for experimental eyes only attaining half of control values. Retinectomy circumvents optical side effects and enables a more precise evaluation of the visual sensitivity of the regenerating retina.

557.17

RETINAL RESISTANCE TO HYPERBARIC OXYGENATION (HBO) IN NEWBORN RATS. **H. WAFAPOOR, D. TORBATI* AND G.A. PEYMAN. LSU Eye Ctr. and Dept. Physiol. New Orleans, LA 70112.**

Retinopathy of prematurity (ROP) can be produced by exposure of newborn animals to normobaric hyperoxia (NH) of 60-80% oxygen for several days. The proposed mechanisms for ROP are either retinal damage by reactive oxygen species (ROS) or a maintained retinal vasoconstriction after NH exposure. We have demonstrated that, unlike adult rats, the newborn rats can survive up to 3 h at 5 atmospheres absolute (ATA) oxygen without mortality or visible morbidity. In the adult rat, however, HBO produces both vasoconstriction and ROS before the onset of CNS oxygen toxicity. Because of sensitivity of premature retina to NH, we hypothetized that exposure of newborn rats to acute HBO may accelerate the rate of production of ROS and/or create a severe retinal vasoconstriction, which later may result in the development of ROP. Four days postnatal litters (9-11 rats each) were subjected to a single exposure at 5 ATA oxygen for either 30, 60 or 90 min. Histopathological evaluation by light microscopy up to two weeks after HBO exposure showed no detectable retinal damage. These preliminary results indicate that contrary to ROP developed following exposure to NH, the retina of the newborn rat is resistant to HBO. Whether retinal resistance to HBO is due to existence of an efficient antioxidant mechanism or because of a reversible but protective retinal vasoconstriction, remains to be clarified. Supported by NEI grants No. EY07541 & EY02377.

558.1

PREDEGENERATED NERVE GRAFTS FOR PRIMARY AND DELAYED REPAIR. J.M. KERNS*, N. DANIELSEN, M. KANJE AND G. LUNDBORG. Dept. Anatomy, Rush Med. Coll., Chicago, IL. and Depts. Hand Surg. Malmö Gen. Hosp., Animal Physiol., Univ. Lund, Sweden.

The early progress of nerve regeneration was studied in rat sciatic nerve using fresh vs. predegenerated autografts in primary vs. delayed nerve repair. Results from the sensory pinch test on alternate postoperative days showed that 10 mm grafts predegenerated for one week by transection/ligation and placed in a fresh contralateral host site were superior to fresh nerve grafts. The initial delay period was reduced from 3.6 to 0.2 days, there were no "failures" (0/40 vs. 13/44), the regeneration rate was slightly increased (1.8 vs. 1.5 mm/day) and there was less individual variability. The presence of regenerating axons in the graft was confirmed by immunocytochemical staining for neurofilament protein. The response of the fresh graft was identical in either a primary or delayed nerve repair. Predegenerated grafts used in delayed repair were better than fresh grafts, but inferior to predegenerated grafts for primary repair. It is suggested that an increased number of Schwann cells is condusive to more immediate and improved nerve regeneration.

558.2

NERVE GRAFTS CAN BE IMPROVED BY PRIOR CRUSH. N. DANIELSEN*, J.M. KERNS, M. KANJE AND G. LUNDBORG. Depts. Hand Surg. Malmö Gen. Hosp., Animal Physiol., Univ. Lund, Sweden and Dept. Anatomy, Rush Med. Coll., Chicago, IL.

In our companion study of rat sciatic nerve grafts, predegeneration by transection/ligation for 7 days improved the regeneration compared to fresh nerve grafts. In the present study we determined whether predegeneration for longer periods and prepared by nerve crush had a similar effect. Results from the sensory pinch test at 2-10 days showed a nearly identical response for grafts predegenerated for 7 days prepared by either transection or crush; both regeneration rates were 1.8 mm/day. The presence of regenerating axons was confirmed by immunocytochemical staining for neurofilament protein. The regeneration distance at 8 dpo was not significantly affected by the duration of the predegeneration (7-28 days) or the type of injury. These data suggest that the presence of newly regenerated axons in the graft predegenerated by nerve crush do not interfere with the enhancement effect produced by activated Schwann cells. The predegeneration enhancement effect can be produced by both types of lesions and can last for at least 28 days.

558.3

PRELABELING OF NEURONAL POOLS TO STUDY ACCURACY OF MOTOR AND SENSORY AXONAL REGENERATION IN THE RAT FEMORAL NERVE. R.D. Madison*[1], S.J. Archibald[2], T.M. Brushart[3], and S.M. Meadows[2]. Depts. of Surgery (Neurosurgery)[1,2], Neurobiology[1], Duke University Medical Center, and Research Service[1], VA Hosp., Durham, NC 27710, and Depts. of Neurology and Orthopaedics[3], Johns Hopkins, and Curtis Hand Center, Baltimore, MD 21218.

Madison and colleagues have recently devised a method of double labeling neuron pools to determine the accuracy of axon regeneration at the single neuron level (J. Neurosci. Meth., 39, 123-129, 1991. The rat femoral nerve divides into a terminal sensory branch (saphenous nerve) and a terminal motor branch to the quadriceps muscle. Motor axons preferentially regenerate into the terminal motor branch following nerve transection and repair proximal to the terminal bifurcation (Brushart, J. Neurosci., 8, 1026-1031,1988). In the present study, adult rats received transection of the femoral nerve either 1 (low) or 4 (high) cm proximal to the terminal bifurcation (N=10 in each group) two weeks after prelabeling of the neuronal pools which contribute to the motor branch (see ref. above). Four weeks later we determined the number of neurons in the original pools which correctly regenerated an axon back into the motor branch by using a second fluorescent label and quantifying double-labeled neurons. Approximately 78% and 61% of the motor neurons were doubled labeled in the low and high transection groups respectively. Approximately 73% and 49% of the sensory neurons to the muscle spindles were doubled labeled in the low and high transection groups respectively. These data show that preferential motor reinnervation takes place regardless of the level of transection, but preferential sensory reinnervation only takes place with a low transection. NS22404-07 and VA Merit Review (RM).

558.4

DISTINCTIVE ABNORMALITIES IN REGENERATED RAT CUTANEOUS AND MUSCLE NERVES AFTER NERVE CRUSH INJURY. C.M. Bowe*[1], N.H. Evans[1] and C. Hildebrand[2], [1]Dept. of Clinical Neurosciences, Brown Univ., Providence, RI 02912 and [2]Dept. of Cell Biology, Univ. of Linköping, Linköping, Sweden.

Regenerated sciatic nerves chronically exhibit a pronounced sensitivity to potassium channel blockade with 4-aminopyridine (4-AP). This has been attributed to the "unmasking" of paranodal potassium channels by myelin sheath remodelling which occurs in regenerated axons. It is not known if these axonal abnormalities are present in all regenerated fibers or if they are restricted to a subpopulation of regenerated axons. The present study examined the morphological and physiological properties of regenerated, myelinated axons in a rat cutaneous and muscle nerve, following unilateral, sciatic nerve crush injury. *In vitro*, whole nerve recordings (CAPs) were performed in regenerated sural (R-SN) and lateral gastrocnemius (R-GN) nerves during superfusion with oxygenated Krebs' solution (NS) and during exposure to NS containing 1 mM 4-AP. Light and electron microscopy were used to determine the morphological characteristics of R-SN and R-GN.

During recording in NS, latencies to peak CAP amplitude in R-SN and R-LN were comparable to values for their respective control (C-) nerves. After 4-AP, a delayed depolarization was noted in R-GN, C-GN and C-SN. In contrast, a pronounced, second negativity with a "rippled" appearance was seen in R-SN exposed to 4-AP and recovery properties were significantly compromised. Decreased internodal distances and axonal diameters were observed in R-SN and R-GN but active myelin sheath remodelling was not prominent in either nerve.

558.5

SENSORY FIBER PATHWAYS IN EMBRYONIC AND REGENERATE CERCI OF THE INSECT, *ACHETA DOMESTICUS*. I.S. Edwards*, W.P. Chan, N.V. Sherbina, and M.R. Meyer. Dept. of Zoology, NJ-15, Univ. of Washington, Seattle, WA 98195.

Using neuronal and other markers, events of neurogenesis and axon outgrowth in the cricket cercus, a caudal sensory appendage, have been determined to investigate sensory pathway formation during both embryonic development and postembryonic regeneration. In the embryonic cercus, a cluster of neurons first appears near the base of the cercal lumen. These cells project axons to establish the first connection with the central nervous system (CNS). Subsequently, a second cluster of neurons appears more distally in the lumen and projects axons toward the basal cluster. Following formation of these distal cells, neurons arise within the cercal epidermis. The epidermal neurons project axons toward the cercal base either along the luminal wall or via pre-established pathways in the lumen.

Following cercal amputation at a postembryonic instar, new neurons and axons are observed in the regenerate cercus after the first postoperative molt. DiI labelling at later stages shows that axons of the regenerate sensory neurons can reestablish connections with the CNS by following either the nearest persistent sensory pathway or the extrinsic cercal motor nerve.

In embryonic cerci, basal lamina expression of glionexin (GX; antigen 5B12), a glia-related extracellular matrix glycoprotein, [Meyer et al., Dev. Biol. 130:374 ('88)], precedes appearance of neuronal structures. GX expression is correlated with the luminal neurons, their projections to the CNS, and other early axon pathways. Similarly, GX is transiently re-expressed on the basal lamina of regenerating cerci during axon outgrowth. These observations suggest elements of pathway formation are common to both the embryonic and regenerate cerci, while other mechanisms may be unique to either state. Supported by NS-07778.

558.6

PATTERN OF AXON REGENERATION FOLLOWING HYPOGLOSSAL NERVE TRANSECTION AND ENTUBULATION REPAIR. C. Timo-Iaria*[1], G. Chadi[2] and C.F. Da-Silva[3]. [1]Laboratories of Clinical Physiology and Experimental Neurology, [2]Dept. of Anatomy and [3]Dept. of Histology, University of São Paulo, São Paulo, Brazil.

The pattern of axon regeneration following unilateral hypoglossal nerve lesion was studied in adult rats. Transected nerves were repaired by entubulation technique with silicone tubes. Three months after the lesion, animals were processed for morphometric analysis. In one series of experiments, both the regenerated cable found within the tubes and the main medial and lateral branches of the hypoglossal nerve were processed for E.M. Quantitative parameters were determined with a computer system (Biographics Inc.) and compared with uninjured control animals. Regenerated animals showed a higher number of myelinated and unmyelinated axons in the distal nerve branches and regenerated cable of the hypoglossal nerve. Ultrastructural analysis demonstrated a decrease in the mean myelinated axon diameters and myelin thicknesses for the regenerated animals compared to the control group. In another series of experiments, HRP was applied to the cut end of 1) the regenerated hypoglossal nerve distal to the tube; 2) the main medial and 3) the main lateral branch of the hypoglossal nerve proximal to their ramifications. The number and distribution of HRP-labeled cell bodies in the hypoglossal nucleus after regeneration were compared with non-operated animals processed in the same fashion. The results demonstrated that, after nerve lesion and entubulation repair, no significant loss of motor neurons occurred, but hypoglossus nucleus somatotopy was not maintained. These findings indicate that after hypoglossal nerve transection and regeneration the normal pattern of axon innervation is only partially restored.

Supported by grants from FAPESP and CNPq.

558.7

SENSORY REINNERVATION OF THE RAT GLABROUS SKIN BY ORTHOTOPICALLY GRAFTED FETAL ALLOGENEIC AND XENOGENEIS DORSAL ROOT GANGLION CELLS. P. Dubovy, C.M. Rosario, T. Carlstedt* and H. Aldskogius . Departments of Anatomy, Medical Faculty, Brno, Czecho-Slovakia and Karolinska Institutet, S-104 01 Stockholm, Sweden.

Sensory reinnervation of the glabrous skin of the hindpaw in adult host rats was examined following orthotopic transplantation of fetal rat or mouse dorsal root ganglia (DRG). Sensory corpuscles were identified by the presence of non-specific cholinesterase (nChE) in their non-neuronal cells. Nerve fibers were identified with antibodies to neurofilament proteins (RT-97) or growth-associated protein, GAP-43. Eight months after allografting to the L4 and L5 DRG levels (L6 removed at the time of grafting, saphenous nerve cut seven days prior to sacrifice), RT-97+ nerve fibers were present, some of which terminated in sensory corpuscles. A few fibers displayed GAP-43 immunoreactivity. In xenografted animals, numerous GAP-43+ nerve fibers were present five weeks after grafting (cf. allograft procedure). Some of these fibers were associated with nChE+ cells of sensory corpuscles.

These findings demonstrate that orthotopically grafted fetal DRG neurons extend axons all the way to the hindpaw and differentiate into terminals of sensory corpuscles.

558.8

SPROUTING IN PARTIAL NIGROSTRIATAL LESIONED RATS FOLLOWING ADRENAL MEDULLA AND SCIATIC NERVE COGRAFTS. Z. Zhang,*[1] M. Bresjanac,[1] J.T. Greenamyre,[2] J.Olschowka[1] and D.M. Gash.[1] Depts. of Neurobiology & Anatomy[1] and Neurology,[2] University of Rochester, Rochester, NY 14642.

We report regenerative sprouting of dopaminergic fibers in the striatum following cografts of adrenal medulla and sciatic nerve in hemiparkinsonian rats. Adult Fischer 344 rats with unilateral 6-OHDA partial lesions were divided into three groups: nongrafted controls, sham-implanted controls and cografted hosts. After surgery, eight animals in each time point were sacrificed on 3, 7, 14, 28 days for immunohistochemical studies and 32 rats used for behavioral evaluation were sacrificed at 3 months and another nine animals sacrificed at 28 days were used for autoradiographic quantitation of high affinity dopamine uptake sites (HADAUS) and D2 receptors. The results of computer image analysis showed that there was a significant increase in the TH positive fibers seen at 3 days in both sham and cografted animals. However, TH fiber density in sham-grafted animals was significantly declined by 7 days and nearly reduced to background levels by 28 days post transplantation. In contrast, TH fiber density remained elevated in cografted animals with no significant differences seen between recipients in the 3, 7, 14 or 28 day test groups. Additionally, a positive correlation was found between the maintenance of TH positive fibers density and the viability of Schwann cells in cografted hosts. The results of amphetamine-induced ipsilateral rotation revealed a 30 to 45% decrease in the cografted group and a slightly increased number of rotations in sham-implanted controls over 3 months compared with the baseline. Finally, autoradiography showed an increase in HADAUS density as well as a decrease in D2 receptors in the cografted striata. All results suggest factors produced by the cografts influenced the dopaminergic regenerative response. Supported by NIH 2 P01 NS25778.

558.9

DEVELOPMENTAL PLASTICITY OF SELECTED BRAINSTEM-SPINAL PATHWAYS. G.F. Martin*, X.M. Wang and X.M. Xu. Department of Anatomy, The Ohio State Univ., Coll. of Med., Columbus, Ohio 43210

Rubral axons grow around a lesion of their spinal pathway at early stages of development in the North American opossum and it is possible that all brainstem axons do so. We have attempted to establish whether reticular and vestibular axons can circumvent a spinal lesion during development and, if so, to determine their critical period(s) for plasticity. We hemisected the thoracic cord and 30 days later made bilateral injections of Fast-Blue caudal to the lesion. At postnatal day (PD)20, rubral neurons were labeled contralateral to the lesion, as expected; but neurons in the medial pontine reticular formation and the dorsal part of the lateral vestibular nucleus were not labeled ipsilateral to it. Rubrospinal axons project contralaterally, whereas the reticular and vestibular axons in question project ipsilaterally. At EPD5, however, neurons were labeled in all of the above areas. We conclude that reticulospinal and vestibulospinal axons are capable of plasticity and that the critical period for their plasticity ends earlier than that for rubrospinal axons. Supported by NS-25095 and 10165.

558.10

AXONAL REGENERATION AND PATHWAY SELECTION BY BRAINSTEM NEURONS AND BEHAVIORAL RECOVERY IN SPINAL-TRANSECTED LAMPREY LARVAE. G.R. Davis* and A.D. McClellan Div. of Biological Sci., Univ. of Missouri, Columbia, MO 65211.

Larval lampreys recover swimming 3-6 wks after a complete spinal cord transection. Our goals are to identify the brainstem regions involved in the recovery of locomotion and to describe the specificity of tract selection in the spinal cord by regenerating axons from the brainstem.

Four months after a complete spinal cord transection at 10% of body length (BL), Petromyzon marinus larvae (n =27) swam with few or no deficits. These animals received additional cord lesions several segments both above and below the original transection. Behavioral tests and EMG's were done 5-7 days after the second lesions, and HRP was applied to the spinal cord at 25% BL. Nine to 14 days later, brains and spinal cords were processed with Hanker-Yates reagents. Labeled cells in wholemounts were classified into 1 diencephalic, 2 mesencephalic, 3 isthmic, 2 bulbar, 1 lateral, and 3 vagal cell groups.

Medial tract lesions typically altered posture and equilibrium but otherwise did not disrupt locomotion. Labeled cells were consistently found in large numbers within subdivisions of the isthmic, bulbar, and vagal groups. Cells were rarely labeled in diencephalic, mesencephalic, and lateral groups. Animals with bilateral lesions of the lateral tracts rarely swam spontaneously or in response to stimulation of the head. Very few labeled braintem cells were found in these animals suggesting that regenerating axons either prefer to grow into the lateral tracts and/or avoid the medial tracts. Most animals with hemisections on the left side had an altered posture and all swam only in small circles to the right. The pattern of cell labeling indicated that the axons of many neurons which normally project to one side of the cord had crossed to the opposite side. Labeled axons were observed crossing the midline only at the site of the healed transection. Most animals with lesions of the medial tracts above and both lateral tracts below the original transection could swim suggesting that some descending lateral tract axons which are important for initiation of locomotion in normal animals (McClellan, 1988) can regenerate into the medial tracts. (Support: NIH NS29043, APA MB1-9108.)

558.11

CHRONICALLY INJURED SUPRASPINAL NEURONS EXHIBIT AXONAL REGENERATION FOLLOWING SPINAL CORD INJURY. J.D. Houlé*, J.W. Wright and M.K. Ziegler. Dept. of Anatomy, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205.

Intraspinal and dorsal root ganglion neurons will regenerate into peripheral nerve (PN) segments grafted 1-2 mos. after injury to the lumbar spinal cord. The lack of regeneration by supraspinal neurons following injury at this level suggested that some neurons may be unable to regrow under chronic injury conditions. To test this True Blue (TB) was injected into the cervical (C6) spinal cord of adult rats. A hemisection lesion cavity including the injection site was created after 7 days, followed 30 days later by apposition of an autologous PN graft to the lesioned surface. The distal tip of the PN graft was exposed to Nuclear Yellow (NY) after 30 days. Chronically injured neurons capable of regeneration contained both TB and NY and were found in several regions of the brainstem, including: the spinal trigeminal nucleus, medullary and pontine reticular formation, ventral raphe, the spinal and lateral vestibular nuclei, several hypothalamic nuclei and nucleus cuneatus. Regions lacking TB/NY labeled neurons included locus coeruleus, red nucleus and motor cortex, although numerous TB labeled neurons were present in all areas. In contrast, PN grafts placed immediately after injury contained regenerated axons from both the locus coeruleus and red nucleus. These results demonstrate differences in the regenerative response of neurons relative to the post trauma interval and level of spinal cord injury. Supported by NIH Grant NS-26380.

558.12

CO-EXISTENCE OF REGENERATING AND SEVERED AXONS IN THE OPTIC NERVE OF THE FROG *LITORIA (HYLA) MOOREI*. S.A. Dunlop* & W.M. Ross, Neurobiology Laboratory, Dept. of Psychology, University of Western Australia, Nedlands 6009.

After extracranial optic nerve crush in the frog *Litoria moorei*, regenerating axons penetrate the crush site, grow along the optic nerve and tract to re-innervate the optic tectum; normal vision is restored within 2-3 months. Using horseradish peroxidase labelling and light microscopy to examine the optic tracts and tecta, we have recently shown a co-exitsence of regenerating axons with the distal stumps of the severed axons that are destined to degenerate (Humphrey, Dunlop, Shimada and Beazley, Exp. Brain Res. *in press*). To investigate the spatial and temporal relationship between the regenerating and severed axons in the optic nerve, we examined animals between 3 and 28 days after optic nerve crush. Animals were perfused with 4% paraformaldehyde and regenerating axons labelled anterogradely with DiI from the optic disk; the distal stumps of the severed axons that are destined to degenerate were labelled retrogradely from the optic brachia with DiA. Nerves were examined as wholemounts and as longitudinal sections. At 3 days regeneration, DiI-labelled regenerating axons had retracted somewhat towards the eye; by 7 days, axons had penetrated the crush site although few had reached the other side. Between 10 and 14 days, a small number of pioneer axons had grown through the crush site to the chiasm; these axons were peripherally located and closely grouped. Between 17 and 28 days, more axons crossed the crush site and occupied both the core and the periphery of the nerve. The DiA-labelled severed axons persisted in the nerve at all stages examined. Severed axons were lost first from the core of the nerve and then the periphery; there was a progressive loss from the crush site towards the brain.
Funded by the National Health & Medical Research Council, Australia.

558.13

RESTORATION OF RETINOCOLLICULAR PATHWAY AFTER SCIATIC NERVE TRANSPLANTATION INTO THE SECTIONED BRACHIUM IN ADULT HAMSTERS. H.Sawai, Y.Fukuda and M.Watanabe*° Dept. Physiol. Osaka Univ. Med. Sch.,Suita 565, °Dept. Physiol., Aichi Pref. Colony, Kasugai 480-03, Japan.

After transection of the brachium of superior colliculus (BSC) we transplanted the autologous sciatic nerve to make a bridge to the deafferented colliculus in 28 anesthetized adult hamsters. Four to 15 weeks later, we examined photic responses (PR) to diffuse flash stimuli applied to the contralateral eye in operated animals. PR of moderate amplitude were obtained from both the graft and the superior colliculus (SC) in 8 grafted animals. The PR were further verified at single unit level in 3 of the 4 grafted animals examined. In the remaining 8 animals, however, the graft came out from the SC and no PR was obtained from the SC. None of the 5 control animals, in which the BSC had been transected without the graft, exhibited any visual response when tested at 5-20 weeks after surgery. With intraocular injection of WGA-HRP, we were able to trace regenerating retinal axons to pass through the graft and terminate in the superficial part of the SC in 4 grafted animals. In other 3 animals the graft was missing and yet some regenerting fibers were found to traverse the cleft of the sectioned BSC. From these results we conclude that the sciatic nerve graft is benificial for the functional and morphological restoration of the retinocollicular pathway after BSC section.

558.14

DEVELOPMENTAL CHANGES IN THE RESPONSE OF OPTIC TRACT AXONS TO TRANSECTION IN THE HAMSTER: SWITCHING FROM REGENERATION TO RETRACTION. L.I. Mortin* and G.E. Schneider. Department of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Following a complete transection of the brachium of the superior colliculus (SC) in Syrian hamsters, optic tract axons can regenerate if the lesion occurs prior to four days postnatal (P4); axons severed in P4 and older animals normally do not regenerate (So et al., Exp. Neurol. 72, 1981). What is the nature of regenerative growth or its failure, and how soon can we differentiate between them? We used the carbocyanine dye DiI to visualize the response of single optic tract axons to transection at P1 and P6, developmental stages permissive and restrictive, respectively, for regeneration. Retinofugal axons were labeled either *in vivo* by injecting a solution of DiI dissolved in N,N-dimethylformamide into the eye or in fixed tissue by placing a crystal of DiI in the optic chiasm. Anterograde labeling with HRP was used to determine the rate of growth or retraction of cut axons.

After transection on P1, optic tract axons grow rapidly across the lesion site, sometimes in large numbers; they can extend up to 800-1000μ in 24 hr. Axons crossing the region of gliosis and beyond are mostly unbranched, and have simple growth cones. Some growth cones remain in the gliotic region longer or grow along the axis of the lesion, perpendicular to their normal pathway. Caudally axon growth appears to slow significantly; nine days after the lesion the SC is still not completely reinnervated. By three weeks the processes of optic tract axons can cover the entire SC.

When the transection occurs on P6, retraction of severed axons can be seen within 12 hr. These axons display retraction bulbs or simple growth cones at their tips. Some axons can remain near the gliotic region of the lesion; some become thinner near the lesion site. Thus, within hours, the response of axons transected at P6 is different from those transected at P1.

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558.15

FORMATION OF NEURAL LOBE-LIKE NEUROVASCULAR CONTACT ZONES IN TISSUES GRAFTED INTO THE HYPOTHALAMO-NEUROHYPOPHYSEAL TRACT IN RATS. J. Carithers* and H.-D. Dellmann. Dept. of Veterinary Anatomy Iowa State Univ., Ames, IA 50011.

Large neural lobe-like neurovascular contact regions developed within 6/7 neural lobe explants (after 30 days in vitro axonal fragments had disappeared, and explants consisted mainly of pituicytes) grafted into contact with neurosecretory axons transected in the lateral retrochiasmatic hypothalamic area. Much smaller contact regions were seen in 4/9 optic nerve grafts and 3/17 sciatic nerve grafts, and none developed in vascular grafts. In neural lobe explants the fine structure of contact regions resembled that of normal neural lobes; i.e., neurosecretory axon terminals associated with pituicytes formed palisades abutting perivascular spaces of capillaries, most of which were fenestrated. In grafts of optic and sciatic nerves, although neurosecretory axon terminals accompanied by astrocytes or neurolemmocytes incompletely invested capillaries, they did not form palisades, and capillaries were non-fenestrated. Moreover, it was not always possible to delineate neurovascular contact regions within optic and sciatic nerve grafts from contact regions that developed in the adjacent hypothalamic neuropil. These results are consonant with our hypothesis that non-neuronal components of neural lobes, most likely pituicytes, promote development of neurovascular contact regions. Supported in part by NSF grant BNS 8919729.

558.16

Fate of CNS neurons afferent to an excitotoxic lesion. S. Marty, N. Le Forestier, J.M. Weinitz and M. Peschanski*, INSERM CJF 91-02, Faculté de médecine, F - 94010 Créteil, France

Injection of an excitotoxic substance into the adult CNS induces a rapid neuronal death, while acutely sparing axons afferent to the lesion. Excitotoxins can thus be used as tools to investigate the dependance of neurons toward their targets. In a previous study we found that in these conditions dorsal column nuclei (DCN) axons formed terminal varicosities in a one month old kainic acid lesion of the adult rat thalamus. The persistance of some of these varicosities one year post-lesion indicated that afferents can survive for long periods of time after loss of their targets. Using wheat germ agglutinin conjugated to peroxidase (WGA-HRP) as an anterograde and retrograde tracer in light microscopy, or electron microscopy, we have now characterized this evolution over time between four days and eight months.

In one group of rats WGA-HRP was injected into the DCN at different times post-lesion, and allowed to be anterogradely transported to the thalamus. Peroxidase was revealed using TMB. In another group WGA-HRP injected into the thalamus at different times post-lesion was allowed to be retrogradely transported to the DCN. Peroxidase was revealed using nickel-enhanced DAB. The third group was directly perfused and brains were processed for electron microscopy.

Major changes occur during the first month. There is an important decrease in axonal density. Terminal enlargments appear as soon as four days and become prominent after two weeks, while presynaptic profiles disappear. During the same period there is an atrophy of DCN neurons and a conspicuous decrease of their retrograde HRP labeling when compared with controlateral controls. After one month the decrease in number of axons is slower so that there are always axons from the DCN in the eight months lesion. These axons always exhibit varicosities, and the retrograde labeling of their cell bodies remains altered.

The present results indicate that during the first month excitotoxicaly induced loss of their targets provokes modifications of adult CNS neurons : axons loose presynaptic specializations and form varicosities, retrograde WGA-HRP labeling is decreased, and cell bodies are atrophic. Some of these modified neurons can survive for months.

TRANSPLANTATION II

559.1

CHONDROITIN SULPHATE AND ASTROGLIAL DISTRIBUTION IN NEURAL TRANSPLANTS AND IMPLANTED GLIOMA. J. Rosenstein* and T. Moody. Depts. of Anatomy, Neurosurgery, Biochemistry Molecular Biology, George Washington University Medical Center, Washington, D.C. 20037.

The advent of an injury to the brain produces potential long term cellular and extracellular changes. To determine the presence of the glycosaminoglycan chondroitin sulphate (CS), a molecule involved in axonal growth by inhibitory mechanisms we examined the expression of the MAb CS-56 in neocortical grafts (2 weeks-18 mos) and C6 gliomas (3 day-3 weeks). Concommittant distribution of astrocytes was also examined by GFAP. Early grafts up to 4 weeks had CS staining only at the host interface after which time it almost entirely disappeared. Gliosis was found around the grafts for at least one year where it overlapped with extracellular glucose transporter immuno-staining. Stab wounds showed similar results. In gliomas, CS stained intensely particularly at the proliferating tumor-brain interface. In gray matter surprisingly little gliosis was observed whereas in white matter GFAP staining was intense. Ultrastructurally, tumor cells supplanted astrocytes at the interface and CS filled the extracellular space. In neural grafts there appears to be transient expression of CS whereas in tumors CS is intensely expressed and reactive astroglia have disparate distribution. (NS-17468) (BNS88-15133).

559.2

MIGRATION PATTERNS OF TRANSPLANTED LABELLED GLIAL CELLS. A. Espinosa*, A. Watabe, M.-S. Zhang and J. de Vellis. UCLA Mental Retardation Research Center, Los Angeles, CA. 90024-1759.

Glial cell plasticity has been investigated in vitro by numerous laboratories. Such plasticity persits when normal cultured glial cells are grafted into perinatal host brains. Fast Blue labeled O2A lineage cells can survive migrate and integrate within normal and abnormal host brains. In the present study we compared the migration patterns of unaffected rat O2A cells to those of C6 glioma cells grafted into normal rat hosts. Migration patterns of FB^+ C6 cells are extensive throughout the brain. These cells migrate continuously and form clusters (1 week post-grafting), and tumors later on. Cells derived from such tumors migrated out to form new tumors. FB label persists with variable intensity within grafts despite intense proliferation. This model is useful to investigate a) the differences in cell migration between normal and transformed cells; b) the early changes in host brain caused by tumoral cells, and graft/host interactions; c) the plasticity of grafted C6 cells vs cultured C6 cells. (Supported by NIH and DOE)

559.3

DIFFERENTIAL SEIZURE SUPPRESSANT EFFECTS OF SOLID NORADRENALINE-PRODUCING LOCUS COERULEUS AND SUPERIOR CERVICAL GANGLION GRAFTS IN KINDLING.

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The noradrenergic locus coeruleus (LC) system exerts a strong inhibitory influence on kindling development. We have previously shown that the facilitation of kindling caused by lesions of this system can be reversed by intrahippocampal LC cell suspension grafts. Similar to intrinsic LC neurons, grafted cells increase their release of noradrenaline (NA) in response to seizures. We investigated whether also solid LC and superior cervical ganglion (SCG) grafts can influence kindling. Rats were given 6-hydroxydopamine (6-OHDA) in the ventricle and were then subjected to a bilateral aspirative fimbria-fornix (FF) lesion and cervical sympathectomy. One week later, fetal LC and autologous SCG grafts were placed in the FF lesion cavity. Lesioned and intact animals served as control. After 10-12 weeks the animals were subjected to kindling, microdialysis and histological analysis. Solid LC grafts significantly retarded the development of kindling in previously 6-OHDA-lesioned animals, whereas SCG grafts had no effect. Both baseline and seizure-induced NA release was significantly higher in the LC grafted animals than in the SCG grafted group. No release of NA was detected in lesioned animals without grafts. Normal or supranormal fiber density was observed in the dorsal hippocampus of LC grafted rats, while the SCG graft derived reinnervation was restricted to certain subfields. These results indicate, that solid LC grafts can suppress seizure development and restore noradrenergic innervation as well as basal and seizure-induced NA release in the hippocampus of NA depleted rats. The lack of effects on the development of kindling in the SCG grafted animals can possibly be accounted for by more limited graft-derived fiber outgrowth and NA release. However, the present data may also suggest that the seizure suppressant action of NA is dependent on a synaptic release, which can be provided by the LC but not the SCG grafts.

559.4

INTEGRATION OF LABELED FETAL HIPPOCAMPAL GRAFTS INTO KAINIC ACID-LESIONED F344 RATS.

G.K. Pyapali*, D.A. Turner and R.D. Madison. Neurosurgery and Neurobiology, DUMC, and VAMC, Durham, NC 27710.

Survival of grafted neural tissue is improved if the graft is placed into a pre-lesioned area of the host. The overall time course and critical factors underlying enhanced survival and synaptic integration of grafts into lesioned hippocampus remains unclear. This study investigated the differential influence of the availability of neurotrophic factors and vacant synaptic sites on the survival and integration of fetal tissue grafts in a kainic acid lesion model.

Dissociated fetal hippocampal and cerebellar cell suspensions were labeled with rhodamine dextran amine and stereotaxically implanted into pre-lesioned hosts. Two-three months later hippocampal slices containing the grafts were assessed for graft survival, integration, migration and the development of individual cells using anatomical and physiological indices. Compared to normal hosts, grafts in lesioned hosts showed enhanced cellular survival, development, migration of cells from the needle track and synaptic interconnections with the host. Survival was significantly increased in early transplants (at 2-4 or 11-12 days) compared to controls, grafts at 6-7 days and late grafts at 14-16 and 28-33 days post lesion. Late grafts demonstrated equally good synaptic interconnection but only moderate survival and enhanced migration of cells away from the needle track. Grafted cells showed considerable development and the processes were mainly confined to the graft region; synaptic potentials could be elicited with host fiber stimulation.

The time course of graft survival and integration indicates that host recovery processes leading to enhanced survival may be separate from factors influencing migration and integration of graft cells within the host. This finding has important implications for the timing of grafts following a lesion and the evolution of lesion recovery mechanisms in the host. Supported by NINDS Grant RO1 NS29482-01, ADRDA and VAMC Merit Review Awards.

559.5

DENDRITIC OUTGROWTH OF TRANSPLANTED DENTATE GRANULE CELLS.

D.I. Legendre, B.P. Vietje and J. Wells*. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

Allogeneic neonatal granule cells were injected into the infragranular cleavage plane of the rat dentate gyrus, simultaneously lesioning and replacing the host granule cells. The transplanted granule cells were intracellularly labeled with Lucifer Yellow in fixed sections. Normal granule cells were similar to those already described in the literature. At one week post-transplant, many of the dendrites that had not reached the hippocampal fissure had growth cones, and their dendritic spines were longer than those on adult granule cells. Virtually all of the dendrites reached the hippocampal fissure by two weeks post-transplantation and the spines looked normal. By eight weeks, the dendrites appeared thicker, more coiled, and more spiny than normal. Some of the dendrites bent back into the molecular layer upon reaching the hippocampal fissure. Between three and eight weeks a return of cytochrome oxidase staining indicated that the transplanted cells formed functional synapses. If transplanted granule cells were placed into the hippocampal fissure, the dendrites developed spines and grew preferentially into the molecular layer rather than CA1.

559.6

INTRACRANIAL TRANSPLANTATION OF HYPOTHALAMIC NEURONS.

Hanna Bergman*[1] George D Prell[2] and Ann-Charlotte Granholm[3]. [1]Dept Cell Biology, Univ. of Linköping, Sweden, [2]Dept Pharmacol., Mount Sinai School of Med, NY, NY 10029 and [3]Dept Basic Science, Univ. of Colorado, HSC, Denver, CO 80262.

Many histamine-containing tuberomammillary neurons of the hypothalamus project to the hippocampus through the fornix. Afferent denervation has been shown to significantly decrease hippocampal levels of histamine. In order to investigate survival and growth of intracranial grafts containing histaminergic neurons, fetal hypothalamic tissue from day E17 was grafted into lesion cavities two weeks after unilateral lesions of the fornix in adult Sprague-Dawley rats. Hippocampal levels of histamine and its metabolites were determined in lesion-free, lesioned and lesioned-grafted rats. Post-transplant immunohistochemical evaluation showed that all animals contained surviving transplants; furthermore, numerous immunoreactive fibers projected from transplanted tissue into host brain, including the hippocampus. In conclusion, we found that histaminergic neurons will survive intracranial grafting and innervate surrounding host brain. Sponsored by the Swedish MRC, Blanceflor Fnd and Magnus Bergvall Fnd and NINDS grant NS-28012 to GDP.

559.7

ADULT OLIVARY AXONS ENLARGE THEIR TERMINAL DOMAIN TO INNERVATE EMBRYONIC PURKINJE CELLS GRAFTED ON THE SURFACE OF THE ADULT RAT CEREBELLUM.

P. Strata, F. Rossi and T. Borsello, Dept. of Human Anatomy and Physiology, I-10125 Turin, Italy. SPON: European Neuroscience Association

It is commonly assumed that neuronal loss in the host brain is necessary for the integration of grafted embryonic neurones. We have studied the capability of transplanted cells to interact with host elements in an intact adult brain. To this aim solid pieces of cerebellar primordia taken from 14-days-old rat embryos, were placed in the fourth ventricle of adult hosts in close contact with the cerebellar surface, where they developed minicerebella. Embryonic Purkinje cells (PC) visualized by anti-D-28k calbindin antibodies were able to leave the graft and penetrate the host molecular layer, where they developed adult structural features. PHA-L tracing of host olivocerebellar axons, revealed that adult climbing fibres (CF) emit collateral sprouts, which elongated through the molecular layer and ended in terminal plexuses impinging upon grafted cells. Such a process was already active at early stages after transplantation, during the migratory phase of grafted PCs development and continued at longer survival times, ending with the formation of fully developed new CFs. In addition, CF sprouts were able to leave the host cerebellum and penetrate the transplant, where they innervated clusters of PCs. These results show that grafted PCs are able to migrate and develop in an adult unlesioned cerebellum and to interact with host elements by eliciting the growth of adult axons. On their part, adult olivary axons, which face an increased target population, are able to enlarge their terminal domain by retaining their adult targets and innervating a number of transplanted cells.

559.8

Effects of Amygdala Grafts from SHR and WKY Donors on Depletion-Induced Water and Saline Intake in Amygdala-Lesioned Wistar Rats.

C.A. Murphy, R. Canbeyli, & B.G. Yongue*. Columbia Univ. and N. Y. S. Psychiatric Inst. New York, NY 10032.

Damage of the amygdala including the central nucleus is known to reduce salt appetite and blood pressure (BP). We have previously shown that transplantation of hypothalamic or cortical tissue from spontaneously hypertensive rat (SHR) fetuses to normotensive Wistar and Wistar-Kyoto (WKY) rats induces elevated BP. This study investigated the effects of fetal amygdala grafts including the central n. in Wistar rats with damage to the central n.

Rats underwent bilateral lesion aimed at the central n. at 25 days of age; 8 were lesioned controls, others received bilateral grafts, in the lesioned area, of amygdala from SHR (n=6) or WKY donor (n=7) fetuses at day 17-18 of gestation. Sham operated controls (n=8) received volume-matched infusions of 0.9% saline. Tail-cuff BP was measured at 30,60,90 & 120 days of age. To assess need-free salt appetite, animals were presented at 60 & 120 days with 1.5% saline, H_2O and low-Na^+ diet for 4 days; depletion-induced consumption was then measured for 2-hours, 24 hours after a diuretic/natriuretic. Direct BP was measured via the tail artery at 180 days of age.

The 4 groups (lesion, sham, SHR- and WKY-amygdala graft) did not differ in BP or need-free saline intake. At 60 days, depletion-induced saline intake was reduced (ns.) in the lesion group (mean 9.2 ml) relative to the shams (16.5 ml). SHR grafts restored intake to levels (14.6 ml) comparable to shams. WKY grafted rats consumed significantly less saline (4.8 ml) than shams. Water intake for the 24-hours after diuretic injection was comparable in the sham and SHR-amygdala grafted groups, which were significantly higher than in the WKY-amygdala grafted group in the second depletion test. The results are interpreted in terms of the differential effects on fluid and mineral intake of amygdala grafts from SHR and WKY; donors with phenotypic differences in salt appetite and BP regulation. (MH00803,MH45951)

559.9

THE DYNAMIC CHARACTERISTICS OF INJECTED MYOBLASTS IN THE HOSTS. H. J. Li*, Q. Fang, M. Chen, T. Goodwin and P. K. Law. Cell Therapy Research Foundation, Memphis, TN 38117.

In animal experiments and clinical trials, various laboratories have demonstrated that injected normal myoblasts survive and fuse with host muscle fibers. However, there has been little description about myoblast distribution in the host muscle. We injected Fluoro-gold (FG) labelled myoblasts (C57BL/6J) into the quadriceps muscle of mice (C57BL/6J). These mice were sacrificed every seven days lasting up to 1.5 months after myoblast injection. Injected muscles were cut with cryostat at 10 μm. Muscle sections were examined under fluorescence microscope. The cell fusion rate was determined. We found that the distribution of injected myoblasts in the host muscle sections varies according to the different ways of injection. A good injection method allowed even distribution of donor myoblasts over the whole muscle cross-section and it increased the chance for the donor cells to fuse with host myofibers. Mosaic myofibers were observed within seven days after cell injection. The highest fusion rates ranged between 62.5%-72.2%. These results are used to improve the efficacy of myoblast transfer therapy. (Supported by PHS NS 26185 PKL)

559.10

IMMUNE REACTIONS FOLLOWING MYOBLAST TRANSPLANTATION IN DUCHENNE MUSCULAR DYSTROPHY PATIENTS. Tremblay, J.P.*, Huard, J., Roy, R., Bouchard, J.P., Malouin, F., Richards, C.L., Lemieux, B. and Langevin, P. Neurobiology Laboratory, Enfant-Jésus Hospital, 1401, 18e Street, Québec (Qc), G1J 1Z4

The discovery of the genetic defect resulting in a protein deletion responsible for Duchenne Muscular Dystrophy (DMD) has led to intense investigation aimed at achieving a therapy for this disorder. A possible therapy is the transplantation of normal myoblasts into DMD muscle. This would result in fusion of the donor myoblasts with host myoblasts to form hybrid muscle fibers which have the ability to produce dystrophin and incorporate it into the sarcolemma. Our research group has transplanted myoblasts obtained from an MHC compatible donor to nine Duchenne patients. Transplantations were done in the biceps, extensor carpi radialis longus and/or tibialis anterior muscles. No immunosuppressive treatment was used during the follow-up period (8-19 month). No clinical sign of rejection (i.e. fever, swelling or pain) was observed. Biopsies done 1 month after myoblast transplantation showed the presence of dystrophin in an higher percentage of muscle fibers in the myoblast injected muscle than in the contralateral muscle in 7 of the nine patients. Antibodies against the donor myoblasts and/or myotubes were observed in five patients. In some case these antibodies were capable of fixing the complement and lyzing the myotubes in vitro. Transient increase of maximum voluntary contraction were observed in only 3 patients. Although some aspect of these experiments are encouraging, they also indicate that myoblast transplantation cannot be done without some immunosuppression even when the donor is immunocompatible.

559.11

VIABILITY, MORPHOLOGY AND IMMUNOGENICITY OF COLD PRESERVED NERVE ALLOGRAFTS. P.J. Evans*, S.E. Mackinnon, J. Wade and R. Midha. Peripheral Nerve Lab., Univ. of Toronto, Toronto, Ontario, Canada, M5S 1A8.

PURPOSE: Nerve banks may provide ready access to tissue and ample time for donor tissue screening. Three week stored nerve allografts may be dead and subsequently non-immunogenic and serve as a connective tissue scaffold for proximal regenerating host axons.

METHODS: Three centimetre ACI rat nerve grafts were harvested and stored in 15 ml of Belzer/UWCSS solution at 5°C for 0-22 weeks. Nerves were prepared for standard light and electron microscopy and for laminin immunostaining. Graft cell viability was assessed by DNA or protein synthesis by incorporation over 24 or 96 hrs of 2 μCi/ml of ^{3}H-leucine or ^{3}H-thymidine respectively, and corrected for total protein by protein spectrophotometry. Immunogenicity was assessed by recipient sera screening utilizing the complement dependent cytotoxicity assay at 16 weeks post-engraftment.

RESULTS: Morphology and laminin staining in grafts stored for up to 22 weeks remained similar to fresh nerve grafts. DNA and protein synthesis was maximal in 3 day *in vivo* Wallerian degenerated nerves, lower in normal and 24 hr stored nerves, but still present in 3 week stored nerves. Nerve allografts stored for up to 5 weeks tested positve for antibody, but freeze-thawed nerve allografts were negative.

CONCLUSION: Stored nerve grafts appear to undergo early Wallerian degeneration, but leave behind an intact connective tissue scaffold rich in laminin. Some cells within the graft remain viable after 3 weeks of storage and grafts stored up to 5 weeks remained immunogenic.

559.12

UNBIASED QUANTITATIVE STEREOLOGICAL ASSESSMENT OF INTRACEREBRAL GRAFT SURVIVAL. D.A. Peterson[1], H. Takayama[1], D. Barba[2], G.R. Chalmers[1], M.H. Tuszynski[1], and F.H. Gage[1]* Departments of Neurosciences[1] and Surgery[2], UC San Diego, La Jolla, CA, 92093-0627

Histology of engrafted neural tissue has largely focussed on qualitative aspects of graft health, integration, morphology, and phenotype. To objectively assess the survival of cografted cells in a variety of paradigms, we found it necessary to perform quantitative assessment to permit statistical evaluation. We present here the application to the intracerebral grafting model of two unbiased stereological techniques: the optical disector estimator of cell numerical density (N_v) and Cavalieri reference volume (V_{ref}) estimator.

Stereology has been applied to estimate cell numbers in normal neuroanatomical structures, and recently to cultured tissue, but it has not been used with intracerebral grafts. The optical disector directly counts cells in a known volume using the formula $N_v=\Sigma Q^-/V_{dis}$ where Q^- is the number of cells counted within the sampling volume (V_{dis}) which is determined by multiplying the *x,y* area of the sampling frame by the sampled depth of focus (*z* axis) into the tissue (measured with an electronic microcator). All cells within the optical disector volume are sampled with equal probability with respect to cell size, shape, or distribution and sampling is therefore without bias and requires no correction factors. Using the Cavalieri estimator formula $V_{ref}=(\Sigma P)(A_p)(t)$, graft volume is quickly calculated from systematically sampled sections knowing only the sum of points (ΣP) from a point-counting grid overlying the graft profiles, the calibrated area associated with each point (A_p), and the section thickness (t). In addition to providing a useful parameter in itself, graft volume (V_{ref}) can be combined with N_v to estimate the total number of cells (N_{abs}) by the equation $N_{abs}=(N_v)(V_{ref})$. Our resulting estimations of absolute graft volume and unbiased total cell number provide a reliable, comparable basis upon which to assess biological significance.

MEMBRANE COMPOSITION AND CELL-SURFACE MACROMOLECULES

560.1

PHOSPHORYLATION OF GAP JUNCTION PROTEIN IN C6 GLIOMA CELLS TRANSFECTED WITH CONNEXIN43 cDNA. M. Deakin, J.F. Bechberger and C.C.G. Naus*. Dept. of Anatomy, The University of Western Ontario, London, Canada N6A 5C1.

Gap Junctions are transmembrane channels which aid in intercellular communication permitting the transfer of ions and low MW molecules between cells. C6 glioma cells transfected with connexin43 (cx43) cDNA were used to examine the association of phosphorylation of cx43 on intracellular localization. The total, membrane and cytoplasmic fractions of these cells were isolated by differential centrifugation, and separated using PAGE and Western Blotting. In these cells, the phosphorylated state of cx43 was found principally in the membranous fractions while the non-phosphorylated state was found principally in the cytoplasmic fractions. Following treatment in culture with either 10 μM or 100 μM forskolin to increase cAMP production and protein phosphorylation, an increase in the amount of cx43 in C6 cells was evident by indirect immunofluorescence. Examination of cx43 mRNA levels revealed a 7 fold increase in mRNA production in C6 cells treated with forskolin, as well as a 5 fold increase in endogenous mRNA production in transfected cells. However, the expression of the transfected cx43 cDNA was not affected by forskolin. Forskolin treatment appears to increase the endogenous mRNA and protein levels of cx43 in the C6 cells. In transfected cells, forskolin induced an increase in the phosphorylation of cx43, suggesting an enhanced insertion of this protein into the membrane. Supported by a grant from the Medical Research Council of Canada.

560.2

TRANSFECTION OF C6 GLIOMA CELLS WITH CONNEXIN43 UNDER HUMAN METALLOTHIONEIN PROMOTER CONTROL: EXPRESSION, DYE COUPLING, AND GROWTH RATE FOLLOWING INDUCTION. J.F. Bechberger*, D. Zhu, G.M. Kidder and C.C.G. Naus. Depts. of Anatomy & Zoology, University of Western Ontario, London, Ontario, Canada, N6A 5C1.

C6 glioma cells express very low levels of the gap junction protein, connexin43 (cx43), but upon transfection with cx43 cDNA in a pLTR expression vector, the amount of cx43 protein is greatly increased with a resultant increase in dye coupling and reduced cell growth rate (Zhu et al., 1991). The pLTR expression vector contains a constitutive promoter, thus individual clones could demonstrate mutational or clonal differences not related to the presence of the exogenous cx43 protein. An expression vector containing a modified metallothionein promoter (pM 2.6) has been engineered which has a very low level of basal activity, but an extremely high inducible expression in the presence of Zn^{2+} (McNeall et al., 1989). The use of this vector enables the transfected clones to be analyzed prior to induction for cellular alterations due to the insertion of the vector and/or clonal selection. Cx43 cDNA was inserted into the pM 2.6 plasmid and stable transfectants were isolated. After 12 hours of Zn^{2+} treatment (100 μM), several clones demonstrated up to a 25 fold induction of cx43 mRNA, as well as an increase in cx43 protein. Dye coupling analysis of these clones demonstrated a significant increase in functional gap junctions. At present, we are determining if the induction of cx43 protein causes a reduction in growth rate. Supported by Medical Research Council of Canada.

560.3

PURIFICATION AND CHARACTERIZATION OF AN ENDOGENOUS PROTEOLYTICALLY-DERIVED SOLUBLE FRAGMENT OF N-CADHERIN WHICH RETAINS ADHESIVE FUNCTION. Nancy E. Paradies* and Gerald B. Grunwald, Dept. of Anatomy, Thomas Jefferson University, Philadelphia, PA.

The expression of N-cadherin is developmentally down-regulated in the neural retina of the chick embryo after day 9. We have previously shown that this is in part due to decreased mRNA levels. However, there is evidence that epigenetic mechanisms are also at work, in that a soluble 90kD fragment of N-cadherin is shed from the cell surface into conditioned medium during retina organ culture. We now provide biochemical evidence from pulse-chase studies that this fragment is indeed derived directly from N-cadherin. This activity is apparently due to a membrane-associated metalloprotease as turnover occurs in isolated membranes. Turnover of N-cadherin also occurs in vivo, as we have purified this fragment from freshly isolated embryonic vitreous humor. The purified protein retains adhesive activity, promoting attachment, spreading, and neurite outgrowth among cultured neural retina cells. Neurites growing on the 90kD fragment are shorter and more highly branched than those growing on laminin. We conclude that an intact transmembrane and cytoskeletal domain may not be essential for cadherin function, and that proteolysis may be an endogenous mechanism for regulating N-cadherin expression and function and generating novel functional forms of the protein. Supported by grants from NIH and NSF.

560.4

MUSCLE CADHERIN IS SPECIFICALLY EXPRESSED IN DEVELOPING SKELETAL MUSCLE OF THE MOUSE EMBRYO R.Moore, F.S.Walsh*, Dept. of Experimental Pathology, UMDS, Guy's Hospital, London SE1 9RT, UK.

We have examined, by *in situ* hybridization, the spatiotemporal pattern of expression of the muscle cadherin (M-cad) molecule (Donalies *et al.*, Proc.Natl.Acad.Sci. USA 88 8024, 1991) during mouse embryogenesis. We compared the M-cad staining with that obtained with neural cadherin (N-cad) and several muscle specific probes applied on serial tissue sections. The probes used were ^{35}S-labelled cRNA riboprobes. We found that at all embryonic ages examined M-cad was expressed in developing skeletal muscle and was absent from all other tissues. The M-cad transcript was found in the skeletal muscle myotome shortly after its formation. However high levels of myogenin transcripts were found in the myotome prior to M-cad at E8.5. The M-cad transcript was also found in muscles derived from the myotome such as those surrounding the vertebrae. Cells immigrating into the forelimb buds, from which the limb musculature develops, did not express M-cad until E11.5 when they did so coordinately with several other muscle specific genes. In contrast the N-cad transcript was found in both the neural tube and the early somite. At later embryonic stages the neural distribution of N-cad predominated as levels decreased in muscle. However transcription of both cadherins was down regulated shortly prior to birth such that at E17.5 neither transcript could be detected. We believe that the tissue specific distribution of M-cad, which has not been described for any other cadherin to date, suggests that it plays a role in cell sorting prior to myoblast fusion.

560.5

CHARACTERIZATION OF cDNAS ENCODING HUMAN CONTACTIN E.O. Berglund and B. Ranscht*, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

We have recently reported the isolation and characterization of a membrane glycoprotein, Gp135, from human brain (Berglund et al., 1991). Amino acid sequence analysis of the amino terminus and of an internal peptide revealed a strong similarity of Gp135 to chicken contactin/F11 and mouse F3. These glycoproteins belong to the immunoglobulin superfamily of cell adhesion molecules in the nervous system.

We now report the isolation and characterization of cDNA clones encoding Gp135. The protein sequence predicts that Gp135 consists of six immunoglobulin-like domains and four fibronectin type III domains and thus shares structural similarity with chick contactin/F11 and mouse F3. At the amino acid level, the sequence identity with these proteins is 78 and 94 %, respectively. The sequence terminates with a hydrophobic region indicating that Gp135 is anchored to the membrane through a glycosylphosphatidylinositol moiety. Surprisingly, we isolated two types of cDNAs that indicate heterogeneity at the amino terminus of the Gp135 protein. The two cDNAs are identical except for the insertion of a 33 basepair stretch at the 5'-end. The cDNAs therefore encode proteins of different length with differences at their extreme amino termini. The amino terminus of the longer form is identical to the amino terminal sequence of purified Gp135 (Berglund et al., 1991) and thus matches the corresponding region of F3 with the exception of a few amino acids. In comparison, the shorter form of Gp135 lacks 11 residues at the amino terminus and is three amino acids shorter than contactin/F11. By Northern analysis, one mRNA species of approximately 6.6 kb is detected in normal human brain.

These data confirm that contactin/F11, F3 and Gp135 are species homologues.

560.6

Distribution of NCAM Isoforms and N-Cadherin on Developing Muscle Cell Surfaces BJ Fredette*, LT Landmesser, U Rutishauser Dept. Physiol. Neuro., U. Connecticut, Storrs, CT and Dept. Genetics, Case Western U., Cleveland, OH.

Myotubes develop in tightly adherent clusters of cells within which myoblasts fuse on the surfaces of primary myotubes to form secondary myotubes. These then separate from the clusters to become independently contracting myofibers. This study describes the temporal and spatial distribution of NCAM and N-cadherin on muscle cells during fusion and myotube separation in the chicken hindlimb. Both LM and EM immunocytochemistry reveal that N-cadherin, and the low and high sialylated forms of NCAM are differentially expressed. N-cadherin is preferentially localized on myoblasts and on newly formed myotubes attached to primary myotubes, but not on the surfaces between more mature myotubes. While NCAM is expressed on all cell surfaces, its polysialylated form is restricted to the free surfaces of muscle cells and is not expressed on surfaces apposed to other cells. Biochemical analysis shows that a major portion of polysialic acid is carried by the 130 kD, lipid-linked NCAM, and that a shift in the predominant expression of 145 kD NCAM during primary myogenesis to 130 kD NCAM during secondary myogenesis corresponds to a rapid and transient increase in immunostaining for polysialic acid. When muscles develop in the absence of nerve activity, myotubes fail to separate from clusters normally. This correlates with a delay in the down-regulation of N-cadherin, a failure of NCAM to be polysialylated, and with a failure in the shift from 145 kD to 130 kD NCAM to occur. Since in other systems NCAM promotes and polysialic acid reduces adhesion, the distributions of N-cadherin and polysialylated NCAM during normal development and the perturbations observed in activity blocked muscles implicate N-cadherin down-regulation and polysialylated NCAM up-regulation in the process of myotube separation from clusters, and indicate that this is a nerve activity dependent process. *Supported by NSF grant BNS 9109529.*

560.7

EFFECTS OF CYTOSKELETAL INHIBITORS ON N2A AND CHO CELL ADHESION TO N-CAM SUBSTRATES. S.D. Storms, D. Yaghmai, J.J. Jensen, and B.A. Murray*. Dept. of Dev. and Cell Biology and Dev. Biology Center, Univ. of CA, Irvine, CA 92717.

The role of the cytoskeleton in N-CAM mediated adhesion was studied using a quantitative centrifugal removal assay. In order to evaluate the effects of cytoskeletal perturbation on time dependent strengthening of adhesion, cells were treated for 30 minutes with 1μM colchicine to inhibit microtubule assembly or with 1μM cytochalasin D to inhibit microfilament formation. Following treatment, N2A and CHO cells were centrifuged onto immobilized N-CAM substrates and allowed to incubate at 37°C for 10-60 minutes prior to the removal assay. Strengthening of adhesion in both treatments was not statistically different from controls at $p<0.05$, although the range of strength of adhesion in both treated groups was more variable than the controls. Adhesion of N2A and CHO cells to laminin was not significantly different in the colchicine treatment but strengthening was significantly slowed by cytochalasin D treatment. This is expected as laminin is bound by receptors in the integrin family which are known to interact with the cytoskeleton through microfilaments. These results suggest that N-CAM binding is independent of the cytoskeleton but may be affected indirectly by cytoskeleton mediated effects on cellular events such as flattening on a substrate.

This project was supported by American Cancer Society grant CD-416.

560.8

A NOVEL IG-SUPERFAMILY CELL SURFACE PROTEIN DIFFERENTIALLY DISTRIBUTED BOTH ON OPTIC FIBERS AND IN THE CEREBELLUM

Jost Vielmetter*, Jon Faiz Kayyem, Janet Roman, Uli Schwarz, and William Dreyer, CalTech, Pasadena CA 91125.

The establishment of axonal projections in the nervous system is believed to be achieved in part by an address system of cell surface recognition molecules. Address molecules must by definition be distributed differentially in space and/or in time during embryogenesis. Of particular interest are those molecules that contain Ig-like domains which are common to many cell adhesion and recognition molecules and whose primary function is specific recognition of molecules. We developed a method that enabeled us to successfully generate monoclonal antibodies against a large number of cell surface proteins belonging to the Ig-superfamily. One interesting molecule was detected with the monoclonal antibody 10-22A8. In the chick retina at embryonic stages E7 to E10, it stains optic fibers of the nasal retina while staining temporal fibers little if at all. The staining on optic fibers disappears at later stages of development (E11-E22), while it persists in the inner and outer plexiform layers. In the cerebellum the external granular cell layer is stained heavily in the most posterior part, whereas staining of this layer is much less conspicious in other regions of the cerebellum. This staining pattern persists throughout embryonic stages E8-E18. Preliminary sequence information of cDNA clones coding for a portion of this protein reveals its homology to members of the Ig-superfamily. It contains at least 4 Ig-domains and 3 fibronectin typeIII repeats. Determination of the entire coding sequence of this complex, multifunctional protein will make it possible for us to design molecular level experiments to study the functions of the various intracellular, extracellular, and alternatively spliced domains.

560.9

HETEROPIHLIC Ng-CAM BINDING: EVIDENCE FOR INTERACTIONS WITH LAMININ. Martin Grumet* and Gerald M. Edelman, NYU Medical Center and Rockefeller University, New York, NY 10016.

Ng-CAM is a cell adhesion molecule mediating neuron-glia and neuron-neuron adhesion via different binding mechanisms. It can bind homophilically as demonstrated by the ability of Ng-CAM coated beads (Covaspheres) to self-aggregate. In the present study, we found that the rate and extent of Ng-CAM Covasphere aggregation were strongly diminished in the presence of approximately stoichiometric concentrations of laminin. Comparisons among different laminin preparations indicated that, in contrast, to aged preparations that inhibited aggregation of Ng-CAM-Covaspheres, non-aged preparations of laminin induced formation of very large aggregates of Ng-CAM-Covaspheres. To analyze whether the ability of laminin to cross-link Ng-CAM-Covaspheres was related to its ability to self-associate, the effects of the molecule were tested after gentle proteolysis with elastase which cleaves the globular A-chain domain that has been implicated in self-association of laminin. This treatment yielded prepartions that inhibited aggregation of Ng-CAM-Covaspheres and contained a major fragment of Mr 130,000 on SDS/PAGE. In addition, laminin-Covaspheres coaggregated with Ng-CAM-Covaspheres, and this binding was inhibited by anti-Ng-CAM and by anti-laminin Fab' fragments. To test whether Ng-CAM present on neurons is involved in binding to laminin, neuronal adhesion to various protein substrates was tested in the presence of specific antibodies. Anti-Ng-CAM Fab' fragments inhibited neuronal binding to laminin. The combined results suggest that Ng-CAM on the surface of neurons may be one of several molecules that bind to laminin.

560.10

CHARACTERIZATION OF FOUR CELL ATTACHMENT SITES IN CYTOTACTIN. A.L. Prieto, C., Andersson-Fisone, K.L. Crossin*. Dept. of Neurobiology, Scripps Res. Inst., La Jolla, CA. 92037

The multidomain structure of the extracellular matrix molecule cytotactin/tenascin, and its restricted distribution suggest that it plays diverse roles during morphogenesis. Cytotactin has domains homologous to epidermal growth factor (EGF), fibronectin type III repeats (FN type III) and fibrinogen (Fg). Previous studies indicate that these domains may represent independent functional units. To search for functions of the various domains, non-overlapping recombinant protein fragments were made that spanned almost the entire molecule. Cell attachment and morphology of fibroblasts, glia, and neurons were examined using several adhesion assays. In contrast to the previous identification of one cell attachment and one counter-adhesive site, at least four non-overlapping sites on the molecule were found to interact with the cell surface. Two sites mediated cell attachment and were located in the proximal FN type III repeats (I-VI in the chicken sequence) and in the Fg domain; two others were located in the EGF region and the distal FN type III repeats (VII-VIII), and were counteradhesive. The quantitation of attachment on these fragments, competition experiments, and differences in adhesion of the different cell types support the hypothesis that cell attachment to different sites on cytotactin is mediated by different cell receptors and may be responsible for differential cell adhesion to intact cytotactin.

560.11

REGULATION OF ASTROCYTIC TENASCIN BY BASIC FIBROBLAST GROWTH FACTOR (bFGF). Sally Meiners, Jennifer L. Rittenhouse, and Herbert M. Geller*, Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854

Extracellular matrix (ECM) molecules have been implicated in the regulation of neurite adhesion and outgrowth both during development and after injury. We have previously shown that astrocytes are heterogenous in terms of expression of the extracellular matrix protein tenascin. High tenascin astrocytes have a reduced ability to support neurite outgrowth. In other experiments, bFGF was shown to cause a significant change in the morphology of cultured neonatal rat cerebral cortical astrocytes and also reduced neuronal adhesion to these astrocytes. In this series of experiments, we tested the hypothesis that bFGF could have increased expression of tenascin by the astrocytes. Cultures of purified protoplasmic astrocytes were established from the cerebral cortex of neonatal rats and subcultured into tissue culture flasks coated with polylysine. Basic FGF was added to test cultures at a concentration of 5 ng/ml, a concentration which was previously shown to cause a significant change in astrocyte morphology and neuronal adhesion. Tenascin levels were evaluated by Western blot analysis of both extracted cells and conditioned media. Tenascin levels began to increase after 24 h and continued to increase throughout 8 days of treatment. The bFGF treatment was discontinued, and the cells were maintained for an additional 8 days in culture. Tenascin levels returned to control values, demonstrating that the bFGF effect is transient. Neuronal adhesion was reduced on bFGF-treated cultures, suggesting that tenascin may be inhibitory to neuronal growth. It is our hypothesis that the action of growth factors during injury may evoke the induction of tenascin on astrocytes, thereby reducing regeneration in the central nervous system.

560.12

Isolation from human brain of large hyaluronate-binding proteoglycan (versican?). G. Perides*, and A. Bignami. Harvard Medical School and Brockton/West Roxbury VA Medical Center, Boston, MA. 02132.

A large proteoglycan (365 kDa), identified with monoclonal antibodies raised against chondroitin sulfate, was isolated from human brain. The isolation required cation and anion chromatography followed by gel filtration through a Sephacryl S-500 column. The proteoglycan bound specifically to [^{3}H]-hyaluronate (HA). The binding was not reduced by high salt concentrations (up to 4M) and was inhibited at low pH (< 4.0). The binding was inhibited specifically by the octamer and the decamer but not the hexamer oligosaccharide of HA. Limited proteolysis of the proteoglycan gave rise to a relatively stable polypeptide (80 kDa). The amino terminal sequence of the 80 kDa polypeptide was identical to the cDNA derived amino terminal sequence of versican, a large fibroblast proteoglycan (Zimmerman and Ruoslahti, EMBO J. 8:2975, 1989) and to previously reported sequences of the 60 kDa glial HA-binding protein (GHAP). The finding suggests that a large aggregating proteoglycan participates with hyaluronate and GHAP in the formation of brain ECM. Supported by NIH grant NS 13034 and by the Veterans Administration.

560.13

pgT1, A LARGE CHONDROITIN SULFATE PROTEOGLYCAN FROM ADULT RAT BRAIN, BINDS TO HYALURONIC ACID.
Mineo Iwata*, Thomas N. Wight^ and Steven S. Carlson, Dept of Physiology and Biophysics and ^Dept of Pathology, University of Washington, Seattle, WA.

We have identified a large chondroitin sulfate proteoglycan (pgT1) that has the characteristics of a general extracellular matrix component of rat brain (Iwata and Carlson, Abst. Soc. Neuro.,16:496,1990). This proteoglycan is identified with a mAb to a rare chondroitin sulfate epitope (T1). pgT1 is immunocytochemically localized throughout the brain in white and gray matter. Like extracellular matrix components from other tissues, pgT1 requires denaturing conditions to be solubilized from brain tissue. pgT1 is distinct from the 'space-filling' proteoglycans, smooth muscle cell versican, nasal cartilage aggrecan and rat brain versican-like proteoglycan. We propose that pgT1 is a general component of adult brain extracellular matrix (Iwata and Carlson, Abst. Soc. Neuro, 17, 575, 1991).

pgT1 has a high affinity for hyaluronic acid. We measured binding affinity of pgT1 to human hyaluronic acid (hHA) by the affinity coelectrophoresis developed by Lee and Lander (P.N.A.S., 88:2768-2772,1991). The Kd of pgT1 for hHA was determined to be 0.4nM. This is somewhat higher than that found for versican of 38nM. pgT1 was also shown to bind to endogenous HA of rat brain by gel filtration. When complexes of pgT1 and HA were visualized by electron microscopy, we observed "bottle brush" structures attached to a thin filamentous strand resembling hyaluronic acid.

We propose that pgT1 binds to hyaluronic acid and forms a part of extracellular matrix in brain.

560.14

PERINEURONAL AND WHITE MATTER LOCALIZATION OF HYALURONIC ACID IN POSTNATAL RAT CNS. H.J.L. Fryer*, G.M. Kelly, R.G. Kalb and S. Hockfield. Sect. of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510

The CNS Cat-301/304 antigen is a high molecular weight chondroitin sulfate proteoglycan (CSPG) related to the cartilage CSPG, aggrecan [Fryer et al., JBC 267:9870]. Both CSPGs aggregate with hyaluronic acid (HA). Purified cow and cat CSPGs are recognized by antibodies Cat-301 and Cat-304. The Cat-301/304 CSPG is associated with the cell surface of neuron cell bodies and proximal dendrites of specific neuronal populations in the mammalian CNS. In this study we have used purified aggrecan to localize candidate receptors for CNS CSPGs. Sections of postnatal and adult rat spinal cord were incubated with bovine aggrecan then stained for bound CSPG using monoclonal antibody Cat-304, which recognizes bovine, but not rodent, CSPG.

At the light microsopic level purified aggrecan binds to neurons and axons in sections of adult spinal cord. In white matter aggrecan binds to spaces surrounding axons, and in grey matter it binds to elements of the neuropil and is also associated with the surface of cell bodies of neurons in the ventral horn. Binding of aggrecan is inhibited in spinal cord sections that are treated with hyaluronidase or chondroitinase ABC (both of which digest HA) prior to incubation of sections with aggrecan. This suggests that aggrecan binding is mediated through HA in tissue sections.

Perineuronal binding of aggrecan increases during postnatal development. At P5 diffuse staining is seen throughout the grey and white matter with no apparent association with neuronal cell surfaces. By P22 surface staining of neurons as well as axonal staining is present. The developmental time course of aggrecan binding to neuronal cell surfaces parallels the time course of expression of the Cat-301 CSPG on neuronal cell surfaces. These results suggest that HA is present on neuron cell surfaces and that the assembly of neuronal extracellular matrix occurs over the first postnatal month in the rodent spinal cord. [Supported by EY06511]

560.15

Proteoglycan Distribution in Developing Retinal Neurons, Arnold G. Hyndman*, Sandra Echeverria, and Nelda Davis. Department of Biological Sciences, Rutgers University, Piscataway, NJ 08855

Proteoglycans are known to play an important role in the development of the nervous system. We examined the distribution of proteoglycans in the developing chick retina using an Alcian Blue staining procedure. Proteoglycan staining was observed at embryonic day 6 (E6) and was present throughout remainder of embryonic development. Photoreceptors, ganglion cells and the nerve fiber layer were most heavily stained. Moderate staining was seen in all other regions. To determine where neurons deposit newly synthesized proteoglycans, isolated retinal neurons were maintained in culture. The incorporation of [^{3}H]-glucosamine into proteoglycans was determined in three fractions. Results indicate that approximately 50% of the newly synthesized proteoglycans were secreted by neurons and 40% remained associated with the cell surface. A remnant of radioactivity was found "tightly" associated with either the intracellular or matrix compartments. In cultures from E8 retina, total proteoglycan synthesis is one-third less than in E11 cultures. Furthermore, transferrin increased the amount of proteoglycan secreted in E11 cultures, but not in E8 cultures.

560.16

GLYPICAN, A PROTEOGLYCAN, IS REGIONALLY EXPRESSED IN THE DEVELOPING RAT BRAIN. E.D. Litwack*, C.S. Stipp, and A.D. Lander, Dept. of Biology and Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139

Proteoglycans (PGs) bind and regulate the function of a variety of proteins, including growth factors (such as bFGF) and extracellular matrix molecules (such as fibronectin and laminin). A major heparan sulfate PG (HSPG) of the rat brain, designated M12 (Herndon and Lander, Neuron, 4:949, 1990), has a protein core with an apparent M_r of 65kD and is associated with cell membranes via a phosphatidylinositol linkage. Rat brain extracts made at E18, P0, and adult all contain this core. The biochemical properties of M12 are similar to those of a human lung fibroblast PG, glypican (GP) (David et al., J. Cell Biol., 111:3165, 1990), raising the possibility that GP is expressed in the rat brain, and is M12. To test this possibility, M12 was purified from neonatal rat brain. N-terminal sequences obtained from tryptic fragments of M12 were all highly similar to sequences in human GP, varying from 72% to 100% identical. To confirm that M12 is rat GP, a rat GP cDNA was obtained as follows: A human GP fragment was obtained by PCR from human foreskin fibroblast RNA. Northern analysis detected an RNA of about 3.7 kb from neonatal rat brain, rat fibroblasts and PC12 cells, a rat neuronal-like cell line which we find contains an M12-like major HSPG. One of the clones detected upon screening a PC12 cDNA library with the GP probe was an approximately 3.0 kb cDNA. Partial sequence indicates that it is 85% similar to human GP. One M12 peptide falls in the region of and is correctly predicted by the partial cDNA sequence. To determine the pattern of expression of GP in the adult rat brain, in situ hybridization was performed with a probe from the rat GP cDNA. Results indicate that GP is regionally expressed, with high levels present in areas such as the thalamus. Further studies will characterize the pattern of GP expression during rat brain development. (Supported by NIH grant NS26862)

560.17

THE 1D1 PROTEOGLYCAN OF BRAIN. U. Rauch, L. Karthikeyan, P. Maurel, R.U. Margolis, and R.K. Margolis* NYU Med. Center, NY, NY 10016, and State Univ. of NY Health Sci. Center, Brooklyn, NY 11203.

Two rat brain proteoglycans (PGs) with core glycoproteins of 150 and 245 kDa were isolated by immunoaffinity chromatography using the 1D1 mAb (Rauch et al., *J. Biol. Chem.*, 266:14785, 1991). Up to ~2 weeks postnatal the PG with the 245 kDa core glycoprotein is the main physiological product, whereas after three weeks the smaller PG becomes predominant and is essentially the only species present in adult brain. Peptide maps of both core proteins indicated that the 150 kDa core glycoprotein is a part of the 245 kDa PG, because all peptides generated from it could also be found in the larger species. The amino acid sequence of the cloned 150 kDa core protein revealed a high degree of homology with versican and aggrecan, based on the presence of two EGF-like domains, a lectin-like domain, and a complement regulatory-like domain in the C-terminal half of the PG. The larger PG has the ability to form aggregates with hyaluronic acid, and peptide and cDNA sequences from this PG revealed the presence of immunoglobulin folds and tandem repeats similar to those present in the hyaluronic acid binding regions of versican and aggrecan which, however, have much longer glycosaminoglycan attachment domains which cannot be accommodated in the 1D1 core glycoprotein. Northern blots demonstrated that only a single message of 7.5 kb was detected in either 4-day or adult brain, and no message was detectable in liver, kidney, muscle, or lung mRNA. Our results therefore indicate that the adult form of this PG is derived by *in vivo* proteolytic processing from the larger species present in early postnatal brain, and that the 1D1 PG is a new, and possibly central nervous tissue-specific, member of the versican/aggrecan PG family.

560.18

ROLE OF GLYCOSPHINGOLIPIDS IN SKELETAL MUSCLE DIFFERENTIATION *IN VITRO*. K.C. Leskawa*, S. Hickerson and L.D. Cambron. Dept. Anat. Sci. & Neurobiol., Sch. of Med., Univ. of Louisville, Louisville, KY 40292.

We have previously reported that when glycosphingolipid (GSL) synthesis is inhibited by PDMP, which blocks the formation of glucosylceramide, clonal muscle cells cease to fuse to form multinucleated myotubes. This inhibition of myogenesis was overcome by addition of exogenous lactosylceramide (LacCer) or ganglioside GM3, suggesting a role for these compounds in myoblast fusion. We have recently explored this phenomenon in greater detail. Myoblasts, either controls or PDMP-treated, were pulsed with radiolabeled LacCer or GM3 and the fate of these added GSLs examined at varying times thereafter. It was found that neither LacCer nor GM3 were converted to any other GSL structure, in either treated or untreated muscle cells, up to 96 hr following exposure.

Whether the reversal of fusion inhibition by LacCer or GM3 was specific, as opposed to a general membrane lipid perturbation effect, was examined by assessing the effects of various other GSLs on PDMP-treated myoblasts. It was found that neither GalCer, GbOse$_3$Cer, ganglioside GM2 nor GM1 were capable of reversing the myoblast fusion block caused by PDMP.

Skeletal muscle cells were also treated with exogenous neuraminidase and ceramide glycanase. Both treatments inhibited myoblast fusion. Myoblasts treated with neuraminidase assumed an entirely different, non-myoblast morphology and exhibited loss of contact inhibition. In contrast, cells treated with ceramide glycanase resembled PDMP-treated cells morphologically, in that they migrated and aligned in parallel arrays.

These studies further support our hypothesis that surface GSLs play an important role in skeletal muscle differentiation. In addition, these results specifically point to a necessary role for lactosylceramide and GM3.

560.19

NEUTRAL GLYCOSPHINGOLIPIDS OF NORMAL ADULT HUMAN BRAIN: MOLECULAR CHARACTERIZATION OF GgOse$_4$Cer. E.L. Hogan* and S. Dasgupta. Neurology, Med Univ SC, Charleston, SC 29425.

The nonpredominant neutral glycosphingolipids (Ngsls) of neuronal tissues of the globo-, ganglio- and lactoseries, accumulate in brain in some sphingolipidoses. Ngsls of normal fetal brain have been characterized as galactosyl/glucosylceramide (48%), lactosylceramide (17%), Gb$_3$Cer, Gg$_3$Cer (9%) and traces of Gb$_4$Cer and Gg$_4$Cer and two additional Ngsls as nLc$_4$Cer (13%) and III^{3}FucnLc$_4$Cer (10%) [Ishikawa et al, J Biochem (Tokyo), 101, 1369, 1987]. FucnLc$_4$Cer, a minor constituent of normal adult brain [Vanier et al, FEBS Lett, 112, 70, 1980], is considered a stage-specific fucolipid antigen. GgOse$_4$Cer (GA1 or asialo-GM1) identified in mouse myelin [Kusunoki et al, Brain Res, 334, 117, 1985], has recently been analytically and immunochemically characterized in bovine brain [Dasgupta et al, FEBS Lett, **in press**, 1992], adult human and rat brain and myelin. We have purified and characterized several Ngsls from human brain as GalCer, GlcCer, LacCer, Gb$_3$Cer, Gg$_3$Cer and possibly Gb$_4$Cer and nLc$_4$Cer. A homogeneous glycolipid band that comigrates with standard GA1 has been purified and characterized as GA1. PMAA-derivative of another partially purified Ngsl with Rf close to GA1 has the possible structure of GbOse$_5$Cer. Employing the DIG-immunostaining [Kniep and Muhlradt, Ann Biochem, 188, 5, 1990] and FACE [Jackson, Biochem J, 270, 705, 1990; Stack et al, Glycoconjugate J, 8, 249, 1991] we have identified several minor long chain (CHO- > 4-5) Ngsls bands in brain and myelin that have not been previously reported. The specific expression of these Ngsls during brain development and myelinogenesis remains to be explored.

560.20

GANGLIOSIDE BINDING MOLECULES OF RAT SKELETAL MUSCLE. R.E. Shapiro*[1] and R.L. Schnaar[2]. Depts. of [1]Neurology and [2]Pharmacology, Johns Hopkins School of Medicine, Baltimore, MD, 21205.

Gangliosides on motoneuron terminals have been proposed as binding sites for several neuropathogenic agents. We hypothesize that motoneuron gangliosides also act as specific receptors for molecules derived from surrounding endogenous tissues including skeletal muscle. We evaluated crude sarcosolic extracts from male rats in a soluble assay for binding activity to a ganglioside ligand (bovine trisialogangliosides (G_{T1b}) covalently and multivalently bound to radioiodinated bovine serum albumin). We found specific binding activity with a rapid on-rate (>75% of max. binding within 1 min.), and which was proportional to the quantity of extract protein in the assay. Binding activity is eliminated by prior boiling of the extract, and is inhibited by free gangliosides (G_{T1b} $IC_{50} \approx 0.5$ μM; $G_{M1}/G_{D1b}/G_{D1a}/G_{M2}/G_{M3}$ IC_{50}'s between 0.5 μM and 5 μM), by phosphatidylcholine ($IC_{50} \geq 50$ μM), or by increased ionic strength (NaCl $IC_{50} \approx 50$ mM), but not by the disaccharides cellobiose or lactose (up to [10 mM]). Binding at pH 7.4 is >90% dependent upon the presence of divalent cations (Ca^{++} or Mg^{++}). Binding activity increased at low pH (specific binding at pH 4.5 was >6 fold that at pH 7.4), but this increased binding activity was ≤50% Ca^{++}-dependent. Scatchard analysis indicated a single class of Ca^{++}- dependent ganglioside ligand binding sites ($K_D \approx 5.6$ nM) at pH 7.4. [NIH grants KO8 NS01518 and HD14010.]

560.21

DOPAMINE β-HYDROXYLASE AND CYTOCHROME B561 ARE PRESENT ON THE AXONAL RETICULUM IN BOVINE SYMPATHETIC NEURONS. J.R. Quatacker, W.G. Annaert, B.J. Miserez and W.P. De Potter. N. Goormaghtigh Inst. Univ. Hosp. Ghent and Lab of Neuropharmacol., Univ. Antwerp, B-9000, Belgium.

The axonal reticulum in sympathetic neurons is considered to be an extension of the secretory pole of the Golgi apparatus. If so it would likely contains the enzymes involved in catecholamine elaboration.

To test this hypothesis the distribution of DβH and cyt b561 was investigated in bovine splenic nerve and nerve terminals in the vas deferens with an immunogold procedure after glycolmethacrylate embedding. Counterstaining was with phosphotungstic acid at low pH.

With antibodies against both enzymes gold labeling was detected over the large dense-cored vesicles, the Golgi-associated axonal reticulum, the reticulum within axons and over the tubular complex at the nerve terminal.

From our results it can be concluded that in sympathetic neurons the axonal reticulum represents a tubular, neurosecretory transport system, spanning the neuronal cell from Golgi apparatus to nerve terminal.

560.22

THE LOCALIZATION OF A NOVEL CA^{2+}-BINDING PROTEIN (CBP-18) IN THE RAT BRAIN. D.P. Wolfer*, H.-P. Lipp, W. X. Qin and C.W. Heizmann. Institute of Anatomy, University of Zürich, CH-8057 Zürich, Switzerland.

The distribution of a novel calcium-binding protein (CBP-18) with a molecular weight of 18 KDa in the rat brain (Manalan & Klee, *J. Biol. Chemistry* **259**, 2047-2050, 1984) was studied by means of immunohistochemistry on cryostat-sectioned tissue and compared with staining patterns of parvalbumin on adjacent sections.

The polyclonal rabbit-derived antibody for CPB-18 showed selective affinity for periglomerular cells and dendrites in the olfactory bulb, and also distinctly stained some cells and dendrites in the anterior olfactory nuclei. Marked but diffuse pericaryal staining of neuropil and of cell bodies (including proximal dendrites) was observed in the retrosplenial cortex, hippocampal rudiment, the septum, area preoptica, hypothalamus, in the parabrachial nuclei and in the cerebellar neuropil of both the molecular and the granule cell layer. Less intense neuropil staining for CPB-18 was found in the neocortex, the remaining basal forebrain, parts of the colliculus superior, and in the entire brain stem. Neuropil staining was barely detectable or missing in the striatum, the hippocampus, the thalamus, the colliculus inferior, the cerebellar Purkinje cell layer, and in the cuneate nucleus. CPB-18 appeared to stain regularly cross-sectioned axons but rarely longitudinal fibers. Thus, CPB-18 shows an unique staining pattern in the CNS different from all other Ca^{2+}-binding proteins studied so far. Supported by Swiss National Science Foundation for Scientific Research (SNF 31-27737.89, 31-9470 and 31-30742).

560.23

PURIFICATION AND CHARACTERIZATION OF CMP-SIALIC ACID:LACCER SIALYLTRANSFERASE (ST-I) FROM RAT BRAIN. Preuss, U., Gu, X. and Yu, R.K.* Dept. of Biochem., Med. Coll. of Virginia, Richmond, VA 23298

Sialyltransferases (STs) are a family of glycosyltransferases which catalyze the transfer of sialic acid (NeuAc) to the non-reducing terminal sugar of glycoproteins and glycolipids. One of the key enzymes in the synthesis of gangliosides is CMP-sialic acid:LacCer sialyltransferase (ST-I) which catalyzes the transfer of sialic acid to lactosylceramide (CDH) to form GM3. In this report we describe the purification and characterization of ST-I from a Triton X-100 extract from rat brain. The enzyme was purified by affinity chromatography on CDP-Sepharose and resolved by NaCl gradient elution from the same adsorbent. Further purification of GM3 synthase was achieved by chromatography on a "CDH-acid"-Sepharose column eluted with CDH. The enzyme activity was highest at pH 6.5 and required Triton CF-54 or Triton X-100 (0.15%) for full activity. The apparent Km value for CMP-sialic acid was 170 μM. These data correpond with the results obtained for ST-I purified from rat liver (1), suggesting that both enzymes may share a common domain structure or may have significant sequence homology. (Supported by USPHS grant NS-11853 and A. von Humboldt Found. to U.P.)

1) Melkerson, L.J. & Sweeley, C.C. (1991). J. Biol. Chem., 266, 4448-4457

560.24

An Estrogen Receptor-Like Antigen on Membranes of GH_3 Cells. T.C. Pappas[1]*, B. Gametchu[2] and C.S. Watson[1]. [1]Univ. of Texas, Med. Br. Galveston Tx 77550, [2]Med. College of Wisconsin, Milwaukee WI 53226.

The mechanisms of rapid actions of estrogen, such as the rapid release of prolactin from GH_3/B6 cells, have not been elucidated. We hypothesize that a membrane form of the estrogen receptor (ER), similar to the intracellular ER may mediate some these actions. A polyclonal antibody (anti-ER) was generated to a peptide corresponding to amino acids 270-284 of rat ER. The antisera recognizes both native (sucrose density gradient) and denatured ER from rat uterine cytosol, and peptide affinity purified antisera recognizes a single species of 67 KD on immunoblots. GH_3/B6 cells were separated by estrogen affinity chromatography using magnetic beads. When these enriched populations were incubated at 2°C with the affinity purified anti-ER, indirect immunocytochemistry revealed heterogeneous membrane labeling. Anti-Actin controls showed that cells were not permeablized by the live-labeling procedure. When cells were brought to 37°C after anti-ER incubation, labeling became "patchy" and decreased in amount, disappearing by 15 min. Nuclear and cytoplasmic labeling were also evident in cells permeabilized with detergent after fixation. Both membrane and intracellular labeling could be competed with the peptide used to generate the antibody.

560.25

DIFFERENTIAL EXPRESSION OF GLUCOSE TRANSPORTERS, GLUT3 AND GLUT1, IN CULTURED CEREBELLAR GRANULE NEURONS: EFFECTS OF POTASSIUM AND NMDA. Frances Maher* and Ian A. Simpson. NIDDK, NIH. Bethesda, MD 20892.

Cerebellar granule neurons in culture express two glucose transporter isoforms, GLUT3 and GLUT1. GLUT3 is the predominant isoform. We investigated the chronic effects of depolarizing stimuli, ie. high potassium concentration and NMDA, on glucose transporter expression in neurons cultured in defined medium. Compared to culture in standard high KCl (25mM; K25), neurons cultured in low KCl (K5 and K15) showed similar morphology but up to 20% decline in cell number. In K5 medium, 2-deoxyglucose transport activity was decreased by 50-60%. This corresponded to decreased glucose transporter expression as determined by immunoblotting with antisera to each isoform; GLUT3 levels decreased by 40-50% and GLUT1 by 20%. Addition of NMDA (100-200μM) to K5 and K15 medium stimulated GLUT3 expression to the levels in K25 but failed to fully restore GLUT1 levels. The effect of NMDA was blocked by APV. Potassium concentration, NMDA and other EAA had no acute effects on glucose transport or GLUT1 and GLUT3 levels. Expression of Na^+K^+ ATPase isoforms was not altered by these culture conditions. In conclusion, potassium depolarization and NMDA receptor activation result in optimal expression of neuronal glucose transporters, the GLUT3 isoform being more sensitive than GLUT1 to chronic regulation by these conditions.

560.26

CHOLINE METABOLISM AND THE CONFORMATIONAL EPITOPE OF *TOR 23*. M.B. Foreman*, S. Wright and P.D. Kushner. ALS Research Center, California Pacific Medical Center, San Francisco, CA 94115

Previously we reported that the epitope defined by the monoclonal antibody *Tor 23* is on a presynaptic form of *Torpedo* acetylcholinesterase (AChE), which associates with the membrane via a glycophosphatidylinositol (GPI) linkage. To confirm the conformational architecture of the epitope we have performed a series of Triton X-100 experiments. Although Triton does not inhibit AChE activity, even trace amounts (0.001%) interfered with the immunoprecipitation of AChE by *Tor 23*. To test whether the lack of precipitated AChE was due to a change in the AChE molecule such that the antibody failed to recognize it, Triton was added to immune precipitates and the pellet and supernatant assayed for AChE activity. Triton released AChE activity from the pellet into the supernatant. In contrast, AChE activity remained in the pellet in untreated samples. These experiments support other data suggesting that *Tor 23* identifies a conformational epitope of presynaptic *Torpedo* AChE.

Although we have no data that *Tor 23* recognizes AChE in mammals, the antibody inhibits high affinity hemicholinium-3 binding, high affinity choline uptake and acetylcholine synthesis in rat neocortex (Evans et al., submitted). Furthermore, our studies of neuroblastoma cell lines using mannosamine suggest that, as in the *Torpedo*, the *Tor 23* antigen has a GPI linkage in mammals. In sum, evidence supports the hypothesis that the *Tor 23* antigen, in both *Torpedo* and mammalian systems, is a GPI-linked molecule involved in cholinergic processing.

This work is supported by the Street Estate.

560.27

PURIFICATION AND IMMUNOCHEMICAL STUDIES OF HUMAN BRAIN NA,K-ATPASE ALPHA SUBUNIT PROTEINS AND THEIR POLYPEPTIDE FRAGMENTS. J.H.F. Peng*, Y.C. Zeng and J.C. Parker, Jr. Department of Pathology, Univ. of Missouri-Kansas City Sch. of Med., Kansas City, MO 64108.

In order to study the properties, structure and function of human Na,K-ATPase, the alpha (α) subunit proteins as well as their polypeptide fragments of the enzyme were purified. Polyclonal antibodies to these antigens were prepared. The enyme was purified from axolemma, which was isolated from brainstem, by selective SDS extraction and followed by sucrose density gradient centrifugation. Partially purified enzyme was resolved by SDS-PAGE, stained with zinc ions, and the stained band(s) corresponding to 100 kDa molecular weight (MW) were excised, with the proteins in the gel being eluted by electroelution. The purified proteins were treated with 88% formic acid, lyophilized, and incubated with the SDS sample buffer. The polypeptide fragments were separated by SDS-PAGE. Protein bands corresponding to 40, 50, and 60 kDa MW were cut out of the gel, and the proteins electroeluted as above. Rabbits were subcutaneously immunized and boosted with 100 µg of antigens, which were emulsified with complete and incomplete Freund's adjuvant, respectively. Seven days after the fourth booster injection, blood samples were collected. The antisera to α proteins, and 60 & 40 kDa polypeptides were obtained and characterized by Western blotting. Antiserum to α proteins cross-reacted with α bands as well as 40, 50, & 60 kDa bands. Antisera to 40 and 60 kDa cross-reacted, respectively, with 40 and 60 kDa band as well as α bands. These antibodies will be valuable reagents for immunoaffinity purification, for cDNA screening during cloning of α1, α2 & α3 isozymes, and for immunohistochemistry studies.

560.28

HETEROGENEITY IN mRNAs ENCODING THE Na/Ca EXCHANGER IN RAT BRAIN. M.Juhaszova[1], P.Kofuji[2], D.Schulze[3], W.J.Lederer[1], S.M.Wang[1], P.J.Yarowsky[2], M.P.Blaustein[1*]. Depts.[1]Physiol., [2]Pharm., [3]Microbiol. Immun., U. of MD Med. Sch., Baltimore, MD 21201.

Northern blot analysis of total RNA from rat brain revealed heterogeneity in mRNAs encoding the Na/Ca exchanger. The blots were probed with an 800 bp 3' end cDNA probe coding for the rat cardiac Na/Ca exchanger and with the full length cDNA probe coding for human heart Na/Ca exchanger (Kofuji et al., *Biophys. J.* **61**:A387, 1992). Blots were washed using medium stringency conditions (0.1% x SSC at 37°C). We identified three Na/Ca messages (~15 kbp, 7 kbp and 3.2 kbp) not only in whole adult rat brain total RNA, but also in the developing rat brain total RNA in late embryonic stage (day 18). Northern blot analysis of total RNA from cultured cortical astrocytes at 10 days in vitro, however, showed only a 7 kbp Na/Ca exchanger mRNA under the same conditions. Immunochemical Western blot analysis of the astrocytes, synaptic plasma membranes and heart sarcolemma proteins using polyclonal antibody raised against purified canine heart Na/Ca exchanger (Ambesi et al., *Biophys. J.* **59**:138a, 1991) revealed the same protein band pattern in all samples: 70, 120 and 140 kDa. These data suggest that the expressed Na/Ca exchanger in whole brain and in astrocytes may be the same protein, highly homologous to heart Na/Ca exchanger. The different-sized mRNAs probably vary from each other only in their untranslated regions.

560.29

AXONAL TRANSPORT OF FOUR SYNAPSINS IN RAT SCIATIC NERVE; DIFFERENT DEGREE OF VESICLE ASSOCIATION. *J.Y. Li*, A. Czernik† and A. Dahlström, Institute of Neurobiology, Medicinareg. 5, Univ. of Göteborg, S-413 90 Göteborg, Sweden, and Dept. of Cell. & Molecular Neurobiology†. The Rockefeller University, New York, 10021 NY, USA*

Previous immunofluorescence work has shown that synapsin I (SYN I) is transported with rapid axonal transport in sciatic nerve and ventral roots of mammals. SYN I-like immunoreactivity (LI) accumulates proximal to the site of a short term crush, but only very little accumulation is observed distally (10-20 % of proximally). Recent studies, using immunofluorescence and cytofluorimetric scanning (CFS), have concentrated on accumulation and transport of the SYN isoforms, SYN Ia, Ib; SYN IIa and IIb, using isoform-specific antisera on consecutive sections of rat crushed (1-8 h) sciatic nerves. The immunofluorescence proximal and distal to the crush region, after incubation with the various antisera, was quantitated, in relation to gelatine standards with purified SYN Ia,b. Also p38, the vesicle marker, was studied, and this protein accumulated bidirectionally, with 60-80% of anterograde amounts present in retrograde accumulations (recycling). Initially (1h) after crush, SYN Ib-LI showed small, distinct accumulations proximal to the crush. However, little or no SYN Ia-LI was seen near the crush. After 8h an increase in both SYN Ia-LI and SYN Ib-LI was evident, but proximal SYN Ib-LI was stronger than SYN Ia-LI ($p<0.001$). Proximal accumulations of SYN IIa-LI and SYN IIb-LI were observed 1h after crush, and at 8h. More SYN IIa-LI than SYN IIb-LI ($p< 0.001$) was seen proximal to the crush. The results suggest that the isoforms may have different affinities to organelles in fast anterograde transport.

560.30

TYROSINE PHOSPHORYLATION OF GP180 IN ADULT AND DEVELOPING BRAIN. **J.W. Gurd*, N. Bissoon, M. Khurgel and J. Soulliere. Scarborough Campus, University of Toronto, West Hill, Ontario, M1C 1A4.**

We have reported the presence of protein tyrosine kinase in isolated postsynaptic densities (PSDs) and demonstrated that the PSD glycoprotein, GP180, is phosphorylated on tyrosine residues (Gurd and Bissoon, J. Neurosci. Res. 25 336-344). We have now used Western blotting of rat brain homogenates with anti-phosphotyrosine antibodies to determine the distribution of ptyr-containing GP180 (PTGP180). GP180 was the only ptyr-containing Con A-binding glycoprotein in PSDs and was the major immunoreactive Con A* glycoprotein present in homogenates. PTGP180 was enriched approximately 20-fold in PSDs relative to homogenate consistent with a postsynaptic localization. Cerebellum and hippocampus contained low levels (less than 15% of forebrain) of PTGP180. PSDs from these regions contain GP180 suggesting regional variation in the activities of postsynaptic tyrosine kinases or ptyr-phosphatases or both. Forebrain homogenates from newborn rats contained less than 5% of the adult level of PTGP180. The amount of PTGP180 increased markedly during the third and fourth weeks of postnatal development before declining to adult values. Incubation of PSDs with ATP increased the tyrosine phosphorylation of GP180 12 to 15-fold, indicating that less than 10% of potential tyrosine phosphorylation sites are occupied in vivo. Supported by the N.S.E.R.C.

560.31

IMMUNOHISTOCHEMICAL STUDY OF C-*kit* RECEPTOR EXPRESSION IN ADULT PRIMATE AND MOUSE BRAINS. Q. Yan*, J. Sun, C. Matheson Neurobiology Program, Amgen, Inc., Amgen Center, Thousand Oaks, CA 91320

The tyrosine kinase receptor c-*kit* is coded by the *W* locus and its ligand, stem cell factor (SCF), is coded by the *Sl* locus in mice. c-*kit* and SCF play a critical role for the development of neural crest-derived melanoblasts, germ cells and hematopioetic stem cells. Using a mouse monoclonal anti-human c-*kit* antibody (SR-1) and a rat monoclonal anti-mouse c-*kit* antibody (ACK2), the expression of c-*kit* was studied in the brains of adult baboon and rhesus monkey with SR-1 and mouse brains with ACK2 by conventional immunohistochemistry. c-*kit* IR was observed in the hippocampus, dentate gyrus, fimbria-fornix and lateral septum. The staining appeared to be on fiber tracts and terminals but not on the hippocampal and dentate pyramidal cells. Distinct c-*kit* IR were observed in many areas including olfactory bulb, cerebral cortex, amygdala, caudate putamen, thalamus, interpeduncular nucleus, and inferior olive. In the brainstem and spinal cord c-*kit* IR seemed associated with the sensory pathways which included dorsal horn in the spinal cord, spinal trigeminal nucleus and tract, gracile and cuneate, vestibulocochlear nucleus and solitary tract and its nucleus. In the cerebellum, very intense staining was on Purkinje cell axon hillock and around Purkinje cell bodies. Staining was also seen in the molecular layer and upper granule layer. This study and recent reports on the expression of mRNAs for both c-*kit* and SCF in mouse brain (Motro et al., Development 113:1207-1221; Morii et al., Dev. Brain Res. 65:123-126) suggest potential functions of these proteins in the nervous system.

560.32

CAROTENOID REPLACEMENT IN *Drosophila*: FREEZE-FRACTURE. W.S.Stark*, G.Brown, D.Hombs, J.S.Christianson & R.White±. Div. Biol. Sci., Univ. of Missouri, Columbia, MO 65211 & ±Dept. of Biology, Univ. of Massachusetts, Boston, MA 02125.

Carotenoid deprivation in *Drosophila* reduces visual pigment, opsin, size of the rhabdomere and P-face particle density; replacement by feeding carrot juice rapidly restores visual pigment (Sapp et al., 1991, *Exp.Eye Res.* **53**:71). Our data indicate that this effect is mediated by retinoid-activated opsin gene transcription (Stark et al., 1992, *Invest. Ophthal. Vis. Sci.* **33**: 1398). Here we report that P-face particle density also increases in rhabdomeric microvilli in the early days of replacement therapy to 3000 particles/μm^2 by 1 day, reaching the control level of over 4000 by day 2. Our vistas reveal a continuity of the microvilli with the adjacent retinula cell plasmalemma between the adhering junction and the rhabdomere. This plasmalemma reflects the rhabdomeric P-face particle density. Freeze-fracture preparations of *Drosophila* photoreceptors also displayed autophagic coated pits budding from bases of microvilli and from plasmalemma as well as multivesicular bodies and Golgi apparatus. Recovery in *Drosophila* is considerably faster and more complete than recovery induced by 11-*cis* retinal in similarly deprived *Manduca* (Bennett & White, 1991, *Vis. Neurosci.* **6**: 473). Further, there are substantial differences in the endomembrane traffic in deprivation vs. replacement. Support: NSF BNS8811062 & NIH EY07192 (WSS) & NSF BNS91 10672 (RW).

560.33

EFFECTS OF ISCHEMIA ON BRAIN LIPID MONOLAYER SURFACE PRESSURE-AREA DIAGRAMS (SPAD). E.M. Nemoto*, M. Chavko, V.R. Hartwell. Department of Anesthesiology/CCM, Univ. of Pittsburgh, Pittsburgh, PA 15261

The rapid rise in brain free fatty acids during ischemia signals the breakdown of membrane lipids which could affect membrane biophysical characteristics and function. We evaluated the effect of ischemia on brain lipids and the impact on their surface-pressure area diagram (SPAD).

Wistar rats on 70% N_2O/30% O_2 were subjected to 0 or 30 min of complete global brain ischemia by neck cuff and arterial hypotension. The brains were frozen in situ and cerebrosides, sulfatides, phospholipids, cholesterol and sphingomyelin in the frontal cortex, hippocampus and basal ganglia were quantitated by HPTLC. SPAD were constructed using the Wilhelmy plate method on lipid monolayers at 25°C.

Thirty min ischemia without recirculation expanded the SPAD of brain lipids such that at a given area/molecule, SP was increased by 10 dynes/cm. SPAD of some lipids were more greatly affected than others. The magnitude of the changes in the SPAD indicate that membrane function should be markedly affected.

GENE STRUCTURE AND FUNCTION VII

561.1

CULTURED POSTMITOTIC NEURONS DO NOT UNDERGO VIRION-MEDIATED HOST CELL SHUTOFF OF PROTEIN SYNTHESIS AFTER INFECTION WITH HERPES SIMPLEX VIRUS-1; A UNIQUE RESPONSE IN NEURONS AND A PROMISING RESULT FOR HSV-1 VECTOR TECHNOLOGY. P. F. Nichol,* J. Y. Chang, L. S. Greenlund, P. Olivo, E. M. Johnson, Jr. Washington University School of Medicine, St. Louis, MO 63110

Herpes simplex virus (HSV)-derived vectors or recombinant HSV have been proposed and preliminarily characterized as vehicles for expression of foreign genes in neurons. A potential factor compromising the utility of such vehicles is the ability of HSV to produce virion-induced host-cell shutoff (VHS), a phenomenon mediated by a virion-associated protein (UL41). Upon infection, the UL41 gene product decreases protein synthesis (20-50% of controls) by disrupting translation, degrading cellular transcripts, and potentially preventing further cellular transcription. If such a shutdown were to occur in neurons, a significant alteration of the cellular physiology would be produced that might affect the ability of a neuron to express and respond to foreign genes. VHS has not been examined in neurons. We demonstrate that shutdown of global protein synthesis does not occur after infection of cultured rat sympathetic neurons with HSV-1 strains KOS and, the replication incompetent mutant, KOS d120. Also, no difference is seen in the rate of protein synthesis in neurons infected with d120 or KOS-derived *vhs*-ΔSma, a deletion mutant that lacks the UL41 gene. Undifferentiated or NGF-differentiated PC-12 cells and Vero cells experienced VHS upon infection with KOS-derived d120 (a decrease in protein synthesis to 40% of controls at an MOI of 20). The results suggest that neurons are resistant to VHS as mediated by the UL41 gene product. This apparent lack of VHS may be important in the ability of HSV to become latent in these neurons. The results indicate that VHS should not be a complicating factor in these, and perhaps all, postmitotic neurons. (Supported by the American Paralysis Association and by the Monsanto Corporation.)

561.2

TRANSCRIPTIONAL ELEMENTS OF THE HUMAN GENE FOR GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP). F. Besnard, K. Masood, Y. Su and M. Brenner*. Lab. of Molec. Biol., NINDS-NIH, Bethesda, MD 20892

To gain insights into how astrocyte functions are regulated, we are studying transcriptional control of the human gene for glial fibrillary acidic protein (GFAP), an intermediate filament protein found almost exclusively in astroctyes. We previously found that the 5'-flanking region of the gene contains three segments, A, B and D, that contribute to its cell-type specific expression in cultured cells; and that the most potent of these is the 124 bp B region, which resides about 1500 bp upstream of the RNA start site [J. Biol. Chem. 266, 18877-18883 (1991)]. We now report that site-directed mutagenesis of the B segment reveals it to contain several active subregions. One of these is a novel 12 bp sequence found also in the other two important regulatory segments, A and D. Another critical sequence matches precisely the consensus AP-1 binding site for the Fos and Jun families of transcription factors. Gel mobility shift assays indicate that both Fos and Jun family members are present in a GFAP-expressing astrocytoma cell line. Demonstration of a functional AP-1 binding site in the GFAP gene provides a focus for future studies of astrocyte regulation during development and reactive gliosis.

561.3

ALTERNATIVE EXONS AND PROMOTER ELEMENT OF THE $GABA_A$ RECEPTOR β3 SUBUNIT GENE.
E.F. Kirkness*, G.A. Hastings and C.M. Fraser. Section on Mol. Neurobiol., LN, NIAAA, Rockville, MD 20852.

The $GABA_A$ receptor β3 subunit is a relatively abundant component of several $GABA_A$ receptor subtypes. The gene encoding the human β3 subunit maps to a region of chromosome 15 that is associated with two imprinted, genetic disorders (Prader-Willi and Angelman syndromes). In order to examine how expression of this gene is regulated, the 5' regions of rat and human β3 subunit genes have been cloned and characterized.

RNase protection and RACE analyses indicate that transcription of the β3 gene starts from multiple sites, 100-200 bp upstream of the putative initiation ATG within exon 1. The RACE analysis of human brain RNA also uncovered the expression of an alternative exon 1 (exon 1a) that is located upstream of exon 1 on the human β3 gene. Exons 1 and 1a appear to encode distinct signal peptides of identical length. Cell lines were identified in which the β3 transcript is present (eg PC12, GT1-7) or absent (HeLa). These cell lines were transfected with CAT-constructs in order to identify regulatory elements within the 5' region of the human β3 gene. A strong promoter activity was detected within a 140 bp region, between exons 1a and 1. Incubation of this minimal promoter with nuclear extracts in a DNase 1-footprinting assay revealed a single protected element of 23 bp. Mobility-shift DNA binding assays indicate that this element binds both Sp1 and at least one additional factor. The activities and specificities of these binding factors have been assessed using transfected CAT-constructs, and by binding studies *in vitro*.

561.4

CHARACTERIZATION OF ISO- AND DIVERSITY-FORMS OF NCAM IN PRIMARY AND TRANSFECTED CELLS
D. Barthels*, U. Hartmann[1], A. Christoph[1], G. Vopper[1], M. Ramos Gomez[2], G. Ramirez[2], and W. Wille*. [1]Institut für Genetik, Universität zu Köln, FRG & [2]Centro de Biologia Molecular, Universidad Autonoma, Madrid, Spain

Alternative RNA splicing of primary transcripts coding for cell adhesion molecules is the main source for immense molecular diversity. A well analyzed example is the neural cell adhesion molecule (NCAM). This Ig-family gene codes for 192 NCAM amino acid sequences (Barthels *et al.* (1992) Eur. J. Neurosci. 4: 327-337). In addition to postranslational modifications, alternative splicing significantly contributes to the diverse population of NCAMs. it is of subtantial interest whether the differences of defined protein domains are important for the function of different NCAMs.

Transfection of NCAM-cDNA sequences into mammalian cells provide a usefull tool to investigate interactions of specific NCAM forms with primary neurons. For this analysis one needs a sensitive detection system for the influence of different NCAM iso- and diversity-forms on neuronal adhesion and fiber outgrowth. One of the *in vitro* systems discussed is the modified 'stripe assay' (after F. Bonhoeffer), in which axons of primary retinal explants are forced into numerous decisions between alternative substrates. In addition, specific antisera and monoclonal ABs against diversity epitopes have been developed in order to identify specific gene products and to interfere with defined functions. The second strategy of investigating the biological relevance of NCAM diversity forms is the analysis of their regulation in defined parts of the CNS. We choose the embryonic chicken retina for this study because of the limited number of neuronal cell types and the well defined developmental pattern.

561.5

DIFFERENT STRUCTURAL REQUIREMENTS FOR MAO A AND B CATALYTIC ACTIVITY. H.F. Wu*, K. Chen, and J.C. Shih , Dept. of Mol. Pharm. and Tox., Sch. of Pharmacy, Univ. of Southern California. Los Angeles, CA 90033.

There are seven conserved cysteines in the deduced amino acid sequences of human liver MAO A and MAO B. Site-directed mutagenesis of these cysteines to serines showed that the MAO catalytic activity was totally lost when FAD-linked cysteine was mutated (Cys 406 and Cys 397 for A & B respectively). Mutant Cys-156 and Cys-356 of MAO B also lost their activities, but the corresponding MAO A mutant Cys-165 remained active.

Chimeric MAO A/B enzyme were also constructed. When the N-terminal 30 amino acids were exchanged between A and B, the catalytic properties remained the same, suggesting that the N-terminus may not be important for enzyme specificities. When the C-terminal 125 amino acids were exchanged, MAO A with MAO B C-terminus has normal MAO A activity, suggesting the C-terminal of MAO A has no effect on the A activity. However MAO B with MAO A C-terminus has no activity for either A or B. This result suggested the C-terminus of MAO B is critical for MAO B activity. Taken together, these results suggest that the tertiary structure for MAO A and B may be different and the requirement for MAO B activity is more stringent. (Supported by NIMH grants R37 MH39085 (Merit Award), K05 MH00796 (Research Scientist Award), R01 MH37020 and Welin professorship).

561.6

DOPAMINE TRANSPORTER MUTANTS SUPPORT DIFFERENTIAL FUNCTIONS FOR TRANSMEMBRANE SEGMENTS 8-12. **J.B. Wang, D. Donovan*, and G. Uhl.** *Lab. Mol. Neurobiol., ARC/NIDA & Depts. Neurol.& Nsci., JHUSM, Box 5180, Baltimore, MD. 21224.*

The dopamine transporter's (DAT) 12 hydrophobic putative transmembrane domains contain polar amino acid residues that are candidates for involvement in recognizing cocaine analogs (eg. CFT), dopamine (DA) and the dopamine neurotoxin MPP^+ (see abstract by Kitayama et al). DAT cDNAs mutated by changing polar amino acids in domains 8-12 to alanine were examined in COS cells for their abilities to mediate DA and MPP^+ uptakes and CFT binding. Mutants in domains 9 and 12 ablate each function. Mutants in domains 8 and 10 and more selective mutations in domain 12 reduce DA and MPP^+ uptakes and CFT binding by 50-80%. Mutations in domain 11 increase DA and MPP^+ uptakes without changing CFT binding.

Differential effects of changes in different domains are consistent with differential contributions of each to DAT function in dopamine transport and cocaine recognition.

561.7

DOPAMINE TRANSPORTER RESIDUES IMPORTANT FOR SUBSTRATE TRANSPORT AND LIGAND RECOGNITION. S. Kitayama*, S. Shimada, D. Donovan & G. Uhl. *Lab. Mol. Neurobiol. ARC/NIDA & Depts. Neurol. & Nsci, JHUSM, Box 5180, Baltimore, MD. 21224.*

Polar amino acids lying within putative transmembrane (TM) regions 1, 7 and 8 of the dopamine transporter (DAT) are analogous to those important for ligand recognition by catecholamine receptors. Their possible functional significance was examined by testing binding and function in COS cells expressing mutant DAT cDNAs. Substitution of aspartate at position 79 in TM 1 with alanine, glycine or glutamate dramatically reduced uptake of [^{3}H] dopamine and [^{3}H] MPP^+ and the mutants' affinity for the cocaine analog [^{3}H] CFT without affecting B_{max}. Replacing serines at positions 356 and 359 in the outer portion of TM 7 by alanine or glycine reduced dopamine and MPP^+ uptake, while [^{3}H] CFT binding was less affected. Mutations in serine residues predicted to lie in the inner half of TM 7, 350 and 353, yield enhanced dopamine and MPP^+ uptake, with little change in [^{3}H] CFT binding B_{max}. Substitution of two serines in TM 8 results in wild-type values for dopamine and MPP^+ uptake and [^{3}H] CFT binding.

The TM1 aspartate residue is thus crucial for cocaine binding and dopamine uptake, while serine residues in TM 7 appear to play larger roles in substrate transport. These data define molecular features differentially important for cocaine binding and for dopamine uptake.

561.8

METHYLATION OF CpG SEQUENCES IN THE GLIAL FIBRILLARY ACIDIC PROTEIN GENE PROMOTER IN RAT ASTROCYTES. B.D. TETER*, H.H. OSTERBERG, C.E. FINCH, Neurogerontology Division, University of Southern California, Los Angeles, CA 90089-0191.

The astrocyte-specific gene encoding glial fibrillary acidic protein (GFAP), an intermediate neurofilament, increases in expression in the brain with age, as well as with hormone manipulations and deafferenting lesions. Analysis of sequence data (Miura *et al.*,1990,*J. Neuroch.*,55:1180) shows that the mouse GFAP gene promoter contains an island of eight CpG sequences (CpG dinucleotides are rare in eukaryotic genomes). Limited sequencing of the rat GFAP promoter shows additional CpG sites; the absence of these sites in the mouse promoter is consistent with their loss by mutation of methyl-cytosine to thymine. CpG sequences are sites of cytosine methylation which is correlated with promoter activity (in general,↑ CpG methylation = ↓ promoter activity). CpG methylation shows a generalized decrease in rat brain with age (Das and Das,1989,*Biochem. Arch.*,5:359), which suggests the hypothesis that demethylation of the GFAP promoter CpG island will correlate with the age-related increase in GFAP expression. We are investigating the pattern of CpG methylation in the GFAP promoter in GFAP-expressing tissues (hippocampus) and non-expressing tissues (liver) using ligation-mediated polymerase chain reaction (LMPCR). We are characterizing the pattern of CpG methylation in C6 glioma cells and any changes with corticosterone treatment (see abstract by C.J. Huang, *et al.*). Results will evaluate whether the pattern of GFAP promoter CpG methylation is corrlated with constitutive and induced GFAP expression and whether a change in the methylation pattern with age is dependent on GFAP expression. Supported by PHS grant AG 00093 and AG 7909

561.9

Extracellular ATP stimulates DNA synthesis in PC12 cells via P_2-purinergic receptor.

A. Takashima*, K. Noguchi, Y. Kudo, K. Inoue, Mitsubishi Kasasei Institute of Life Science, and National Institute of Hygenic Science, Tokyo, Japan.

ATP (0.1 mM) and [*methyl*-^{3}H]-thymidine(20 Ci/mmol) were added to the culture medium, and the incorporated ^{3}H-thymidine into DNA of PC12 cells were measured as an acid insoluble precipitation. We investigated the stimulant-evoked DNA synthesis. Only ATP showed the stimulation of the DNA synthesis in PC12 cells. Even if the ATP was washed out from medium after the stimulation of cells for 10 min, the DNA synthesis could be observed at after 2 hr incubation. The DNA synthesis of PC12 cells was stimulated by ATP, or ADP, but not by AMP, or adenosine. Moreover, suramine, a specific antagonist of P2 purinergic receptor, inhibited ATP-evoked DNA synthesis. These results suggested ATP-evoked DNA synthesis was stimulated through P_2 purinergic receptor. This DNA synthesis was insensitive to aphidicholine treatment(0.1 ng/ml), a DNA replication inhibitor, and the growth rate of ATP treated PC12 cells decreased to a half of control cells. Thus, the ATP was not recognized as a mitogenic stimulant for PC12 cells. The ATP-evoked DNA synthesis was insensitive to cycloheximide treatment, a protein synthesizes inhibitor, but was completely inhibited by actinomycine D treatment(0.1 ug/ml), which is an RNA synthesize inhibitor. These results suggested that the ATP-evoked DNA synthesis occurred through RNA dependent manner. This type of DNA synthesis was observed in the differentiated PC12 cells and in the primary culture of rat hippocampus.

561.10

***In Vitro* Pre-mRNA Splicing of the Amyloid Precursor Protein Gene** L.C. Kalef and G.A. Higgins. Molecular Neurobiology Section, NIA/NIH, Baltimore, MD 21224.

Alzheimer's disease (AD) and Down's syndrome (DS) are characterized by the extracellular deposition of amyloid in the form of senile plaques and cerebrovascular amyloid. The major component of these neuropathological markers is a 4 kDa protein called the ß/A4 protein, which is derived from a larger 110 to 135 kD precursor protein. The amyloid protein precursor (APP) gene consists of at least 18 exons, which undergo alternative RNA splicing to produce at least six different precursor protein products. APP-695 is the primary form found in the brain and lacks two exons (E) (E7 and E8), which are present in the predominant forms (APP-751 and APP-770) of APP found in most other tissues. Changes in the differential expression of the APP gene, through alternative splicing, may play a role in the deposition of the ß/A4 peptide found in the senile plaques and cerebrovasculature of AD and DS brains. We have developed a model to identify and characterize the splicing mechanism of the APP gene *in vitro*, using multiple minigene constructs of E6 through E9 (E6/7, E6/9, E7/8, E7/9, E8/9, E6/7/9, and E6/7/8/9). The hierarchy of splicing reaction intermediates and products of minigene constructs for E6/7, E6/9, and E8/9 have been directly analyzed in a HeLa cell *in vitro* splicing system. Branchpoints are being identified using primer extension analysis of lariat intermediates of the *in vitro* splicing reactions. A subcloned fragment of a full-length cDNA for APP-770 has been used in RNA protection analysis to quantify the relative levels of the wild-type APP transcripts normally produced by HeLa cells. Future studies involve development of a cellular model to study splicing of our mininigene constructs, and mutagenisis is being used to identify critical cis-acting elements required for correct splice site selection.

561.11

ALTERNATIVE SPLICING OF A HUMAN AMYLOID PRECUSOR PROTEIN MINI-GENE CONSTRUCT IN MOUSE N2A NEUROBLASTOMA CELLS. D. Willoughby*, S. A. Johnson, and C. E. Finch. Andrus Gerontology Center and Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089

In Alzheimer's disease, the amyloid precursor protein (APP) is cleaved abnormally to produce a 42-43 amino acid ß-amyloid peptide that accumulates extracellularly as a major component of senile plaques. Alternatively spliced APP-mRNAs encode 695, 751 and 770 amino acid polypeptides. This alteration in coding potential of APP-mRNAs is conferred by the variable inclusion of exon 7 and exon 8 of the APP gene. Exon 7 encodes a 57 amino acid Kunitz protease inhibitor domain, while exon 8 encodes a 19 amino acid motif with similarity to OX2, a neuron and thymocyte cell surface marker. Alterations in the relative abundance of alternatively spliced APP mRNAs were observed in neurons undergoing differentiation and as an in vivo neuronal response to neurotoxic lesions. We constructed a plasmid ($pRSV^{6-9}$), which contains in series the exons 6/7/8/9 of the human APP gene. Each exon is flanked by 0.1Kb to 1Kb of intron sequence except exon 6, which is fused at the 5' end to the Rous sarcoma virus LTR. Similarly exon 9 is fused directly to SV40 3' sequences.

$pRSV^{6-9}$ was transfected into N2A neuroblastoma cells. RNA transcripts derived from the transfected construct were assayed by reverse transcription-PCR. Processed mRNA containing exons 6/7/8/9, exons 6/7/9 and exons 6/9 were detected. A related plasmid $pRSV^{6-9\Delta 8}$, which lacks exon 8, gave rise to splice variants both containing and lacking exon 7. Alternatively spliced mRNAs derived from $pRSV^{6-9}$ were also detected in transfected rat C6 glioma cells. $pRSV^{6-9}$ in transfected neuroblastoma cells can be used to explore the mechaism(s) of APP RNA differential splicing during neuronal differentiation and in response to neuronal trauma. DW was supported by TG-AG00037-13; this work was supported by AG7909 to C. E. F.

561.12

SYNTHESIS OF cRNA PROBES FROM PCR-GENERATED MINIGENES. J. Logel*, D. Dill, C. Drebing, S. Leonard. Denver Veterans Administration Medical Center, Denver, CO 80220.

Many molecular biological techniques such as northern, RNase protection and *in situ* hybridization analyses are dependent on the use of radioactive probes. High specific activity cRNA probes offer greater sensitivity and more stable duplexes, but they require the availability of cloned cDNAs in vectors containing T7, T3 and SP6 RNA promoter sequences.

We report a method for *in vitro* transcription of cRNA probes using PCR generated DNA fragments or minigenes. Sense oligonucleotide primers, specific for mouse acidic fibroblast growth factor (aFGF), were synthesized with 5'-extensions containing sequences for the T7, T3 and SP6 polymerase promoters. A common antisense primer was used with each of the promoter/aFGF primers to prepare PCR-generated DNA fragments (minigenes). *In vitro* transcription efficiency for each of these constructs was evaluated by incorporation of radioactivity into the cRNA products. We find that both the T7 and T3 promoters can direct the synthesis of cRNA probes of high specific activity from a PCR-generated DNA fragment, but the SP6 promoter can not.

Antisense cRNA probes, transcribed from minigene constructs for aFGF were used for northern blot hybridization. *In vitro* transcription of minigene constructs for β-nerve growth factor (β-NGF) were used to synthesize antisense and sense cRNA probes for *in situ* hybridization of human hippocampal tissue sections.

GENE STRUCTURE AND FUNCTION VIII

562.1

REGULATION OF THE MAJOR NEURONAL GROWTH ASSOCIATED α-TUBULIN GENE, Tα1, BY NGF. J.G. Toma, Y. Ma*, and F.D. Miller, Dept. of Anat. & Cell Biol., University of Alberta, Edmonton, Canada T6G 2H7.

We have previously demonstrated that two members of the α-tubulin multigene family that encode virtually identical proteins are differentially regulated in mammalian neurons; expression of Tα1 mRNA is specifically correlated with process outgrowth, whereas expression of T26 mRNA is constitutive. Furthermore, expression of Tα1 mRNA is upregulated by NGF both *in vivo*, and in cultured sympathetic neurons, whereas T26 mRNA levels are not altered. In order to address the regulatory elements responsible for inducing Tα1 mRNA in response to NGF, we have isolated the promoter region from a rat genomic library. The 5' promoter region thus isolated has been fused to lacZ and CAT reporter genes. We have used PC12 cells to start to map the elements responsible for NGF-inducibility. The results obtained so far indicate that, in PC12 cells, endogenous Tα1 mRNA levels were increased within 2-6 hours following NGF addition, while levels of T26 mRNA were not altered. The observed increases in Tα1 mRNA occurred at the transcriptional level, as determined using nuclear run-offs. Transient transfection experiments indicate that 1.1 kb of the Tα1 promoter is sufficient to confer NGF-inducibility in PC12 cells. To determine whether this promoter fragment confers NGF-inducibility *in vivo*, we have introduced a similar construct into transgenic mice. Analysis of these mice is currently underway.

562.2

REVERSIBLE INTERRUPTION OF FAST AXONAL TRANSPORT IN VIVO LEADS TO ALTERATIONS IN GENE EXPRESSION IN BOTH NEURONS AND SCHWANN CELLS. W. Wu, T.C. Mathew, J.G. Toma, and F.D. Miller*, Dept. Anat. & Cell Biol., University of Alberta, Edmonton, Canada T6G 2H7.

Following nerve injury, axotomized neurons respond by inducing a number of genes associated with neuronal growth, and Schwann cells distal to the site of injury down-regulate genes associated with myelination. We hypothesized that at least some of these alterations were due to the loss of ongoing homeostatic signals that were transduced as a function of fast axonal transport. To directly address this hypothesis, we selectively blocked fast axonal transport in vivo by locally-cooling nerves to 3-8° C (a cold block). Immunocytochemistry for the antigens OX-42 or ED1 demonstrated that macrophages do not accumulate at the site of a cold block. Furthermore, neurons rapidly regained the ability to retrogradely transport tracers upon removal of the cold block. Thus, any effects of the cold block were not likely due to nerve injury. To determine whether blocking fast axonal transport was sufficient to induce a neuronal "axotomy" response, we examined expression of Tα1 α-tubulin and p75 NGF receptor mRNAs in facial motoneurons. In situ hybridization and image analysis revealed that both of these mRNAs were induced to a similar degree 36-60 hours following either a cold block or nerve transection. To determine whether blocking fast axonal transport also affected any nonneuronal cells distal to the cold block, we examined expression of Po and p75 NGF receptor, both of which are regulated as a function of Schwann cell:axon contact. Levels of p75 NGF receptor mRNA and protein were unaffected by the cold block. In contrast, levels of Po mRNA were decreased in the distal nerve in a fashion similar to that observed following axotomy. These data therefore suggest a) that neurons normally monitor the status of their axons and connections as a function of fast axonal transport, and b) that alterations in expression of Po and p75 NGF receptors result from two different aspects of Schwann cell:axon communication, one of which involves fast axonal transport.

562.3

THE Tα1 α-TUBULIN PROMOTER DIRECTS GENE EXPRESSION TO DEVELOPING NEURONS IN TRANSGENIC MICE. F.D. Miller, A. Speelman, and J.G. Toma*, Dept. Anat. & Cell Biol., University of Alberta, Edmonton, Canada T6G 2H7.

The Tα1 α-tubulin gene is one member of the α-tubulin multigene family that is regulated as a function of neuronal growth in both developing and mature mammalian neurons. During development, Tα1 mRNA is abundantly expressed in newly-differentiated neurons during the period of morphological growth, and is subsequently downregulated as a functional of target contact and neuronal maturation. We have isolated the Tα1 gene, and have fused 1100 nucleotides of the upstream putative promoter region to a nuclear β-galactosidase reporter gene. Transient transfection assays demonstrated that this upstream fragment was sufficient for basal expression of the marker gene in cultured PC12 cells. To determine whether this fragment was sufficient to direct appropriate expression *in vivo*, transgenic mice were generated. At embryonic days 14 and 16, a developmental period when expression of the endogenous gene is maximal, the transgene is expressed throughout the developing peripheral nervous system. The transgene is also expressed at high levels in some regions of the developing central nervous system, but is not globally expressed. By postnatal day 1 expression of the transgene is undetectable in the brain and in peripheral ganglia. At all timepoints the transgene appears to be specific to the nervous system. Thus, 1100 nucleotides of the Tα1 promoter is sufficient to developmentally regulated gene expression to at least some populations of central and peripheral neurons.

562.4

AMPHETAMINE INDUCED ROTATIONAL BEHAVIOR IN NON-LESIONED RATS: A ROLE FOR *C-FOS* EXPRESSION IN THE STRIATUM. B.J. Chiasson*, M.L. Hooper and H.A. Robertson. Dept. of Pharmacology, Dalhousie University, Halifax, N.S. Canada B3H 4H7.

Ungerstedt (1971a) reported that rats having had a unilateral lesion to the nigrostriatal pathway responded to a D-amphetamine (A) challenge by rotating toward the damaged side. More recently, it has been demonstrated that A can influence the expression of immediate-early genes (IEGs), such as *c-fos*, in normal animals (Graybiel, Moratalla & Robertson, 1990). Here we ask whether the expression of *c-fos* might be important in modulating the functional output of the striatal system in normal non-lesioned animals following an A challenge. To test this possibility we used oligodeoxynucleotide (ODN) technology to selectively inhibit mRNA translation of *c-fos*. Under stereotaxic guidance sense (S) and antisense (AS) ODN's were infused unilaterally into the striatum of normal rats. At varying times post-infusion, 10 and 22 h, animals were given 5mg/Kg A (i.p.) and their rotational behavior was monitored for two hours. Subsequently, animals were sacrificed and immunocytochemistry for Fos-like protein was performed on striatal sections.

Striatum infused with AS ODN at 10h prior to the injection of A showed a marked reduction in the number of Fos positive neurons when compared to the S infused side. These animals rotated 400-500 times in the direction of the AS over a 2h period. The animals that received AS infusions 22h prior to A demonstrated little or no difference in the number of Fos positive neurons in the striatum compared to the S infused side. Unlike the previous group these animals rotated only 30-50 times in the same 2 h period and showed no directional preference in their rotational behavior. Thus, it appears that striatal *c-fos* expression may regulate the sensitivity of the striatum to indirect acting dopamine agonists. Supported by MRC (Canada) & The Savoy Foundation.

562.5

DIFFERENTIAL *C-FOS* ACTIVATION BY PATTERNED STIMULATION H.Z.Sheng*, R.D.Fields, and P.G.Nelson. Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892

The pattern of electrical activation is important for such examples of neuronal plasticity as LTP or regulation of muscle contraction properties. To test whether immediate early gene expression is sensitive to the pattern of electrical stimulation, dissociated neurons of mouse dorsal root ganglion (DRG) were stimulated with electrical pulses organized in various patterns. The expression of c-*fos* mRNA by DRG neurons was monitored in a semi-quantitative PCR assay with optical density reading of ethidium bromide stained gel. A constant number of stimuli (180) were delivered in 3 different patterns for 30 min: 1) steady 0.1 Hz; 2) bursts of 6 stimuli at 10 Hz delivered every minute; 3) 12 stimuli at 10 Hz delivered every 2 minutes. The steady 0.1 Hz produced a small increase in c-*fos* expression over control, unstimulated values (69%, $p<0.02$; 22±5.8 vs. 13±2.8, mean O.D.±SD), while bursts of 6 pulses at 10 Hz produced a significantly larger increase (168%, $p<0.001$; 34.8±3.8). By contrast, the relatively infrequent 12 pulse bursts at 10 Hz produced no significant increase in c-*fos* expression (14.8±1.9). These results show that different patterns of stimulation can differentially regulate transcriptional events in neurons. Hence our data suggest that the cascade leading to c-*fos* activation could represent a mechanism by which the information in patterned electrical activity is decoded.

562.6

SPATIAL AND TEMPORAL DIFFERENCES IN THE DISTRIBUTION OF mRNA ENCODING HEAT SHOCK PROTEIN 70 IN THE RABBIT BRAIN IN RESPONSE TO HYPERTHERMIA. P. Manzerra and I.R. Brown* Dept of Zoology, Univ of Toronto, Scarborough Campus, West Hill, Ontario M1C 1A4, Canada.

Our previous studies have shown that hyperthermia induces the expression of a heat shock gene (hsp70) in the rabbit brain with striking regional differences (for review see J. Neurosci Res., 27, 247-255, 1990). In situ hybridization studies using riboprobes which can distinguish between constitutive and inducible members of the hsp70 gene family revealed the prominent induction of a 2.7 kb hyperthermia-inducible mRNA species at 1 hr in glial-enriched areas of the rabbit brain with little induction in neuronal enriched areas such as the hippocampus. Further studies have revealed that hsp70 gene expression is not only regulated in a spatial manner but temporal differences in expression are also observed. Several neuronal populations including hippocampal and cortical neurons show delayed induction of the hyperthermia-inducible hsp70 mRNA species at 5 hrs post-heat shock with decreases by 10 and 24 hrs. This temporal study has also been extended to the protein level using Western blot and immunocytochemical procedures. Hsps may play important roles in cellular repair and/or protective mechanisms in the nervous system. Supported by grants from MRC Canada.

562.7

EXPRESSION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS IN HUMAN RETINAL PIGMENT EPITHELIAL CELLS. A. Randolph*, D. Yee[1] and E. Feldman. Univ. of Michigan Med. Center, Ann Arbor, MI 48109, [1]Univ. of Texas Health Sci. Center, San Antonio, TX 78284.

Human retinal pigment epithelial (HRPE) cells provide a good *in vitro* model for studying the action of insulin-like growth factor I (IGF I), a polypeptide with growth-promoting and insulin-like activity. HRPE cells express both IGF I and the type I and II IGF receptors. In HRPE cells, IGF I action is modulated by a series of high affinity binding proteins, designated IGFBPs. IGFBPs regulate the tissue compartmentalization of IGF I and its binding to the type I IGF receptor. Glucose, insulin and IGF I alter hepatic IGFBP secretion. In this study we examined the regulation of IGFBP gene and protein expression in HRPE cells by glucose, IGF I, and serum. HRPE cells were grown in MEM in the presence of either 5 to 300 mM glucose, IGF I, IGF I plus the IGF I receptor antibody (αIR_3) or serum. After collecting conditioned media, total RNA was isolated from each condition. Human cDNA clones were obtained from Dr. S. Shimasaki, La Jolla, CA (IGFBP 2,3,5) and from Dr. D. Powell, Houston, TX (IGFBP 1,4). RNA was analyzed by Northern blotting and RNase protection. The IGFBPs present in conditioned media were characterized by ligand blotting. Under all conditions, IGFBP 3 mRNA constituted the major transcript in HRPE cells. Ligand blotting demonstrated the presence of glycosylated IGFBP 3 forms at 42-49 kDa. There was no effect on basal IGFBP 3 gene expression by 1) physiologic concentrations of glucose; 2) IGF I; or 3) αIR_3, added to cells to block potential effects of constitutive IGF I production by HRPE cells. In contrast, IGFBP 3 gene expression was up-regulated, in a dose dependent fashion, by calf serum. These findings suggest that IGF I alone cannot regulate the production of its binding protein(s) and implicate a role for other trophic factors in the IGF I-IGFBP axis. Supported by NIH grant NS01381 (EF) and R29CA52592 (DY).

562.8

EZRIN AND OSTEONECTIN, TWO PROTEINS WHICH INFLUENCE CELL SHAPE AND GROWTH, ARE ENRICHED IN THE LOCUS COERULEUS. C. Bergson*, H. Zhao, K. Saijoh, R. S. Duman and E. J. Nestler. Dept. of Psychiatry and Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06508.

In a first attempt to better characterize the noradrenergic neurons of the locus coeruleus (LC) at the molecular level, we screened a bovine LC cDNA library with a bovine LC minus cerebellum subtracted cDNA probe. Two of the cDNA's isolated show enrichment in LC compared to other regions tested on bovine brain regional northern blots. DNA sequence analysis and GenBank database searches indicate that the more LC-specific clone probably encodes the bovine homolog of ezrin, a phosphoprotein found in cytoskeleton of microvilli, and that the more ubiquitous clone encodes osteonectin, an extracellular Ca2+-binding glycoprotein. *In situ* hybridization studies in sections of rat brains at the level of the LC show that the mRNA's for these two genes are concentrated in LC noradrenergic neurons, but are also present in certain other cell bodies, including motor neurons of the trigeminal nerve. Ezrin-and osteonectin-like immunoreactivity are also enriched in LC noradrenergic neurons. It will be interesting to determine the functions subserved by ezrin and osteonectin in LC neurons.

562.9

INCREASED EXPRESSION OF RAT ADRENAL TYROSINE HYDROXYLASE GENE BY IMMOBILIZATION STRESS. E.L. Sabban*[1], R. Kvetnansky[2], A. McMahon[1], W.G. Frankle[1], B.B. Nankova[1], K.Fukuhara[2], E. Viskupic[2] and I.J. Kopin[2]. [1]Dept. Biochem. & Mol. Biol., New York Med. Coll. Valhalla, NY 10595 and [2]NINDS, Nat. Inst. of Health, Bethesda, MD 20892.

Repeated immobilization (IMO) stress has been shown to elevate circulating catecholamines and the activity, as well as mRNA levels, of their biosynthetic enzymes, especially tyrosine hydroxylase (TH). A single 2 hr IMO of Sprague Dawley rats greatly elevated adrenal TH mRNA levels, with maximal induction up to 12 hours after the IMO. Wistar-Kyoto, Lewis and Fisher rats also increased adrenal TH mRNA levels of 4-10-fold after a single 2 hr IMO. After a 30 min IMO, TH mRNA was not elevated immediately, however 90 min later TH mRNA was increased as much as with continuous 2 hr IMO.

The splanchnic nerve is known to be crucial for the biochemical responses of the adrenal to prolonged IMO. The neuronal output to the adrenal is cholinergic, although other neurotransmitters, such as VIP are co-released. We therefore, examined the effect of a nicotinic cholinergic antagonist, chlorisondamine and of a VIP antagonist. Chlorisondamine (10 mg/kg), prevented the IMO-induced rise in plasma norepinephrine and reduced plasma epinephrine. Surprisingly, chlorisondamine had little effect on the rise in adrenal TH mRNA levels, while the VIP antagonist partially inhibited the rise in the TH mRNA levels following a single IMO.

Actinomycin D prevented the rise in TH mRNA by 2 hr IMO, suggesting transcriptional activation. The IMO induced transcription factor interaction with upstream elements of the rat TH gene is being studied by gel shift assays using adrenal nuclear extracts from IMO rats.

562.10

SULFATED GLYCOPROTEIN-2 (SGP-2, CLUSTERIN) AND THE CENTRAL NERVOUS SYSTEM (CNS): DETECTION OF SGP-2 mRNA IN THE NEVROGLIA AND DISCRETE POPULATIONS OF NEURONS. J.-G. Chabot*, M. Danik, D. Hassan-Gonzalez and R. Quirion. Douglas Hospital Research Centre, Department of Psychiatry, McGill University, Verdun and Maisonneuve-Rosemont Hospital Research Centre, Department of Medicine, Montreal University, Montreal, Quebec, Canada.

We recently reported on the expression of SGP-2 mRNA in human gliomas, epileptic foci and rat brain tissues (Danik *et al.*, PNAS 88, 8577-81, 1991). We now extended these findings and report an extensive analysis of the cellular distribution of SGP-2/clusterin transcripts in the adult rat CNS. The overall pattern was one of widespread expression with several regions showing strong hybridization signals. Resolution at the cellular level revealed the differential labeling of distinct cell types. Among the non neuronal elements expressing SGP-2 mRNA, ependymal cells forming the walls of brain ventricles were strongly labeled. Although glial cells showed a diffuse labeling throughout the neuropil, scattered highly labeled cells were observed in the optic nerve, the corpus callosum and the hippocampal fissure. Specific mRNA labeling was also seen in neurons of the habenular complex, substantia nigra, cerebellum, several hypothalamic and brainstem nuclei, and the gray matter of the spinal cord. Strong labeling was especially concentrated in the motoneurons of the brainstem and the ventral horn of the spinal cord. It is thus clear that SGP-2 gene expression is not restricted to glial cells but is seen in specific populations of neurons. This suggests that SGP-2 (also known as clusterin) may be a multifunctional protein which may be implicated in events such as the packaging, processing and transport of neurotransmitters and neuropeptides, as well as in the lipid metabolism. Supported by MRC Canada.

562.11

REGIONAL DISTRIBUTION OF ALPHA 1 SUBUNIT ISOFORM OF (Na,K) - ATPase IN THE RAT SPINAL CORD. S. Sayers, R. Shahid, M. Dauzvardis, T. Khan*, L. Farber and G. Siegel. Rehab. R&D Center and Neurology Service, Hines VA Hospital, Hines, IL 60141 and Loyola University Medical School, Maywood, IL 60153

(Na,K)-ATPase is the membrane bound enzyme that produces active cation transport, and therefore is responsible for maintenance of the resting membrane potential. It is a heterogeneous oligomer of a catalytic (alpha subunit) and a glycoprotein (beta subunit). Three isoforms of the alpha subunit have been identified in the central nervous system. Both alpha 1 and alpha 3 mRNAs were found in neurons of the CNS but their proportions vary in different regions of the brain. Alpha 1 mRNA was found predominantly in cerebral cortex, dentate gyrus of the hippocampus, specific isolated brain stem nuclei such as the locus ceruleus and motor nuclei V and VII, and in discrete subsets of anterior horn cells in the lumbar spinal cord. In the present study, mRNA levels for the alpha 1 isoform of (Na,K)-ATPase were measured from five levels of the rat spinal cord.

The spinal cords of 5 anesthetized young adult rats were rapidly harvested and divided into five sections each; Section 1 (C1-C8), Section 2 (T1-T4), Section 3 (T5-T8), Section 4 (T9-T13), and Section 5 (L1-L5). Total RNA from each section was isolated using the method of Chomczynski and Sacchi, slot-blotted onto membranes, and hybridized with 32P-labeled riboprobe transcribed from the cDNA clone which codes for the alpha 1 isoform of (Na,K)-ATPase. The membranes were subjected to autoradiography, the autoradiographs were scanned, linear regression lines were plotted, and the slopes of the lines were compared.

The amount of total RNA/g of tissue varied in the five different spinal cord sections. After normalizing for the total µg of RNA, the alpha 1 mRNA levels were also found to vary. Assigning a value of 1 to the slope obtained from Section 1, Section 2 was 2.6 times higher, Section 3 was 4.5 times higher, Section 4 was 2.6 times higher, and Section 5 was 1.7 times higher. Our results show that alpha 1 mRNA levels are highest in the mid-thoracic level (T5-T8). *In situ* hybridization experiments are underway to further localize expression of alpha 1 mRNA in the rat spinal cord.

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562.12

INDUCTION OF CALBINDIN-D_{28k} mRNA IN MEDULLOBLASTOMA CELLS BY RETINOIC ACID AND IN HIPPOCAMPUS AFTER KAINIC ACID INDUCED SEIZURES IS PRECEDED BY THE INDUCTION OF ZIF/268 mRNA. Y. Wang, S. Lee, and S. Christakos*. Dept. of Biochemistry and Molecular Biology, UMDNJ-New Jersey Medical School, Newark, NJ 07103.

Previously, we reported that calbindin-D_{28k} can be induced by retinoic acid (RA) in B104 neuroblastoma cells (which do not contain calbindin endogenously) after transfection with a retinoic acid receptor α expression vector. We recently found that the human medulloblastoma cell line D283 contains calbindin endogenously and that calbindin protein and mRNA can be induced 8-10 fold in these cells by 10^{-8} M RA. These findings are the first evidence of the presence of calbindin, which can be regulated, in a neuronal cell line. Regulation by RA suggests a role for calbindin in neuronal differentiation. The time course of response indicated that the first significant increase in calbindin mRNA is at 3 hrs with a plateau of calbindin mRNA induction at 48 hours after RA treatment. Co-treatment of D283 cells with RA and either cycloheximide or actinomycin D completely blocked the increase in calbindin mRNA, suggesting that the induction of calbindin by RA requires both RNA and protein synthesis. Induction of calbindin mRNA by RA was preceded by the induction of the early response gene zif/268 (also known as EGR1 and NGFI-A) at 30 min. and 1 hr. after RA treatment. In addition, we have observed that the induction of rat hippocampal calbindin mRNA by kainic acid (12 mg/kg i.p.), which is maximal at 6 hours, is also preceded by the induction of zif/268 mRNA. These findings suggest that the early response gene zif/268 may be involved in calbindin-D_{28k} transcriptional regulation. (Supported by NIH NS-20270)

562.13

CORTICOSTERONE MODULATES THE EXPRESSION OF THE RAT GLIAL FIBRILLARY ACIDIC PROTEIN GENE AND ITS PROMOTER ACTIVITY IN THE C6 GLIOMA. C.J. Huang, N.J. Laping, Y. Su, D.G. Morgan*, C.E. Finch. Neurogerontology Division, Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089-0191.

The intermediate neurofilament, glial fibrillary acidic protein (GFAP), is an astrocyte marker that increases in the brain, following injury, deafferentation, adrenalectomy, or with aging. In this study, the hormone regulatory mechanisms of GFAP gene expression were investigated by linking the 2 kb of upstream region of rat GFAP gene to the chloramphenicol acetyl-transferase (CAT) gene in the promoterless pUC vector. The modified vector was transfected into an astrocyte tumor cell line (C6 glioma) via electroporation. Doses of corticosterone (CORT) and dibutyryl cyclic AMP (dbcAMP) were introduced to transfected and non-transfected C6 glioma. Endogenous GFAP mRNA changes were detected by Northern blotting. The GFAP promoter activity was monitored by CAT assay. CORT (1 uM) and dbcAMP (0.25 mM) alone or in combination increased CAT activity (2-3 fold) in the transfected C6 glioma and increased GFAP mRNA in the non-transfected C6 glioma. CORT in a range of 0.1 - 10 uM increased CAT activity and GFAP mRNA. However, 100 uM CORT depressed CAT activity and GFAP mRNA transcription from the endogenous gene. We note that CORT has the opposite effect on GFAP in vivo (O'Callaghan et al., Brain Res.494:159-161, 1989; Nichols et al., Mol. Brain Res.7:1-7,1990; Laping et al., Mol. Brain Res.10:291-297,1991). These results indicate that the transfected C6 glioma responded to the CORT treatment in a dose dependent manner, and the regulation of GFAP mRNA is mediated at the level of GFAP promoter activity.

Supported by PHS grant AG 07909, The J. D. and C. T. MacArthur Foundation Network in Successful Aging, and NRSA grant AG 05528 to N.J.L.

562.14

TRANSCRIPTIONAL REGULATION OF GLIAL FIBRILLARY ACIDIC PROTEIN mRNA BY GLUCOCORTICOIDS. N.J. Laping*, C.J. Huang, S.A. Johnson, J.R. Day, and C.E. Finch. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089-0191.

Glial fibrillary acidic protein (GFAP) and its mRNA is decreased by glucocorticoids in vivo (O'Callaghan et al., Brain Res.494:159-161, 1989; Nichols et al., Mol. Brain Res.7:1-7, 1990; Laping et al., Mol. Brain Res.10:291-297, 1991). This study examined if this regulation occurs at the transcriptional level. Adult male F344 rats received systemic injections of 1 mg glucocorticoids (GC) and were sacrificed 8 hours later. Nuclei were isolated from cortex or pooled hippocampus. Nascent transcript levels for GFAP mRNA were determined by nuclear run-on assay. GC elevated GFAP transcription rate 3-4 fold in the hippocampus over oil injected controls. In the cortex, GC also elevated GFAP transcription 4 fold ($p<0.05$, $n=4$).

GC also increases the GFAP promoter activity in vitro. GC (1 μM) increased chloramphenicol transferase (CAT) activity 2-3 fold in C6 glioma transfected transiently with a construct containing the rat GFAP promoter attached to the CAT reporter gene. These findings suggest that decreased GFAP mRNA levels in vivo seen after GC treatment are not due to decreased transcription initiation and are probably due to altered mRNA stability or translocation to the cytoplasm. However, decreased mRNA transcripts with intact polyadenylation sequences might also be due to RNA polymerase pausing within the gene, thus overriding the increased transcription initiation induced by GC. This hypothesis will be tested using specific probes for the 3' untranslated region of the GFAP mRNA.

Supported by PHS grant AG 07909, The J. D. and C. T. MacArthur Foundation Network in Successful Aging, and NRSA grant AG 05528 to N.J.L.

562.15

IDENTIFICATION OF INSERTION MUTANTS OF VASOPRESSIN mRNA IN THE HOMOZYGOUS BRATTLEBORO RAT.

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A single base deletion in the vasopressin (VP) gene is the cause of diabetes insipidus (di) in the homozygous (di/di) Brattleboro rat. The di/di rat expresses a mutant VP precursor with an altered C-terminus. A striking feature of the di/di rat is the appearance of apparently normal VP gene products together with the mutant precursor protein in solitary hypothalamic neurons, indicating a heterozygous (+/di) phenotype of these cells. In new-born animals only 0.1% of the VP neurons displays the +/di phenotype, but the number increases proportional to age to 3% in old animals.

The +/di phenotype of these VP neurons in the di/di rat suggests the existence of VP mRNAs which code for the normal VP precursor protein. The aim of the present study was to elucidate the nucleotide sequence of these VP mRNAs of the di/di rat. The strategy used included specific amplification of VP cDNAs by PCR, followed by cloning of VP cDNAs in an expression vector. Clones containing VP cDNAs with restored reading frame were selected by immunoscreening using a rat glycopeptide antiserum. Sequence data, obtained so far, indicate that different, single nucleotide insertions are present in the mutated VP mRNAs. These insertions occur in the same region as the original deletion, which suggests this region is sensitive to mutations.

562.16

ISOLATION OF A DROSOPHILA CUT-LIKE HOMEOPROTEIN WHICH BINDS THE MINIMAL TISSUE-SPECIFIC ELEMENT OF THE RAT TYROSINE HYDROXYLASE GENE. S.O. Yoon* and D.M.Chikaraishi. Dept. of Molecular Biol. and Microbiol., and Program in Neuroscience, Tufts Univ. Sch. of Med., Boston, MA 02111.

The minimal tissue-specific element of the rat tyrosine hydroxylase (TH) gene consists of the AP1 motif and an overlapping E-box containing dyad symmetry element (Yoon and Chikaraishi, Neuron, in press). In conjunction with the AP1 motif, the dyad element activates transcription in TH-positive PC cells, but suppresses it in TH-negative neuroblastomas cells. Therefore, the dyad element was used to probe a cDNA expression library from TH-positive Pc8b cells to clone factors involved in TH expression.

We isolated a partial 3.3 Kb clone, TAD, which binds the TH-dyad in a sequence-specific manner; it fails to bind another closely-related E-box motif (PAN). Surprisingly, TAD also binds the AP1 motif, but fails to bind the CRE which is only one base different from the AP1 site. By sequence analysis, TAD contains a homeodomain and two regions which are strikingly homologous to the "cut" repeat, a region repeated three times in the Dosophila cut gene involved in sensory neuron determination. Neufeld et al. (Nature Genetics 1: 50-55, 1992) have recently cloned a cDNA, CDP, from Hela cells which contains cut repeats and a homeodomain. This gene and TAD are almost identical except for several very divergent regions, which may represent alternative exons. Therefore, TAD along with CDP, may belong to a new family of transcription factors that contain homeodomains and cut repeats.

562.17

REGIONAL DISTRIBUTION OF ACETYLCHOLINE RECEPTOR ALPHA-SUBUNIT mRNA IN THE RAT SPINAL CORD. M. Dauzvardis*, S. Sayers, R. Shahid, C. Trausch, T. Khan. Rehabilitation R&D Center, Hines VA Hospital, Hines IL 60141

A cDNA clone for the alpha-subunit of the acetylcholine receptor (AChR) has been previously identified by J. Boulter (J. Neurosci. 5: 2553, 1985). Variations in the distribution of this specific mRNA have been studied in hindlimb muscles of the rat following transection of the sciatic nerve. The present study demonstrates the presence and distribution of AChR alpha-subunit mRNA in the different levels of the spinal cord

The spinal cords of five anesthetized young adult rats were rapidly harvested and divided into five sections each; Section 1 (C1-C8), Section 2 (T1-T4), Section 3 (T5-T8), Section 4 (T9-T13), and Section 5 (L1-L5). Total RNA was prepared using the method of Chomczynski and Sacchi. The total RNA was slot-blotted onto Gene Screen Plus membranes and the membranes were subsequently hybridized with 32P-labeled riboprobe transcribed from the cDNA clone which codes for the AChR alpha-subunit. The membranes were subjected to autoradiography, the autoradiographs were scanned, linear regression lines were plotted (µg total RNA vs. integration units obtained from scanning the autoradiographs), and then the slopes of the lines were compared.

The amount of total RNA/g of tissue varied in the five different spinal cord sections. After normalizing for the total µg of RNA, the AChR alpha-subunit mRNA levels were also found to vary. Assigning a value of 1 to the slope obtained from Section 1, Section 2 was 3.8 times higher, Section 3 was 9.8 times higher, Section 4 was 1.8 times higher, and Section 5 was 5.3 times higher (see diagram below). Our results show that AChR alpha-subunit mRNA levels are highest in the mid-thoracic level (T5-T8). *In situ* hybridization experiments are underway to further localize expression of AChR alpha-subunit mRNA in the rat spinal cord.

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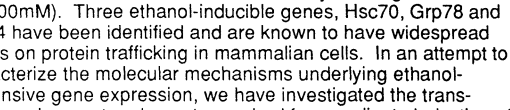

562.18

EFFECTS OF AF64A ON ChAT ACTIVITY AND N-*MYC* EXPRESSION IN THE LA-N-2 HUMAN NEUROBLASTOMA CELL LINE.

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AF64A, which shares structural similarity to choline, may enter cholinergic cells via the high affinity uptake system (HAChT) to exert its cytotoxic effects. In order to further investigate the mechanism of action of AF64A in an *in vitro* model, we used the LA-N-2 cholinergic human neuroblastoma cell line to study the effects of AF64A on choline acetyltransferase (ChAT) activity and on the steady state expression of the N-*myc* gene. ChAT: Following 1 hr exposure to 25, 50 and 100 µM doses of AF64A, ChAT activity was significantly decreased by approximately 15, 25 and 30 % ($p < .05$), respectively. The addition of 1 mM choline or hemicholinium-3 (HC-3) inhibited the decrease of ChAT activity observed at 25, 50 and 100 µM doses of AF64A. The protective effects may be explained by competition (choline) and inhibition (HC-3) of the HAChT system by which AF64A enters cholinergic cells. N-*myc*: Using an S1 nuclease protection assay and doses of 50, 100 and 250 µM AF64A, we observed decreased steady state levels of N-*myc* mRNA at 3 and 6 hours after drug removal. At t=3 hr, N-*myc* mRNA levels were significantly reduced by 40 and 75% ($p < .05$) at doses of 100 and 250 µM, respectively. At the lower doses (50 and 100 µM), the levels of N-*myc* mRNA began to recover at t=6 hr to their initial steady state levels. We are currently examining the possibility that AF64A-induced changes in ChAT activity may be due to AF64A's effect on genes critical for the maintenance of a cholinergic phenotype.

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562.19

REGULATION OF ETHANOL-RESPONSIVE GENES IN NEURAL CELLS. M.W. Sganga, N. Wilke, G. Gayer, W. Chin, S. Barhite, and M.F. Miles*. Ernest Gallo Research Center and Clinic, UCSF, San Francisco, CA 94110

We are currently exploring the hypothesis that changes in neuronal gene expression may underlie the phenomena of tolerance to and dependence on ethanol seen in chronic alcoholics. Our lab has identified a number of genes which are induced or repressed 50-300% in cultured neuroblastoma cells exposed to levels of ethanol seen in actively drinking alcoholics (25-100mM). Three ethanol-inducible genes, Hsc70, Grp78 and Grp94 have been identified and are known to have widespread effects on protein trafficking in mammalian cells. In an attempt to characterize the molecular mechanisms underlying ethanol-responsive gene expression, we have investigated the trans-acting and promoter elements required for coordinate induction of these genes by ethanol. Transient transfection analyses of NG108-15 cells have been used to identify ethanol-responsive cis-acting elements in the Hsc70, Grp78 and Grp94 promoters. Subsequent in vitro studies have confirmed the existence of a specific transcription factor which binds to these sequences. Finally, we have also performed dose-response and mixing studies comparing ethanol and other known inducers of Hsc70, Grp78 and Grp94. Our studies may add substantially to the understanding of central nervous system adaptation to ethanol as seen in alcoholism.

562.20

HIGH LEVELS OF EXPRESSION OF UNIQUE mRNAs ENCODING A NUCLEAR MATRIX PROTEIN IN SPECIFIC REGIONS IN THE BRAIN AND TESTIS. S. Vijayaraghavan, G. Nilaver[1], D.T. Stephens, S.R. Nagalla[2], and M.H. Melner * Div. Reprod. Sciences. and Div. of Neurosciences[2], Oregon Regional Primate Research Center, Beaverton, OR 97006, Dept. of Neurology[1], Oregon Health Sciences Univ., Portland, OR 97201.

The nuclear matrix protein network is thought to be involved in the control and regulation of DNA replication and gene expression. One of these proteins, matrin 3, was recently cloned from a rat insuloma cDNA library (Belgrader et al. 1991, JBC, 266:9893). Here we report the presence of unique forms of matrin 3 in bovine testis. We have isolated 15 cDNA clones from bovine testis that encode unique and distinct forms of this protein. Sequence analysis showed 90% homology at the amino acid level with matrin 3. However, a significant difference in the testis form was found in the region containing the putative nuclear location signal (KKKLKK). In the testis, this signal sequence is embedded in an unique 53 amino acid segment with no homology to matrin 3 suggesting alternative splicing or an alternate exon in the testis. The cDNA inserts of the 15 clones could be categorized into at least 5 distinct classes based on their unique 3' untranslated regions and at least 3 distinct classes based on restriction site analysis. Northern blot analysis detects high levels of expression of a ~4 kb message in the testis and brain. *In situ* hybridization localized high levels of expression of these mRNAs in testis interstitial cells and the brain hippocampus, suggesting specific differentiation or hormone dependent gene expression in these regions.

SYNAPTIC STRUCTURE AND FUNCTION I

563.1

COMPARISON OF DIVALENT CATION BINDING TO BRAIN AND ERYTHROCYTE SPECTRINS. J. A. Babitch*, C. J. Wallis and E. F. Wenegieme. Chemistry Dept., Texas Christian Univ., Fort Worth, TX 76129.

Previously we examined calcium binding to brain spectrin [J. Biol. Chem. 267 (1992) 4333]. Here we report differences in divalent cation binding between brain and erythrocyte spectrins which appear to relate to differences in spectrin function in these two tissues. Flow dialysis and equilibrium dialysis of erythrocyte spectrin revealed two binding components: high affinity, calcium-specific sites with $K_d = 4x10^{-7}$M and n = 100±20 per dimer and a low affinity (millimolar) divalent cation component. The entropy increase upon binding suggests that calcium stabilizes the native conformation of repeat structures. These data support the hypothesis that calcium binding to red blood cell spectrin has become specialized to stabilize the cytoskeletal network and the cell under the stressful conditions of blood circulation, whereas brain spectrin, having fewer high affinity sites, responds to fluctuating calcium levels by altering its interactions with other proteins. Supported by NIH (NS-26518).

563.2

NEURAL DEPENDENCE AND INDEPENDENCE OF THE EXPRESSION OF A NMJ-ASSOCIATED ANTIGEN. Stephanie H. Astrow*, Young Jin Son and Wesley J. Thompson, Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

Our lab has generated a monoclonal antibody (mAb 3G2) that recognizes a subsarcolemmal, Z-disc-associated component of neuromuscular and myotendinous junctions in adult rats. On immunoblots, mAb 3G2 reacts with a relatively insoluble protein of 41 kD. To examine the neural regulation of synaptic localization, we have induced novel neuromuscular junctions in adult muscles. The soleus muscle was cross-innervated by implanting the deep branch of the peroneal nerve onto the muscle surface. Two weeks later, the muscle was denervated by tibial nerve resection. This procedure resulted in the formation of novel, ectopic neuromuscular junctions (as revealed by α-bungarotoxin labeling) at the site of implantation of the foreign nerve, 2-3 weeks after denervation. Ectopic junctions were immunoreactive with mAb 3G2. Thus, the nerve is able to induce expression of the mAb 3G2 epitope at synapses in adult animals. This result further suggests that the effects of innervation on the accumulation of mAb 3G2 immunoreactivity are localized to the site of contact. In contrast, innervation-independent mAb 3G2 immunoreactivity was observed in myotubes formed *in vitro* from rat cell lines and primary cultures. In the rat myogenic cell line, L6, mAb 3G2 labels a filamentous network in the cytoplasm of differentiated cells, confirming previous observations that the epitope is ubiquitously expressed in nascent muscle fibers. Primary cultures also contain filamentous labeling that is particularly prominent at the ends of well-fused myotubes, reminiscent of the myotendinous labeling observed *in vivo*.

563.3

THE RECEPTOR-ASSOCIATED PROTEIN GEPHYRIN IS A COMPONENT OF MANY SYNAPSES IN THE RAT CENTRAL NERVOUS SYSTEM.

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Gephyrin was originally identified as peripheral membrane protein which is localized at the cytoplasmic face of glycinergic postsynaptic membranes of the rat spinal cord. Recent immunofluorescence studies using two different monoclonal antibodies against gephyrin demonstrate its widespread distribution at supramedullary synapses. Tubulin overlay and co-polymerization studies revealed a high-affinity interaction of gephyrin with microtubules (MTs). From cDNA clones, the primary structure of different gephyrin variants has been deduced; these are distinguished by the presence of different inserts (C1-C4) within the amino-terminal half of the protein. Co-polymerization experiments using *in vitro* translated deletion mutants of gephyrin suggest that the invariant carboxy-terminal region is implicated in the MT interaction. Our data suggest an important role of this polypeptide in postsynaptic receptor topology and architecture.

563.4

OCCURRENCE AND ENRICHMENT OF PROTEIN TYROSINE PHOSPHATASE (PTPase) IN A POSTSYNAPTIC DENSITY (PSD) FRACTION ISOLATED FROM ADULT RAT BRAIN.

X.N. Wang[1,2], K. Wu[1,2*], J.L. Xu[2], Y. Huang[3], T.W. Kim[1,2] and I.B. Black[1,2], [1]Graduate Program in Physiology and Neurobiology, Rutgers-The State University of New Jersey, New Brunswick, N.J. 08854, [2]Dept. of Neuroscience and Cell Biology, UMDNJ/Robert Wood Johnson Medical School, Piscataway, N. J. 08854 and [3]Div. of Neuroscience, NYSPI, New York, N.Y. 10032.

Protein tyrosine phosphorylation plays an important role in the regulation of cell growth and differentiation in developing brain. In addition, recent studies showing high levels of protein tyrosine kinases (PTKs) at synapses suggest that tyrosine phosphorylation may be involved in the modulation of synaptic communication and plasticity. The regulation of tyrosine phosphorylation of substrate proteins is achieved by a balance of the activities of PTKs which phosphorylate and PTPase which dephosphorylates the substrate proteins. So, remarkably, synaptic PTPase remains to be identified and characterized. Since PTKs were found to be enriched in the PSD (a functionally important, disc-shaped proteinaceous structure attached to inner surface of postsynaptic membrane), we examined the existence of PTPase in this structure with Western blot analysis, using a highly specific antibody against a 37-kDa human recombinant T-cell truncated protein tyrosine phosphatase (TCΔC11PTPase). Our results revealed that the antibody specifically recognized a 60 kDa polypeptide in total homogenate (H), synaptic membrane (SM) and postsynaptic density (PSD) isolated from cerebellum (CBL), olfactory bulb (OB) and cerebral cortex (CTX) of adult rat brain. There was at least a 20-fold enrichment of the 60 kDa species in the PSD over H and SM, suggesting that the enzyme may play a role in postsynaptic mechanisms. Moreover, the 60 kDa protein exhibited differential expression in CTX, CBL and OB. We conclude that the PTPase-like 60 kDa protein is a PSD component that may play a regulatory role in synaptic function.

563.5

IMMUNOREACTIVITY OF ß-AMYLOID PROTEIN PRECURSOR (ß-APP) SEQUENCES AT THE POSTSYNAPTIC DOMAIN OF HUMAN NEUROMUSCULAR JUNCTIONS (NMJs).

R.B. Alvarez, V. Askanas, W.K. Engel*. USC Neuromuscular Center, Los Angeles, CA 90017-1969.

The amyloidogenic fragment of ß-APP, ß-amyloid protein (ß-AP) in the brain has received attention for its putative role in the pathogenesis of Alzheimer's disease. Outside the brain, we recently demonstrated pathologic accumulation of ßAP in vacuolated muscle fibers of patients with inclusion-body myositis (Askanas et al., Lancet 339:560-561, 1992). We have now immunolocalized 3 sequences of ßAPP, C-terminal, amino-acids 676-695 (C-ßAPP), N-terminal sequence 45-62 (N-ßAPP) and ßAP sequence, at the NMJs in 16 normal human muscle biopsies (total 200 NMJs), using four well-characterized antibodies. In all biopsies, all the NMJs identified by alpha-bungarotoxin (α-BT) binding had very strong immunoreactivity (IR) for all 3 ßAPP sequences. N-ßAPP-IR compared exactly to co-localized bound α-BT and dystrophin-IR. ß-AP-IR and C-ßAPP-IR extended slightly deeper into the muscle fiber end-plate region than bound α-BT. By immunogold-EM, C-ßAPP-IR was localized in the muscle fiber postsynaptic domain a) in small tufts along the muscle-fiber side of the folds and b) in the form of clusters on small bits of membrane scattered throughout the end-plate region. We suggest ßAPP may have an important role in normal junctional biology, and possibly in some diseases affecting NMJs. (We are grateful to GG Glenner, DJ Selkoe, B Frangione and D Levartovsky for generous gifts of antibodies.)

563.6

A RAT POSTSYNAPTIC DENSITY PROTEIN, PSD-95, IS A HOMOLOGUE OF THE DROSOPHILA DISCS-LARGE PROTEIN AND CONTAINS A GUANYLATE KINASE DOMAIN.

K.-O. Cho, C. A. Hunt, J. L. Rawlings and M. B. Kennedy*. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

To understand the structure and function of the postsynaptic density (PSD), we set out to study biochemical and molecular properties of several proteins in the PSD fraction from rat brain. We isolated cDNA clones encoding a protein of apparent M_S 95 kDal that is tightly associated with the PSD by screening a rat brain cDNA library with DNA probes designed from tryptic peptide sequences of the protein. The cDNAs encode a protein of 724 residues with a MW of 80,465. The deduced PSD-95 protein sequence has high similarity to the *Drosophila* tumor suppressor protein, lethal (1)discs-large-1 (*dlg*), and lower similarity to human erythrocyte protein p55. All three proteins contain an SH3 domain and a guanylate kinase domain that can catalyze synthesis of GDP from GMP. PSD-95 and *dlg* also contain three internal repeats of a motif termed "GLGF repeats". In Drosophila imaginal discs, the *dlg* protein is associated with septate junctions. Immunoblots with a polyclonal antiserum generated against bacterially expressed PSD-95 show that it is enriched in PSD fractions. The same antibody stains dendrites of neurons in the cortex and hippocampus of rat brain. PSD-95 mRNA was only detected in brain, suggesting that PSD-95 is a brain-specific guanylate kinase. The phenotype of Drosophila *dlg* mutants suggests that GDP synthesis by these proteins may be regulated by external signals and that the locally regulated GDP:GTP ratio in turn regulates G proteins such as ras.

563.7

DISTRIBUTION OF THE POSTSYNAPTIC DENSITY PROTEIN, PSD-95, IN RAT BRAIN.

C.A. Hunt*, K.-O. Cho, and M.B. Kennedy. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

This laboratory has recently cloned a cDNA encoding a prominent protein in rat postsynaptic density fractions, of apparent molecular weight 95Kd, termed PSD-95. It is highly similar to the Drosophila lethal(1)disc-large-1 (*dlg*) tumor suppressor protein, which is associated with septate junctions in developing flies. Confocal immunofluorecense and peroxidase immunohistochemistry with an affinity-purified rabbit polyclonal antiserum to PSD-95 have shown that the protein is present in neurons in many regions of rat brain. Thionin-counterstaining of 15μ immunostained sections suggests that not all neurons contain PSD-95. In hippocampus, dentate granule cells and pyramidal neurons in CA1 and CA3 are stained, as well as mossy fibers in CA3, while most interneurons appear unstained. In neocortex, PSD-95-positive neurons are present throughout laminae II-III and V-VI; staining is particularly intense in layer V pyramidal neurons. Fewer immunoreactive neurons are present in layer IV. In both hippocampus and neocortex, PSD-95 immunoreactivity is absent from cell nuclei and is more intense in dendrites than in somata. Staining is not homogeneous in dendrites, but is concentrated in small discrete spots, consistent with the possibility that PSD-95 is highly concentrated in postsynaptic densities. In cerebellum, PSD-95 does not stain Purkinje cell somata, but is present in somata and dendrites of stellate cells in the molecular layer. In addition, immunostaining is very intense in a plexus surrounding each Purkinje cell and concentrated at its basal end, suggesting that basket cell axons synapsing onto Purkinje cells contain high levels of PSD-95.

563.8

PRESYNAPTIC CONTRIBUTION OF A 130 kDA PROTEIN TO POSTSYNAPTIC DENSITY PREPARATIONS.

J.A. Garner*, USC School of Medicine, Los Angeles, CA 90033

The morphological correlate of vertebrate CNS synapses, synaptosomes, can be enriched by subjecting homogenized brain tissue to sucrose gradient density fractionation. The isolated synaptosomes contain most pre- and postsynaptic features of synapses, and release neurotransmitter in a calcium-dependent fashion. A subcomponent of the synapse, which morphologically appears to be the postsynaptic density, can be further enriched by detergent extraction of synaptosomes. Newly synthesized radioactive proteins in guinea pig retinal ganglion cells were axonally transported as slow component b (SCb) to their terminals. Synaptosomes were prepared from the radiolabeled terminal regions, and subjected to the detergent fractionation scheme developed for enriching the postsynaptic density. A single labeled (presynaptic) protein at ~130 kDa, was found in the fraction enriched for postsynaptic density structures. Whether this protein is actually incorporated within the postsynaptic structure, or is a presynaptic remnant of the synaptic cleft peripherally associated with the postsynaptic density is not yet clear. Triton-x-114 extraction of transported proteins in the presynaptic axons reveals approximately equal distribution of this protein in the aqueous and detergent phases. Once the protein reaches the terminal regions, it is found almost exclusively in the detergent or hydrophobic phase.

563.9

EXCLUSIVELY NEURONAL LOCALIZATION OF THE SYNAPSE PROTEIN SNAP IN *DROSOPHILA MELANOGASTER*.

C. Risinger*, V. A. Pieribone#, D. Nässel§, A. Lambertsson¶, L. Brodin#, and D. Larhammar. Dept of Medical Genetics, Box 589, Uppsala University, S-751 23 Uppsala, Sweden. #Nobel Inst. f. Neurophysiolgy, KI, Stockholm, Sweden. §Dept. of Zool., Stockholm Univ., Sweden. ¶Dept. of Biology, Oslo Univ., Norway.

SNAP (synaptosome-associated protein) is a 25-kDa protein which is expressed exclusively by neurons in mammals. It is localized to presynaptic nerve terminals and it is associated with the inner side of the cell membrane in presynaptic nerve terminals. Its expression starts at the time of synaptogenesis.

We have previously shown that SNAP is an extremely well-conserved protein which implies important functions. The chicken and mouse SNAP proteins are identical throughout the 206 amino acids (S. Catsicas et al., PNAS 88, 785-789, 1991), and SNAP of goldfish, ray *(Torpedo marmorata)*, river lamprey, and *Drosophila melanogaster* show extensive similarity to the mouse protein (Abstract 154.14, NM 1990). The *Drosophila* SNAP gene has a complex structure with eight exons spanning more than 65 kbp (Abstract 458.6, NM 1991).

We have now started to explore the anatomical and temporal aspects of SNAP expression in *Drosophila*. Northern blot analysis reveals a single mRNA of approximately 2.5 kbp in both larvae and adult flies. In situ hybridization to *Drosophila* tissue sections gives specific labelling to neuronal cell bodies in the brain and in the ventral ganglion. No specific binding to the intestine or the muscles was observed.

Thus, SNAP is a highly conserved protein exclusively expressed by neurons in *Drosophila* . The large size of the gene may indicate an important role in the development of the nervous system.

563.10

EXPRESSION AND GENETIC ANALYSIS OF *DROSOPHILA* SYNAPTOTAGMIN.

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Synaptotagmin, an integral membrane protein of synaptic vesicles, is well conserved from invertebrate through vertebrate evolution and has been suggested to play a role in synaptic vesicle docking and fusion. To investigate synaptotagmin function further, we have examined synaptotagmin's developmental expression in *Drosophila* and have initiated a genetic analysis of the gene. Low stringency screens to identify additional synaptotagmin cDNAs derived from *Drosophila* embryos failed to isolate isoforms differing from the adult message, suggesting the presence of a single synaptotagmin gene. Whole mount *in situ* hybridization of *Drosophila* embryos indicate that synaptotagmin transcription begins in the central nervous system during stage 13, and in the peripheral nervous system during stage 15. Synaptotagmin message appears in all neurons of the PNS, and in most, if not all, CNS neurons. Antibodies prepared to bacterially produced recombinant *Drosophila* synaptotagmin and to an amino-terminal synaptotagmin peptide, recognize a 70 kD protein on western blots of crude *Drosophila* synaptic vesicles. Antibody staining of embryos indicate that protein expression begins in the CNS and PNS during stages 14 and 16, respectively. Antibodies to synaptotagmin strongly label the two longitudinal tracts in the ventral nerve cord and brain, as well as many other areas of the CNS. In addition, the antibody recognizes many peripheral axons, with intense staining localized to the neuromuscular junction. We have localized the synaptotagmin gene to the second chromosome at 23B-C, and have identified a deficiency (23A1-3-23C) to which synaptotagmin maps. Embryos or first instar larva with this deficiency are homozygous lethal and are the subject of further work to define the consequences of loss of synapotagmin expression on neurotransmission. These data indicate that synaptotagmin expression in *Drosophila* is widespread in both the peripheral and central nervous systems and correlates to a period of development when synaptic activity begins to occur.

563.11

SYNAPTIC VESICLE PROTEINS IN DROSOPHILA.

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We are undertaking a genetic analysis in Drosophila to understand the function of several synaptic vesicle proteins. Drosophila has homologues of at least three mammalian vesicle proteins, p65 (synaptotagmin) [Perin *et al.*, JBC 266, 615, 1991], Rab3a [Johnston *et al.*, Neuron 7, 101, 1991], and VAMP (synaptobrevin) [Sudhof *et al.*, Neuron 2, 1475, 1989]. We have recently identified a novel Drosophila VAMP. This VAMP as well as the p65 and Rab3A homologues are localized to the nervous system as shown by Northern analysis and *in situ* localization. The previously published Drosophila VAMP is not localized to the nervous system [Chin *et al.*, mns. in prep.] In addition, immunocytochemistry with a rat serum raised to rat p65 [gift of Scheller lab] demonstrates that the Drosophila p65 protein is localized to the neuropil. These data suggest that these proteins' functions may have been conserved from Drosophila to mammals.

To study the function of these proteins we are in the process of making mutations in each. Large chromosome deficiencies have been obtained that remove each gene. We are saturating these regions for lethal complimentation groups by EMS mutagenesis. The study of the mutant phenotype for each gene should allow an analysis of the function of each protein and a starting point for a search for interacting genes.

563.12

STRUCTURAL COMPOSITION OF SYNAPTIC VESICLE PROTEOGLYCAN.

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Proteoglycan constitutes about 25% of the protein in cholinergic synaptic vesicles obtained from the electric organ of Torpedo. In order to study domain structure, the proteoglycan (PG) was purified and subjected to partial degradations. Western blot analysis indicates that anti-SV1 and anti-SV2 recognize carbohydrate epitopes of PG that are removed by keratanase digestion. Additionally, chemical deglycosylation of PG with HF resulted in a complete loss of PG immunoreactivity. Incubation of PG with other glycosaminoglycan degrading enzymes revealed a heterogeneous composition of PG sugars. PG treated with HF or trifluoromethanesulfonic acid generated 62 kDal and 5 kDal polypeptides. The 5 kDal polypeptide is susceptible to proteinase K but not trypsin. The complex nature of PG demonstrated by these experiments suggests that the protein subunits are assembled post-translationally or derived from a larger precursor.

563.13

SYNAPTIC VESICLES IN GLUTAMATE, GABA AND GLYCINE-IMMUNORECTIVE SYNAPSES IN THE LAMPREY SPINAL CORD HAVE DISTINCT MORPOLOGICAL CHARACTERISTICS

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The lamprey spinal cord is particularly useful to study the regional distribution of amino acids in synapses, as synaptic vesicle clusters and axoplasmic compartments in most axons can be anatomically separated. The ultrastructural distribution of glutamate, aspartate, GABA and glycine immunoreactivity was analysed in spinal cords fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, using the immunogold postembedding technique. An accumulation of immunoreactivity over clusters of synaptic vesicles was revealed for GABA, glutamate and glycine in the respective immunoreactive axons, but not for aspartate. Hence, only the former three amino acids can be assumed to act as neurotransmitters. The labelled synaptic junctions displayed distinct morphological characteristics. Axons with GABA-ir vesicle clusters contained spherical to pleomorphic vesicles, numerous glycogen granules and some dense core vesicles, while glycine-ir vesicle clusters contained flattened vesicles. Axons with glutamate-ir vesicle clusters contained spherical vesicles. Glutamate-ir axons formed asymmetrical synapses, which often contained gap junctions. Glycine-ir synapses were symmetrical, while GABA-ir terminals established both symmetrical and asymmetrical synaptic junctions.

The results emphasize that axons utilizing different amino acids as neurotransmitters can have distinct ultrastructural features in glutaraldehyde fixed tissue. The distinct shape of vesicles in these axons will significantly simplify the structural analysis of neuronal circuits in the lamprey spinal cord.

563.14

SYNAPTIC ULTRASTRUCTURE ON TRIGEMINAL MESENCEPHALIC NEURONS

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Sensory endings of masticatory muscle spindles and of many periodontal ligament receptors are innervated by central neurons located in the mesencephalic nucleus of the trigeminal nerve (Vmes). Although these are primary afferent neurons, they possess several features not generally recognized in peripheral sensory ganglia. Synaptic boutons on Vmes somata were demonstrated by Hinrichsen (1968). The purpose of this study was to compare the morphology of boutons on Vmes spindle and periodontal afferent neurons in cats (n=3) and rats (n=6). Peripheral injections of horseradish peroxidase (HRP; Sigma VI) were made into the mandibular periodontal ligaments or the masseter muscle. Periodontal and spindle afferent somata were identified by the presence of reaction product following diaminobenzidine (DAB) histochemistry for demonstration of transported HRP. Labeled Vmes neurons were prepared for and examined with transmission electron microscopy. One to three boutons were observed along the perimeter of most labeled cells in both species. There were no apparent differences related to the peripheral structures innervated nor between species. Most of the boutons contained a relatively dense accumulation of spherical synaptic vesicles although boutons with a few dense-cored vesicles were also observed. Pre- and postsynaptic densities, indicative of synaptic contact, were not always obvious, but they did occur and examination of serial sections would probably reveal such specializations at most boutons. Although the synaptic density along Vmes neurons is relatively low, and these boutons are likely to originate from several sources, some of these contacts may account for the inhibition of Vmes spindle afferent activity that has been reported following electrical stimulation of the ventral diencephalon (Dessem et al., 1991). Supported by DE06027 and DE10132.

563.15

MEMBRANE CYCLING AND ENDOSOMES IN CELL BODIES AND TERMINALS OF FROG RETINAL PHOTORECEPTORS.
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Membrane cycling between cell surfaces and intracellular compartments in neuronal cell bodies is poorly understood. We find that Brefeldin A does not prevent access of endocytosed Wheat Germ Agglutinin to trans-Golgi related systems, probably reflecting interactions of the persistent trans-Golgi elements with endosomes. We also find that some of the endocytic structures that form rapidly in the cell bodies contain synaptophysin, strengthening our suspicion that cycling of synaptophysin via endosomes and Golgi structures in the cell bodies coexists with recycling in the presynaptic terminals.

Vacuolation by weak bases is one line of evidence for participation of endosomes in recycling in terminals (J. Neurocytol. 18:529). We find that weak bases which are only weakly vacuologenic by themselves can engender prominent vacuolation when administered with elevated K gluconate, which also is only mildly vacuologenic. This synergy supports our view that the terminals contain acidified endocytic compartments comparable to endosomes of other cell types.

NEI 03168; NS07258; Am. Psych. Assn.; Danforth.

SYNAPTIC STRUCTURE AND FUNCTION II

564.1

SEGREGATION OF CHEMICALLY-DEFINED VARICOSITIES UPON PARTICULAR DENDRITES OF A NEURON: A NOVEL MORPHOLOGICAL BASIS FOR NEURONAL MODULATION?
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The segregation of chemically-defined synaptic inputs to neurons based upon their proximity to the cell soma has been examined in several studies. However, we are unaware of any reports documenting the segregation of such inputs on particular dendrites of a cell. Here we report selective substance P (SP) innervation of portions of the dendritic tree of neurons in the periaqueductal gray (PAG). Retrogradely labeled PAG neurons were injected iontophoretically with Lucifer yellow (LY), and SP immunoreactivity was detected in the same sections using fluorescence immunocytochemistry. Separate series of optical sections of injected cells and of SP-immunoreactive (SP-IR) varicosities were produced using confocal laser microscopy. Individual, coplanar images from each series were merged and the site of apposition of each SP-IR varicosity was marked on LY-filled neuronal structures. LY images were them merged to produce a projection of the entire neuron. Some cells and the corresponding series of SP images were also reconstructed into stereo pairs, merged, and appositions identified independently on the three dimensional images. Among the 10 cells thus far studied in detail, three have exhibited SP-IR appositions that appear to be preferentially associated with one or more dendrites. Some dendrites of each cell exhibited a high innervation density ($\bar{x}$=5.7 appositions/100μm), while other dendrites exhibited no SP innervation or low innervation density ($\bar{x}$=1.4 appositions/100 μm). These results suggest that chemically-defined inputs to neurons may be spatially segregated by selectively contacting certain dendrites of an individual cell. This pattern may be of functional significance in providing a morphological basis for differential modulation of cellular activity. Alternatively, it may represent the most efficient configuration for providing multiple contacts with adjacent cells.
Supported by DA06687, DE06682 and DC10806.

564.2

AUDITORY IMPRINTING LEADS TO NEURON SPECIFIC ULTRASTRUCTURAL CHANGES IN THE MNH OF CHICKS
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In the rostral forebrain of chicks an area called medio-lateral Neostriatum and Hyperstriatum ventrale (MNH) is relevant for auditory filial imprinting. Wallhäußer and Scheich (Dev. Brain Res.31; 29-44, 1987) characterized 3 types of Golgi-impregnated neurons and noticed a large reduction (47 %) of spines for the largest neurons (type I) after successful imprinting. These events on the postsynaptic side suggest a synaptic selection process but events on the presynaptic side, which could support this hypothesis, remained to be determined. In order to resolve this issue a Golgi-technique for combined light- and electron microscopic studies was applied. The Golgi-Colonnier method was modified for chicks and LM-identified isolated type I-neurons were prepared with a gold-toning procedure for EM. In series of more than 100 sections it was possible to identify and reconstruct spines. With an interactive image analyzing computer system which was adjusted to the electron microscope we investigated several ultrastructural parameters. It was found that all spines of type I-neurons from isolated control chicks carried immature-like synapses. All spines on type I-neurons of imprinted chicks of the same age also carried synapses but exhibited larger presynaptic areas with more vesicles, longer postsynaptic densities and frequently with one mitochondrium. For the type II- neurons no significant subsynaptic changes were demonstrated. Consequently, there exists a correlation between the loss of spines and the loss of synapses during imprinting. Moreover, there is no numerical shift from spine synapses to shaft synapses during imprinting. The ratio for control chicks came to 3:1 and for imprinted chicks it was 2,4:1. This implies that even shaft synapses are lost during the synaptic selection process.
Supported by DFG, SPP-Sche 132, 13/2

564.3

CONFOCAL IMAGING OF THORNY EXCRESCENCES ON HIPPOCAMPAL PYRAMIDAL NEURONS.
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The function of dendritic spines is not known, although spines have long been proposed to participate in mechanisms of long-lasting forms of synaptic plasticity, such as long-term potentiation (LTP). The mossy fibers synapse onto proximal dendritic spines of CA3 pyramidal neurons, referred to as thorny excrescences or thorns, that are perhaps the largest in the mammalian CNS. Their large size and close proximity to the soma makes this synapse ideal for studying the morphology and biophysical function of a living dendritic spine.

We have used the high-spatial resolution of confocal laser scanning microscopy (LSCM) to image mossy fiber thorns on CA3 pyramidal neurons within thick (400 μm) rat hippocampal slices. Droplets of DiI-oil solution (Fine et al., *Soc. Neurosci. Abstr.*, 17, 1991) were placed within stratum pyramidale or stratum oriens of area CA3. Following 30-60 minutes, fluorescence was detected at low-power in pyramidal neurons within the vicinity of the oil droplets. Mossy fiber thorns were then visualized using a long-working distance (500 μm) 63X objective (NA=1.25).

Large dendritic spines (1-3 μm) were identified on the proximal apical dendrites corresponding to stratum lucidum. On some cells, spines were also observed on the proximal basal dendrites. These structures clearly originated and were readily distinguished from the dendritic shaft. We are currently using LSCM to measure directly changes in $[Ca]_i$ within thorny excrescences using long-wavelength Ca-sensitive dyes. *Supported by NIH and ONR.*

564.4

VISUALIZATION OF HIPPOCAMPAL MOSSY FIBER SYNAPSES IN LIVING BRAIN SLICES.
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Granule cells of the dentate gyrus send their mossy-fiber (mf) axons to the CA3 region, where they make synapses onto the proximal dendrites of the pyramidal neurons. The electrotonic proximity of the mf synapses to the cell soma (Siegel et al, *Soc. Neurosci. Abstr. 18*, in press) makes them ideal for voltage-clamp analysis of synaptic microphysiology and use-dependent plasticity (Xiang et al, *Soc. Neurosci. Abstr. 18*, in press; Greenwood et al, *Soc. Neurosci. Abstr. 18*, in press).

We have been interested in microscopic techniques that enable good visualization of the mf synapses in living brain slices (Keenan et al, *Soc. Neurosci. Abstr. 16*, 660, 1990). Here we describe progress using laser scanning confocal microscopy (LSCM) applied to relatively thick (400 - 450 μm) rat hippocampal slices, which were cut perpendicular to the long axis of the hippocampus or else at 15 - 30° from the perpendicular. Slices were attached to a glass coverslip that forms the bottom of our recording/observation chamber (Keenan et al, *Brain Res. Bull. 21*, 373, 1988). Dyes that we explored included dil or diA, FM1-43, and Fluo-3. Images were obtained with a Bio-Rad MRC 600 LSCM system using a long working distance (500 μm) 63X objective (NA = 1.25).

The presynaptic mf boutons, which could be clearly identified after staining with dil or diA, exhibited a trimodal size distribution. When the dye was injected into the stratum granulosum and the slices were sliced perpendicularly, almost all of the stained mf axons were cut before reaching region CA3. Fewer mf axons were cut in the non-perpendicular slices. Using FM1-43, we are currently attempting to visualize vesicle recycling, as previously observed in the neuromuscular junction (Betz and Bewick, *Science 255*, 133, 1992). *Supported by NIH and ONR.*

564.5

SYNAPTIC ENHANCEMENT BY LOCAL PRESYNAPTIC STIMULATION AFFECTS THE SHORT-TERM PLASTICITY OF INDIVIDUAL RETICULOSPINAL EPSPs DIFFERENTLY

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The short-term plasticity of mixed electrochemical EPSPs evoked in lamprey spinal neurons by single giant reticulospinal nerve cells was studied using paired-pulse stimulation (interval 65 ms). During stimulation of cell bodies the amplitude of the second chemical EPSP (kainate/AMPA-mediated, AP-5 present) was always larger than the first EPSP, i.e. facilitation predominated over depression, while the electrical component was unaltered. The degree of facilitation of the chemical EPSP varied considerably in different spinal neurons, from a slight increase to more than a two-fold increase, even among EPSPs evoked by the same presynaptic neuron. When axons were impaled and stimulated close to the synaptic area (about 100-500 μm), the postsynaptic response was found to depend strongly on the stimulation parameters. The amplitude of the chemical EPSP increased if the depolarizing current pulse used to trigger action potentials was increased, or if the distance between the stimulating electrode and the synapse was decreased. This synaptic enhancement could alter the EPSP plasticity, such that less paired-pulse facilitation occurred. In many cases the second EPSP even had a lower amplitude than the first, i.e. depression predominated over facilitation, while both electrical components had the same amplitude. However, an enhancement of the EPSP did not always alter the EPSP plasticity. In some cases a pronounced facilitation remained, albeit the amplitude of the first EPSP was markedly increased. These data imply that individual reticulospinal synapses have different plasticity properties which, at least in part, depend on mechanisms linked to the presynaptic element.

564.6

SIZE AND NUMBER OF ACTIVE ZONES IN MAUTHNER CELL INHIBITORY AFFERENTS INCREASE FROM SOMA TO DENDRITE.

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Morphological characteristics of inhibitory active zones were analysed at the level of Mauthner cell's (M-cell) axon-cap, soma, and the medial and distal parts of the lateral dendrite (150-200 and 350-450 μm away from axon-cap, respectively). For this purpose, presynaptic grids were stained with ethanolic phosphotungstic acid (EPTA). Both glycine and GABA were visualized by post-embedding immunogold labeling on semi-thin (0.5 μm) sections. In the axon-cap and soma, the afferent boutons have small sized glycinergic presynaptic grids with respective areas of 0.066 ± 0.02 μm^2, (mean ± S.D., n = 30), and 0.075 ± 0.03 μm^2, (n = 46). 96% of these terminals in the axon-cap and 82% of these on the soma have only one active zone; the rest are mainly boutons with two grids. On the dendrite, both glycinergic and gabaergic dendritic afferents have larger release sites. Specifically, the surface of each active zone is increased to 0.135 ± 0.08 μm^2, (n = 113; GABA), 0.141 ± 0.08 μm^2, (n = 148; glycine) on the middle portion and 0.139 ± 0.08 μm^2, (n = 125; GABA), 0.147 ± 0.1 μm^2, (n = 115; glycine), on the distal dendrite. These values are similar for both gabaergic and glycinergic boutons, and are significantly different from somatic ones (Student t tests). Furthemore, the proportion of profiles displaying two (31% for GABA; 30% for glycine) and three to four (4% for GABA; 7% for glycine) active zones, is greater on the dendrite than on the soma. These results suggest that the probability of transmitter release is higher at dendritic inhibitory afferents than at somatic ones. Such could also be the case for the size of quanta when occurring at more widespread postsynaptic receptor aggregates.

564.7

BACKFIRING AT MIXED SYNAPSES ON THE MAUTHNER (M) CELL.

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Single eighth nerve afferents terminate on the M-cell's lateral dendrite as large myelinated club endings which have both gap junctions and chemical synapses with the cell. Low threshold extracellular stimulation of the posterior branch of the eighth nerve produces a mixed excitatory post-synaptic potential (VIIIth EPSP) consisting of a fast electrotonic potential, followed by a chemical glutamatergic component. Intracellular recordings of these afferents were obtained, alone or while simultaneously recording from the M-cell. Membrane potential ranged from -65 to -73 mV. Coupling potentials can be recorded from these afferents either when the M-cell is activated antidromically by stimulation of its axon in the spinal cord or when an VIIIth stimulus is subthreshold for the impaled axon. In the latter case, an attenuated composite VIIIth nerve evoked EPSP is recorded in the afferent. Backfiring of these afferent fibers was obtained in a number of situations: i), following stimulation of the VIIIth nerve at a strength subthreshold for the recorded axon, ii), pairing a weaker VIIIth nerve stimulus with antidromic activation of the M-cell, and iii), pairing the antidromic stimulus with a presynaptic depolarizing current. We also found that the coupling potentials recorded from a fiber are voltage dependent, increasing with fiber depolarization and decreasing with hyperpolarization. Current/voltage relationships obtained from the fibers suggest that both membrane and junctional properties may be involved. Backfiring associated with physiological activation of eighth nerve afferents could serve as a positive feedback, recruiting additional fibers and enhancing the EPSP in the M-cell. It could also impart a significant non-linearity to the input-output relationship of the population response.

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564.8

TEMPORAL INTERACTION OF SYNAPTIC INPUTS IN VAGAL MOTONEURONS.

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Temporal synaptic interactions in motoneurons from the dorsal motor vagal nucleus (DMVN) of guinea pigs were studied in brain stem slices. The submerged slice technique and conventional intracellular recordings were used in this study. Synaptic responses were elicited by stimulating non-specific presynaptic axons at the nucleus surroundings (peri-vagal stimulus, Yarom et al. *Neurosciense* 16:4, 719-737, 1985). The temporal interactions were studied by analyzing the responses to trains of stimuli at various inter-train intervals that where delivered at different frequencies. At inter-train intervals longer than 10 msec and a frequency of 0.2 Hz, the peaks of all synaptic potentials in a train tend to reach the same level. Summation of synaptic potentials occurs only at shorter inter-train interval. The decay phase of the response to a train of stimuli (in most cells) is governed by two time constants (20msec, 100msec). Both are slower then the passive membrane time constant (τ_0=10msec). This finding indicates that the train of stimuli activates a long lasting conductance change. Trains of stimuli, delivered at frequencies of 0.25 Hz or higher, induced synaptic potentiation that lasts for 30 sec. Ten trains of 5 stimuli each, elicited at a frequency of 0.25Hz, were sufficient to double the amplitude of the synaptic potential but did not change the pattern of the temporal summation. A detailed model of DMVN motoneurons (Nitzan et al. *J. Neurophysiol.* 63: 333-346, 1990) was used to study temporal interaction in these cells, assuming passive dendrites. Using this model we estimated the non-linearity of synaptic interactions in these motoneurons by comparing the temporal summation as preformed by the actual cell and that expected in a passive model. The results suggest that a bursting input to DMVN motoneurons is more effective than a single input only if the bursting occurs at a frequency around 0.2 Hz, where the response is potentiated.

564.9

MINIATURE EXCITATORY SYNAPTIC CURRENTS (mEPSCs) IN MOTONEURONS (MN) OF ORGANOTYPIC RAT SPINAL CORD DORSAL ROOT GANGLION COCULTURE.

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In order to study the quantal events at central synapses mEPSCs were recorded from voltage clamped MNs in whole cell configuration after bath application of 3 μM Tetrodotoxin, 3 μM Strychnine, 10 μM Bicuculline and 100 μM D-2-Amino-5-phosphonopentanoic acid (D-APV) in 2mM Ca^{++}. Cells were identified morphologically and immunohistochemically with a monoclonal antibody against Choline acetyl-transferase. The mEPSCs were reversible blocked with 10 μM of the competitive non-NMDA receptor antagonist 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX). A detection algorithm selected individual currents from continuous recordings sampled at 10 kHz. Most mEPSCs could be fitted by the sum of two exponentials of which amplitude, halfwidth and risetime were calculated. Amplitude histograms were skewed towards larger events. Individual peaks were not unambiguously resolvable. The mean of the modes was -19 pA (SD=7.7 pA, n=8) with a maximal range of -4 pA to -160 pA. Amplitude distributions compiled from currents with identical risetime or from the whole population were similar. We conclude that the broad range of mEPSCs amplitudes cannot be explained by electrotonic attenuation of currents from different dendritic locations. (SNF 31-27553.89)

564.10

MODULATION OF SPONTANEOUS EPSPS IN RAT MOSSY CELLS.

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We have carried out a series of experiments to characterize spontaneous postsynaptic potentials (PSPs) in rat mossy cells and their modulation by intracellular depolarization. Spontaneous PSPs appear to be almost exclusively excitatory since they are blocked by the non-NMDA receptor antagonist, CNQX (10-50 uM) and alterations of the membrane potential consistently fail to reveal hyperpolarizing synaptic potentials. Previous studies suggested that at least some spontaneous EPSPs may be due to active granule cell terminals, discharging independently of the usually hyperpolarized soma. In four mossy cells, we observed that bath application of TTX (1 uM) dramatically reduced (but did not abolish completely) the frequency of spontaneous EPSPs, suggesting that granule cell axons and/or terminals can generate sodium spikes spontaneously. However, unlike in hippocampal pyramidal cells, TTX-resistant EPSPs in mossy cells were quite large (3-4 mV), of a magnitude similar to that observed before treatment (up to 10-15 mV).

We also have obtained evidence that spontaneous EPSPs can be modulated by depolarization of the postsynaptic neuron. We previously demonstrated that treatments which result in depolarization of mossy cells, including direct current injection into a single neuron, can potentiate both the amplitude and frequency of spontaneous EPSPs for prolonged periods. We now report that intracellular injection of the calcium chelator, BAPTA, significantly deceases the basal level of spontaneous EPSPs, and that strong (1-2 nA) depolarizing current pulses (similar to those which potentiated spontaneous EPSPs) evoke calcium spikes. These data support a role for postsynaptic calcium levels in the modulation of spontaneous EPSPs in mossy cells.

Supported by NIH grants NS20482 and NS07097.

564.11

LOCAL CIRCUIT, SINGLE AXON EXCITATORY POSTSYNAPTIC POTENTIALS (EPSPs) IN DEEP LAYER NEOCORTICAL PYRAMIDAL NEURONES.

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Previous studies of single axon pyramidal-pyramidal connections in neocortex concentrated on layers II/III and IV. EPSPs exhibited a relatively rapid time course, despite an unconventional voltage relation and frequency-dependent potentiation with postsynaptic depolarization beyond -70mV and limited fluctuations in amplitude. The depression in average amplitude with increases in presynaptic firing rate or with paired pulse depression, were consistent with a decrease in presynaptic release probability. The present study aimed to determine whether the properties of connections between deeper pyramidal neurones were similar. Simultaneous intracellular recordings were obtained from pairs of neurones in rat neocortical slices. Pyramidal neurones were identified electrophysiologically and subsequently, morphologically on histological examination. All properties typical of superficial local circuit EPSPs were apparent. The only clear difference, at this stage, was the higher probability with which EPSPs of >1mV average amplitude were encountered in deeper layers. This was approximately 1 in 50, compared with 1 in 100. In addition, the largest events could be 7mV in amplitude.

564.12

NEUROMUSCULAR COMMUNICATION: INFERENCES FROM COMPUTER SIMULATIONS OF MINIATURE ENDPLATE CURRENTS. J.R. Stiles, T.M. Bartol, H.L. Fernandez*, E.E. Salpeter and M.M. Salpeter. Section of Neurobiology & Behavior, Cornell University, Ithaca, NY 14853.

Lizard (*Anolis carolinensis*) intercostal muscle miniature endplate currents (MEPC's) were recorded under 3 conditions: untreated, acetylcholinesterase (AChE) inhibition, and AChE inhibition + ~70% nicotinic acetylcholine receptor (nAChR) binding site blockade. Mean amplitude (A_c), 20-80% rise time (t_r), and e-fold fall time (t_f) for each condition were reproduced (± 5%) by Monte Carlo MEPC computer simulations. Fixed model features included: geometry of 1° cleft and junctional folds; nAChR site density, identical ACh binding sites, and single bi-liganded open state; rate constants for AChE association and hydrolysis. We performed simulations iteratively to determine parameter sets corresponding to different assumed values of $g = \beta/(\alpha + \beta)$, where α and β are rate constants for channel closing and opening, respectively. Each parameter set included: quantal size (N); ACh diffusion coefficient; rate constants for nAChR association (k+) and dissociation (k-); (α+β); AChE site density (σ_e). For each parameter set, we also quantified molecular binding, unbinding, opening and closing events. For $0.8 \leq g < 1$, parameter set values all fell within suggested literature ranges, but nonetheless varied between sets from < 20% (σ_e) to > 20-fold (k-). Based on preliminary MEPC shape analysis, the optimal value for g is ≥ 0.95. With AChE active t_f exceeded mean burst duration by less than 30%, yet single ACh molecules bound nAChR multiple times (e.g. ~2.4 times at g = 0.8, and ~9.0 times at g = 0.975). Clearly, channel opening probability ($\beta/[\beta + 2k_-]$) decreased dramatically as g approached 1, even though MEPC efficiency (A_c/N) increased. Furthermore, ACh rebinding frequency was sensitive to σ_e variation in the range where A_c, t_r, and t_f were little affected. This suggests that trophic ACh/nAChR interactions could be regulated through σ_e without compromising efficacy of electrical signal transduction.

Supported by NIH grants NS09315 (M.M.S.) and 1F32NS09126-01 (J.R.S.). Simulations were conducted at the Cornell National Supercomputer Facility.

564.13

ISOLATED SNAKE NEUROMUSCULAR BOUTONS RELEASE 2-3 QUANTA WHEN ACTIVATED. R.S. Wilkinson* and S.D. Lunin. Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110.

Despite their importance as the anatomical substrate of quantal release, little is known about the function of individual synaptic boutons, in part because axon terminals typically comprise many synchronously active boutons. We have developed a technique which permits dissociation of living boutons from both their motor nerve terminal and their postsynaptic (endplate) site. The individual boutons may be studied either in isolation, which provides access to the presynaptic membrane, or after attachment to vacant endplate sites, for quantal analysis of 'reconstructed' one-bouton NMJs.

Boutons became nearly spherical when micromanipulated from their endplate site with a glass suction pipette. A second pipette (loose patch configuration) was used for stimulation and recording. Boutons remained healthy for >1 hr, as evidenced by uptake of the supravital probes 4-Di-2-ASP (mitochondrial) and sulforhodamine 101 (activity-dependent), and by spontaneous quantal release (MEPPs recorded from reconstructed NMJs). When stimulated, boutons exhibited 4-aminopyridine-sensitive terminal currents similar to those recorded from boutons of intact NMJs. Transmitter release (0.2 Hz stimulus rate) underwent quantal fluctuations about a mean of 2-3 quanta, with occasional failures. Maximum observed quantal content was 7. Serial EM of similar boutons (from intact NMJs) revealed ~6-7 active zones, suggesting that the probability of release was ~0.3-0.4. Supported by NIH grant NS24752 and the MDA.

564.14

HOW MANY VESICLES ARE RELEASED AT A CENTRAL SYNAPSE? N. Hessler*, R. Malinow. Neuroscience Program and Dept. of Physiology and Biophysics, University of Iowa.

We are addressing this question by looking at the AMPA and NMDA components of elicited excitatory postsynaptic currents in hippocampal slices under various release conditions. Under normal conditions we measure the NMDA/AMPA ratio (synaptic amplitude at 40 ms / 5 ms). We apply an APV concentration to produce a 50% decrease in this ratio. After washout of APV, we add an agent to the bath that increases the release of transmitter (Ca^{++}, phorbol esters, adenosine antagonist). We then measure the NMDA/AMPA ratio under increased release conditions. If multiple vesicles are released at a synapse, and NMDA receptors are saturated, then one would expect a decrease in the NMDA/AMPA ratio with increased transmitter release. We then apply APV at a concentration that produced a 50% reduction in the NMDA/AMPA ratio under normal release conditions. If NMDA receptors are saturated we expect less than a 50% reduction in the NMDA/AMPA ratio during increased release conditions. On the other hand, if a single vesicle is released per synapse, then increased release probability will merely recruit more synapses, and the NMDA/AMPA ratio should not change. APV should have the same relative effect in normal as high release conditions.

564.15

TRANSMISSION-DEPENDENT DEPRESSION COEXISTS WITH FACILITATION AT HIPPOCAMPAL EXCITATORY SYNAPSES

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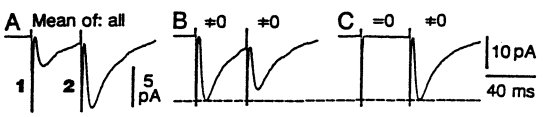
Hippocampal synapses show facilitation, which appears to be presynaptic since it is independent of prior synaptic transmission (Neurosci.Abstr.17, p.1486). Here I report that these synapses also show depression, which is dependent on prior transmission, and usually masked by the facilitation.

CA1 pyramidal cells in slices from young rats were voltage-clamped (whole-cell) at -70 to -85 mV, with 2 mM Ca and 10 µM bicuculline in the bath. Pairs of minimal stimuli (STIM1 and 2; interval 40-60 ms) from a glass pipette in str. radiatum, evoked synaptic currents (EPSC1 and 2) of variable amplitude, alternating with ~50 % clear failures. Normally the average EPSC2 was larger than EPSC1 (**facilitation**) when the failures were included in the average (**A**). In contrast, when all failures were omitted, the mean EPSC2 was smaller (**depression**, in ~20% of the cells; **B**) or equal to EPSC1. Unlike the facilitation, the depression was use-dependent, i.e. it disappeared when EPSC1 failed (**C**). Thus, the mean of EPSC1 (**B**) and the mean of all EPSC2s preceded by a failure (**C**), were equal. The depression may be due to smaller quanta (e.g. receptor desensitization, dissipation of ionic gradients in the spine, or less transmitter per vesicle), or fewer quanta (e.g. depletion of transmitter). The reduced number of failures seems to argue against depletion and for a reduction in quantal size. [Supported by NAVF/RMF]



564.16

QUANTAL ANALYSIS OF EPSCs RECORDED FROM SMALL NUMBERS OF SYNAPSES IN RAT HIPPOCAMPAL CULTURES.

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Current evidence supports the notion of quantal synaptic transmission in central neurons, as revealed by the presence of discrete peaks in histograms of synaptic amplitudes. However, we were unable to resolve such peaks when recording EPSCs from pairs of profusely-connected hippocampal pyramidal neurons in culture (*Nature* 341, p 230, 1989). A possible explanation is that quantal size is variable across synapses in culture, and recording from many synapses obscures the underlying quantal nature of transmission. Accordingly, we attempted to minimize possible synaptic heterogeneity by performing quantal analyses on small numbers of active synapses.

Dual whole-cell recordings were made from pairs of CA1 neurons. The bath solution omitted calcium, to block transmission at the majority of synapses, while a puffer-suction arrangement was used to locally apply bath solution containing 5 mM calcium to short lengths of dendrites. After many sweeps were recorded, a separate puffer was used to apply hypertonic bath solution to the same region, eliciting miniature EPSCs. Finally, Synapsin I antibody was used to count the number of synapses within the activated region.

The synapse count ranged from 5 to 20 in 13 experiments. Peaks were often visible in the amplitude histograms of evoked and miniature EPSCs. The general shapes of evoked histograms were well-fitted by assuming a binomial release process and a quantal size distribution given by the observed distribution of miniature EPSC amplitudes. We conclude that the quantal model is applicable to small numbers of synapses in culture.

564.17

BAYESIAN INFERENCE TECHNIQUES FOR SEPARATION OF SYNAPTIC POTENTIAL COMPONENTS IN HIPPOCAMPUS. D.A. Turner*, M. West and G. Cao. Neurosurgery, Neurobiology and Institute of Statistics and Decision Sciences, Duke University and Durham VAMC, Durham, NC 27710.

Bayesian inference has been applied to the analysis of fluctuations of synaptic potentials. The statistical model assumes the synaptic signal to be composed of a mixture of Gaussian components, with unknown means and noise SD (s_N). Component attributes include arbitrary spacing and probability, representing synaptic sites at varying electrotonic distances with nonuniform amplitudes. The analysis was applied to minimal EPSPs evoked in CA1 hippocampal neurons.

Datasets of peak EPSP values were tested for trends and stationarity and normality of noise data was confirmed (χ^2 test). Analysis output included: 1) component mean±SD values; 2) conditional probability of numbers of components; 3) relative probability of each component; 4) predictive pdf and cdf. A proximal EPSP (n=500, s_N=0.18 mV) analyzed to 3 primary components and sums (0.60 mV/16%, 1.32 mV/50%, 1.71 mV/24%). A distal EPSP (n=500, s_N=0.12) also showed 3 primary components (0.12 mV/22%, 0.21 mV/55%, 0.26 mV/20%) and sums. The predictive pdf/cdf were not different from the raw histogram (χ^2 test: p=0.99). These results were typical for CA1 EPSPs.

The advantages of Bayesian inference over MLE/deconvolution of a mixture distribution include: 1) parameter information can be included in a prior distribution; 2) the conditional probability of the number of components is estimated; 3) the s_N value is only an initial estimate of the component variances; 4) the component parameters include uncertainty estimates; and 5) the predictive distributions include variability. However, component separation remains dependent on the s_N value, to an extent. Further theoretical work will include more accurate noise description and simulation of known distributions with noise perturbations. Supported by NINDS RO1 NS29482-01 and VAMC.

POSTSYNAPTIC MECHANISMS I

565.1

THE EFFECT OF MEMBRANE POTENTIAL ON CALCIUM ACTIVATED POTASSIUM CURRENTS. A. R. Martin* and P. A. Fuchs. Dept. of Physiology, Univ. of Colorado Sch. of Med., Denver, CO 80262.

Previous experiments on chick cochlear hair cells have shown that calcium entering though synaptic channels activates calcium-dependent potassium currents (Fuchs and Murrow, *J. Neurosci.* **12**:800-809, 1992). In voltage clamp experiments the potassium currents increased with membrane depolarization and then decreased, approaching zero at about +20 mV, more than 170 mV negative to the calcium equilibrium potential. To account for this unexpected decrease in potassium current at small positive potentials we present a model in which calcium accumulates in a restricted space under the postsynaptic membrane. A second model is also presented to describe the voltage dependence of extrasynaptic potassium currents activated by calcium entry through voltage sensitive calcium channels in chick hair cells (Fuchs, Nagai and Evans, *J. Neurosci.* **8**:2460-2467, 1988). In both models the marked diminution of the potassium currents at positive membrane potentials can be accounted for by (1) an exponential dependence on membrane potential of calcium entry through open channels and (2) a dependence of the potassium current on the fourth power of the intracellular calcium concentration. With these factors the theoretical calculations provide an accurate description of the previous experimental results. (Supported by NIH Grants NS09660 and DC00276)

565.2

ADENOSINE MODULATES A TRANSIENT OUTWARD CURRENT IN RAT LOCUS COERULEUS NEURONS. W. J. Pan* and S. A. Shefner. Dept. of Physiology and Biophysics, University of Illinois, College of Medicine, Chicago, IL 60612.

We have previously shown that adenosine decreases the duration of the action potential in locus coeruleus (LC) neurons. In the present study, the mechanism by which adenosine affects the shape of the action potential was examined. Intracellular recordings from LC neurons were obtained in a submerged rat brain slice preparation. All drugs were administered by bath application. LC neurons fired spontaneously at rates of 1.0 to 3.5 Hz. Ba^{2+} (1 mM) was administered to prolong the duration of the action potential. Adenosine (100-300 μM) reduced the duration of the Ba^{2+} spike measured at 33% of the peak amplitude by 11.5±2.8% (n=7). In these same cells, administration of 4-aminopyridine (4-AP) (30-100 μM) in addition to Ba^{2+} further increased the duration of the action potential. Administration of adenosine then failed to decrease the duration of the Ba^{2+} and 4-AP enhanced action potential.

An I_A-like transient outward current was investigated using single-electrode voltage clamp in the presence of 0.5 μM TTX and 20 mM Mg^{2+}. Inactivation curves were obtained in 5 cells; I_A was measured following a 1 sec prepulse to a holding potential between -100 and -40 mV, followed by a 200 ms depolarization to between 0 and +10 mV. This outward current was reduced by 30-100 μM 4-AP and blocked by 1 mM 4-AP. On the average, the I_A-like current was 50% inactivated at a holding potential of -60±1.0 mV, and 80% inactivated at a holding potential of -56±0.9 mV, which is very near the threshold for LC neurons. At threshold, adenosine (300 μM) shifted the inactivation curve in a parallel manner in the positive direction by 4.7±0.5 mV, and increased I_A by 132±23%. These data suggest that adenosine decreases the duration of the action potential in LC neurons by potentiating an I_A-like current.

Grant support: PHS AA05846-09 to S.A.S.

565.3

MUSCARINE-SENSITIVE, INWARDLY-RECTIFYING K^+ CONDUCTANCE IN A SYMPATHETIC NEURONE. D.E. Kurenny, H. Chen & P.A. Smith*, Dept. Pharmacol., Univ. Alberta, Edmonton, Canada T6G 2H7.

The inwardly-rectifying K^+ conductance activated in submucosal neurones by noradrenaline, somatostatin and [Met^5] enkephalin involves 30-65, 120-160 and 220-260pS channels (Shen *et al.,* J. Physiol., **445**:581,1992) whereas that activated by acetylcholine in atrial cells involves a single type of unitary conductance (20pS 'I_{ACh}' channel; Clark *et al.,* J. Physiol., **424**:229,1990). A question therefore arises as to whether the conductance activated by muscarinic agonists in neurones involves one type of unitary conductance or whether multiple unitary conductances are involved. Also, the mechanism of this inward rectification remains to be elucidated. We studied the action of 10μM muscarine on the small (C) cells of bullfrog sympathetic ganglion using whole-cell and single channel recording techniques (*see* Selyanko *et al.,* J. Physiol., **425**:471,1990). The activation curve for the muscarine-sensitive conductance was fitted by Boltzmann-type kinetics (potential for half activation = -53mV; valency = -2.3). The extent of inward rectification was increased by elevating $[K^+]_o$ but was not influenced by changing $[Mg^{2+}]_i$ or $[Na^+]_i$. Thus, the rectification does not result from the internal blocking of the channel by Mg^{2+} or Na^+ (Vandenberg, Proc. Natl. Acad. Sci. USA., **84**:2560,1987). Muscarine increased the activity of at least two types of channel in outside-out patches (20pS and 50pS with $[K^+]_o$ = 20mM; $[K^+]_i$ = 110mM). The 20pS channel exhibited linear I-V characteristics. Possible interpretations of these data are that some channels are controlled by a voltage-sensitive gate which is opened by hyperpolarization or that rectification involves blockade of the channel by the outward movement of K^+. In these aspects, the inhibitory action of muscarine on peripheral neurones resembles that of adrenoceptor and peptidergic agonists. Supported by the MRC of Canada.

565.4

IS DIACYLGLYCEROL INVOLVED IN THE EFFECT OF MUSCARINE ON SYMPATHETIC GANGLION CELLS? H. Chen*, D.E. Kurenny and P.A. Smith. Dept. Pharmacol., Univ. Alberta, Edmonton, T6G 2H7, Canada.

Muscarinic agonists depolarize frog sympathetic ganglion cells and this effect involves suppression of a voltage-dependent, non-inactivating K^+-current known as the M-current (I_M, Brown & Adams Nature **283**:673,1980). We used whole-cell patch-clamp recording techniques to investigate the transduction mechanism for this response. Although muscarine (0.5-1μM), phorbol-12-myristrate-13-acetate (2-20μM) and phorbol dibutyrate (1-10μM) all inhibited I_M, their effects were insensitive to the kinase inhibitor, H-7 (1-(5-Isoquinolinylsulfonyl)-2-methylpiperazine; 50-75μM). Responses to 1-oleoyl-2-acetyl-*sn*-glycerol (5-20μM) were weak and inconsistant. Since muscarinic agonists promote hydrolysis of membrane phospholipid in these cells (Pfaffinger *et al.,* Neuron **1**:477,1988) and the action of muscarine seems independent of membrane phosphorylation (Chen & Smith, Br. J. Pharmacol., **105**:329,1992), these data raise the possibility that diacylglycerol (DAG) functions as a second messenger for I_M suppression which acts *directly on the channel without the involvement of protein kinase C.* This possibility is unlikely because inhibition of DAG catabolism with the DAG kinase inhibitor, R 59022 (20-50μM, Janssen Biotech) and/or the non-specific lipase inhibitor, quinacrine (up to 2mM) failed to potentiate the effects of muscarine. Also, high concentrations of phorbol esters (up to 20μM) could not produce the same maximal response as muscarine.

Supported by the MRC of Canada.

565.5

EFFECTS OF MUSCARINIC AGONISTS ON INTRACELLULAR CALCIUM CHANGES IN ACUTELY DISSOCIATED SYMPATHETIC NEURONS. S. Foucart* and R.J. Miller. Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637, USA.

In this study, we evaluated the effects of carbachol (Cch) and oxotremorine (OXO) on intracellular calcium $[Ca^{2+}]_i$ in neurons acutely dissociated from the superior cervical ganglion of adult rats. The neurons were dissociated enzymatically, plated on poly-l-lysine treated coverslips and used within the next 20 hours. The cells were loaded with Fura2-AM (3 μM, 30 min) and mounted in a perfusion chamber. The perfusion medium contained 2 mM $CaCl_2$, 138 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 10 mM HEPES and 10 mM glucose, (7.4). The $[Ca^{2+}]_i$ was estimated by microfluorometry. The basal $[Ca^{2+}]_i$ was 52 ± 3 nM (n=90). Application of Cch (10 μM) or OXO (10 μM) increased the $[Ca^{2+}]_i$ by 86 ± 7 nM and 38 ± 10 nM, respectively. The effects of Cch and OXO were blocked by atropine (1 μM) but not by hexamethonium (10-50 μM). Caffeine sensitive store depletion, pertussis toxin treatment and application of staurosporine (1 μM) or thapsigargin (0.1 μM) did not affect the actions of the muscarinic agonists but omission of Ca^{2+} from the perfusion medium as well as Ca^{2+} channel block with TA 3090 (50 μM) abolished it. These results suggest that the increase in $[Ca^{2+}]_i$ produce by muscarinic agonists does not involve the mobilization of intracellular calcium stores, but rather is mediated by Ca^{2+} influx through voltage-sensitive calcium channels.

565.6

CHOLINERGIC MODIFICATION OF SYNAPTICALLY INDUCED Ca TRANSIENTS IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS. H.Miyakawa*, K.Ito, I.Nakamura, H.Kato, Dept. of Physiology, Yamagata Univ. Sch. of Med., Yamagata, 990-23 JAPAN

Hippocampal CA1 pyramidal neurons receive cholinergic inputs from septal area. Although this input is thought to play a crucial role in memory, the specific nature of the role is not understood. If cholinergic input to hippocampus is really important in memory, cholinergic agonists might be expected to modify long-term potentiation (LTP) because LTP is believed to underlie memory mechanism. Indeed, it has been reported that cholinergic agonists enhance LTP (Blitzer et.al., Shulz & Johnston). Then, what is the mechanism by which cholinergic agonists enhance LTP? Here we report that cholinergic agonists prevent synaptically induce Ca transients from quickly returning back to basal level.

We prepared guinea-pig hippocampal brain slices, injected Ca indicator dye fura-2 into a CA1 pyramidal neuron through an intracellular pipette, and monitored intracellular Ca level at its dendrites and the soma while recording membrane potential from the soma. In standard medium, Schaffer collateral synaptic stimulation evoked a transient Ca rise both at dendrites and at the soma. The Ca rise was linked to synaptically evoked action potentials. The Ca level started to decline immediately after the input and quickly recovered to basal level. In contrast, in the presence of 5-10μM carbachol, the decay time of Ca transients became twice as slow as in the standard medium. Although evoked action potentials became wider in carbachol, amplitude of Ca increase driven by supra-threshold synaptic input was not significantly different in both conditions. These facts imply that some secondary processes are responsible for the prolonged Ca transients.

We surmise that prolonged duration of elevated Ca level after conditioning inputs could be the mechanism by which cholinergic inputs enhance LTP. (supported by MESC grant #02259105 and HFSP)

565.7

PATTERN OF CAFFEINE-INDUCED CALCIUM RELEASE IN CULTURED MOUSE HIPPOCAMPAL NEURONS. K. J. Seymour-Laurent* and M. E. Barish. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

The spatial and temporal pattern of ryanodine-sensitive caffeine-induced calcium release have been investigated using calcium-sensitive dyes (Fluo-3 and Calcium Green) and confocal microscopy. Mouse hippocampal neurons were dissociated on embryonic days 15-17 and studied after 1-12 days in culture. Cells were continually perfused with normal Hank's BSS containing 0.3 μM glutamate and 10 μM glycine. Changes in [Ca]i, measured when 10 mM caffeine was puffed onto single cells, were spatially non-uniform. In most pyramidally-shaped cells the caffeine response was first seen in the apical region of the cell within 2 seconds of initiation of the caffeine pulse, irrespective of pipette position. It then spread to the remaining parts of the cell over a period of 3 to 5 seconds. The nucleus was the last area to show elevated [Ca]i, and [Ca]i in the nucleus remained high after the cytoplasm had returned to resting levels. The change of [Ca]i in the nucleus usually exceeded that in the cytoplasm, and thin (1 μm), rapidly aquired (0.4 seconds/frame) optical sections revealed radial invasion of the nucleus. Organelle-specific dyes showed that endoplasmic reticulum and mitochondia are concentrated in the cell's apical region. We suggest that caffeine initiates propagating calcium release in regions of highest endoplasmic reticulum density and that nuclear invasion may be due to diffusion from the cytoplasm.

565.8

CALCIUM SIGNALLING IN POSTSYNAPTIC SPINES VISUALIZED WITH CONFOCAL MICROSCOPY. A. Hernandez-Cruz*, M.P. Charlton and G.J. Augustine. Dept. Neurosciencias, UNAM, Dept. Physiology, Univ. Toronto, Dept. Neurobiology, Duke Medical Center and MBL, Woods Hole.

We have used the Noran real-time confocal microscope to measure dynamic changes in postsynaptic Ca concentration produced by synaptic activity. The fluorescent Ca indicator, Ca green, was microinjected into the postsynaptic neuron of the squid giant synapse. This neuron forms synaptic contacts at small spines at the end of branched dendritic processes. Brief trains of presynaptic activity (50 Hz, 1-5 s) produced prompt rises in postsynaptic Ca concentration that decayed over several seconds. No such changes occurred during directly evoked postsynaptic action potentials, indicating that Ca was entering through transmitter-activated (CNQX-sensitive) channels rather than through voltage-activated channels. The synaptically-induced rises in Ca were largest in the spine tips, but smaller changes were observed throughout the entire dendritic structure. No measurable changes in Ca could be detected in other regions of the postsynaptic neuron, probably because the large volume of this neuron diluted the Ca signal. In summary, postsynaptic Ca signals are not as acutely compartmentalized in these spines as in hippocampal spines (e.g. *Nature* 354: 73&76, 1991). Supported by a Kuffler Fellowship and grants from CONACyT, MRC and NIH.

565.9

Does intracellular Ca^{2+} play a role in diazepam- or ethanol-induced potentiation of $GABA_A$ currents in hippocampal neurons?

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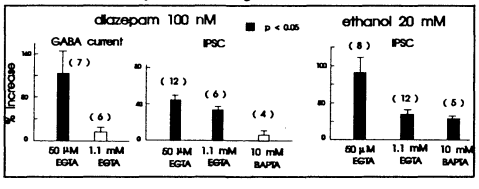
We studied $GABA_A$-mediated IPSCs and GABA currents in rat CA1 hippocampal neurons using whole-cell patch recordings in brain slices. Our standard internal solution contained in mM: K-gluconate 130, KCl 20, Mg-ATP 2, GTP 0.2, HEPES 10, EGTA 1.1, $CaCl_2$ 0.1. In neurons dialysed with the standard internal solution or an internal solution containing 50 μM EGTA, bath application of 100 nM diazepam, midazolam, or 20 mM ethanol significantly potentiated the $GABA_A$-mediated responses. In neurons dialysed with the internal solution in which EGTA/Ca was replaced by 10 mM BAPTA, the potentiation of $GABA_A$-mediated responses by diazepam or ethanol, but not by midazolam, was much less pronounced (see graph below). The underlying mechanisms are currently under investigation.



565.10

REBOUND POTENTIATION OF INHIBITORY SYNAPTIC CURRENTS IN CEREBELLAR PURKINJE CELLS.
M. Kano, J. Dreessen, U. Rexhausen and A. Konnerth*. Max-Planck-Inst. biophys. Chemie, 3400 Göttingen, Germany.

The best known form of synaptic plasticity in cerebellar Purkinje cells (PCs) is a long-term depression (LTD) of excitatory parallel fiber (PF) synapses, resulting from repetitive and conjunctive stimulation of PFs and climbing fibers (CFs). Here we report a new form of synaptic plasticity in cerebellar PCs in which stimulation of the excitatory CF synaptic input leads to a long-lasting (several hours) potentiation of $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs), a phenomenon which we termed rebound potentiation (RP). By using simultaneous whole-cell patch-clamp recordings and fura-2 video-imaging of intracellular calcium concentration ($[Ca^{2+}]_i$), we found that a CF-induced transient increase in postsynaptic $[Ca^{2+}]_i$ triggers RP. Several lines of evidence indicate that RP is caused by a Ca-dependent up-regulation of postsynaptic $GABA_A$ receptor function in PCs. The possible role of other second messengers for this up-regulation will be discussed. We propose that RP of IPSCs is a cellular mechanism which, in addition to the LTD of excitatory PF synapses, contributes to motor learning in the cerebellum.

565.11

CHANGES IN pH MODULATE NMDA- AND HIGH-$[K^+]_o$-EVOKED RISES IN CYTOSOLIC FREE CALCIUM CONCENTRATION IN RAT HIPPOCAMPAL PYRAMIDAL NEURONS. K. Abdel-Hamid* and J. Church. Depts. of Physiology and Anatomy, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1Z3.

NMDA receptor-mediated responses are sensitive to changes in $[H^+]_o$, which might in part account for the anticonvulsant and neuroprotective effects of cerebral acidosis. Ca^{2+} entry via voltage-gated Ca^{2+} channels may also be important for the genesis of convulsive and neurodegenerative phenomena. We have investigated the effects of changes in pH_o and pH_i on rises in $[Ca^{2+}]_i$ evoked by NMDA and high-$[K^+]_o$ in Fura-2-loaded rat hippocampal pyramidal neurons prepared from 18-day-old embryos. In HCO_3^--buffered media, pH was altered by changing $[HCO_3^-]_o$ at a constant P_{CO_2} (5%); in HCO_3^-/CO_2-free, HEPES-buffered media, pH was manipulated with NaOH. All ionic changes were made by substitution for NaCl on an equimolar basis.

In both HCO_3^-/CO_2- and HEPES-buffered media reducing pH to 6.9 attenuated, and raising pH to 7.8 enhanced, rises in $[Ca^{2+}]_i$ evoked by NMDA or high-$[K^+]_o$, compared to responses obtained at pH 7.4. Experiments with methylsulfate-substituted media (pH 7.4) demonstrated that the effects of perfusion with high-HCO_3^- did not reflect changes in $[Cl^-]_o$. Reducing or raising pH_i (at a constant pH_o) by perfusion with media containing weak acids (propionate, butyrate) or a weak base (trimethylamine), respectively, had little effect on either NMDA or high-$[K^+]_o$-evoked responses although small falls and small rises in resting $[Ca^{2+}]_i$ were often noted in response to intracellular acidification and alkalinization, respectively.

The results suggest that changes in pH_o may modulate Ca^{2+} influx via voltage-gated, as well as NMDA receptor-operated, channels.

565.12

NMDA INDUCED REGENERATIVE CALCIUM INFLUX IN HIPPOCAMPAL PYRAMIDAL CELLS. Q.X. Chen* and R.K.S. Wong. Dept. of Pharmacology, SUNY/HSC, Brooklyn, NY 11203.

Whole-cell voltage-clamp was carried out in internally perfused neurons acutely dissociated from the CA1 region of the hippocampus of adult guinea-pigs. Introduction of elevated Ca^{2+} containing medium into the cell activated an outward potassium current followed by an inward current. The Ca^{2+}-dependent inward current has the following properties: (1) It is activated by intracellular Ca^{2+} $\{[Ca^{2+}]_i\}$. (2) Its activation is not voltage-dependent. (3) It can be blocked by extracellular cadmium (1 mM). (4) It is in part carried by Ca^{2+}. We termed this inward current $I_{in(Ca^{2+})}$. Because of the dependency on $[Ca^{2+}]_i$ and its Ca^{2+} permeability, we found that $I_{in(Ca^{2+})}$, once induced, became self-sustaining and regenerative. That $[Ca^{2+}]_i$ level progressively increased following the induction of $I_{in(Ca^{2+})}$ is indicated by the growth in amplitude of the Ca^{2+}-dependent K^+-current. Intense stimulation of NMDA receptors, via NMDA bath application (100 uM, >5s), in the adult hippocampal cells activated $I_{in(Ca^{2+})}$. In contrast, activation of other glutamatergic receptors by kainate and QUIS to the same magnitude did not elicit $I_{in(Ca^{2+})}$. Our previous studies show that $GABA_A$-responses in the hippocampal pyramidal cells are suppressed by a dephosphorylation process when $[Ca^{2+}]_i$ is elevated (Chen et al 1989, *J. Physiol.* 420). We now demonstrate that through this mechanism, intense neuronal excitation via NMDA-receptor can suppress $GABA_A$-receptor mediated reponses of the same neuron following the induction of $I_{in(Ca^{2+})}$.

565.13

NMDA and non-NMDA Receptors Mediate Extracellular Alkalinizations in the Rat Hippocampal Slice. J. C. T. Chen* and M. Chesler; Dept of Physiology & Biophysics and Dept of Neurosurgery, NYU Medical Center, 550 First Ave. N. Y., NY 10016.

Stimulation of Schaeffer collaterals or local application of glutamate evokes picrotoxin-insensitive alkalinizations in area CA1 (1). We have extended our studies of these responses using pH-sensitive microelectrodes.

Cd^{2+} (100μM), TTX (1μM), and zero Ca media did not affect glutamate-evoked alkalinizations, suggesting that Na channels and Ca-dependent mechanisms do not directly mediate these responses. Dihydrokainate (200μM) had no effect on afferent stimulus-evoked alkalinizations, excluding a role for glutamate uptake.

In normal media, CNQX (10μM) blocked Schaeffer collateral-evoked alkalinizations while APV (20-50μM) had no effect. During CNQX superfusion, removal of voltage-dependent block of NMDA channels by withdrawal of Mg^{2+} revealed an APV-sensitive alkalinization similar in magnitude to control. These results suggest that NMDA receptors can also contribute to stimulus-evoked alkalinizations.

We tested whether involvement of both receptor subtypes was simply due to the firing of the CA1 population. Pure antidromic activation of the CA1 cells (by stimulation of subicular alveus during superfusion with CNQX, APV and PTX) evoked extremely small alkalinizations. Despite a population spike of comparable size, orthodromic stimulation evoked 5-fold greater alkalinizations. These data indicate that synaptically-evoked alkaline shifts cannot be attributed to cell firing per se.

Our data indicate that the mechanism underlying the stimulus-evoked alkaline shift is closely linked to the activation of excitatory amino acid receptors. These results support the hypothesis that this phenomenon is generated by passage of proton equivalents through ligand-gated ion channels.

1) JCT Chen and M Chesler, *J Neurophys*, **67(1)**:29 (1992)

565.14

SPONTANEOUS TO EVOKED NMDA CURRENTS IN CEREBELLAR GRANULE CELLS. E.D'Angelo, P.Rossi, E.Parati*, V.Taglietti, Institute of General Physiology, University of Pavia, 27100 Pavia, Italy. *Istituto Neurologico C. Besta, 20133 Milano, Italy.

The aim of present work is to compare spontaneous to evoked NMDA current kinetics. Conventional whole-cell recordings of excitatory currents were obtained from granule cells in cerebellar slices at P10-P14 . Spontaneous NMDA currents could be observed in the presence of the non-NMDA receptor inhibitor 10 μM CNQX, and were suppressed by 50 μM APV (n=19). Individual currents were small (<5pA at -40 mV) and noisy, and in virtual Mg^{++}-free solution (n=6) showed evident single channel transitions generating irregular shapes. In order to compare spontaneous to evoked NMDA currents, unstimulated current traces were displayed (off-line) on computer monitor, a baseline was selected and spontaneous events were picked-up semi-automatically (signals with signal-to-noise ratio <2 were rejected). When averagings were obtained by aligning individual currents on their starting or half-rise point, good correlation was found with the (slow) time-course of evoked NMDA currents in the same cells. However, a fast initial component (10-90% rise-time <1ms) was often generated by synchronizing spontaneous events at their peak. This fast component should be distinguished by the fast non-NMDA currents, which were virtually observed in isolation at -80 mV and presented 200-400 μs 10-90% rise-times.

565.15

IPSPs STRONGLY INHIBIT CLIMBING FIBER ACTIVATED $[Ca^{2+}]_i$ INCREASES IN THE DENDRITES OF CEREBELLAR PURKINJE NEURONS. J.C. Callaway*, W.N. Ross and N. Lasser-Ross. Dept. of Physiology, New York Medical College, Valhalla, NY 10595.

The interaction between IPSPs and climbing fiber (CF) evoked EPSPs was analyzed in the guinea pig cerebellar slice preparation using intracellular recording and high speed fluorescence imaging of intracellularly injected fura-2. All-or-none CF responses were evoked by stimulation of the white matter; IPSPs, blockable by 10 μM bicuculline, were generated by off-beam stimulation in the molecular layer.

CF responses generated large, transient $[Ca^{2+}]_i$ increases due to Ca spikes which usually extended over all dendritic regions. When IPSPs and CF responses were activated simultaneously the Ca transients were reduced in amplitude, sometimes by more than 90%. Most often the Ca transients in the distal dendrites were reduced more than those in the proximal dendrites. The peak amplitude of the somatically recorded CF potential was not reduced by this interaction. The most significant change in the electrical response was a small reduction in the shoulder which followed the peak potential.

If the inhibitory stimulation preceded the CF response by 10-20 msec (the rise time of the IPSP) the Ca transients were still inhibited, but earlier stimulation had no effect. Somatic hyperpolarization by double the IPSP amplitude did not reduce the CF induced Ca transients. These experiments suggest that the increased inhibitory conductance, not the potential, caused the reduction by preventing the generation and/or propagation of dendritic Ca spikes.

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565.16

FUNCTIONAL TOPOGRAPHY OF INHIBITION IN RAT SOMATOSENSORY CORTEX. P.A. Salin and D.A. Prince*. Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA, 94305.

Postsynaptic inhibition is an important mechanism for regulating neuronal excitability and synchronization in normal cortex, however functional details regarding its laminar distribution are incomplete. We used the whole cell patch clamp technique to record postsynaptic inhibitory currents (IPSCs) mediated by $GABA_A$ receptors in identified pyramidal neurons of layers 2-3 and 5 in adult rat somatosensory cortex (S1). The frequency (3.4-35 Hz) and the amplitude (5-310 pA, for V_{hold}= 0 mV and E_{Cl}= -45 mV) of spontaneous IPSCs was highly variable. The mean amplitude of 19.7 pA would correspond to an inhibitory potential of -3.9 mV under the above experimental conditions (R_N= 200 MΩ). In most cases IPSCs decayed with a single time constant close to 7 msec. Preliminary results indicate that inhibitory input varies in part with the cortical laminar organization since layer 5 cells generated a higher frequency of spontaneous IPSCs (mean: 20.2 Hz, n= 8) than those in layer 2-3 (mean: 10.5 Hz, n= 10, p<0.02, Mann-Whitney U test). The topography of inhibitory cortical circuits was examined by applying focal electrical stimulation at different measured distances from the recorded neuron. In solutions containing D-APV (100μM) and CNQX (10μM), threshold monosynaptic IPSCs were evoked in layer 2-3 and 5 cells when stimulating electrodes were in layer 1. Stimuli delivered up to 670 μm lateral to the layer 5 cell, outside the barrel field of the recorded neuron, could evoke IPSCs. These results demonstrate that somatosensory cortex pyramids receive extensive functional inhibitory contacts that may be differentially expressed in cells of different laminae. The strength of the inhibition could play a key role in synchronization mechanisms in layer 5. (supported by NIH grants NS12151, NS07280 from NINDS. P.S. is a fellow of the Fyssen Foundation).

565.17

A LOW-FREQUENCY SUBTHRESHOLD RESONANCE IN NEOCORTICAL NEURONS GENERATED MAINLY BY I_h. B. Hutcheon* and E. Puil, Department of Pharmacology & Therapeutics, University of British Columbia, Vancouver, B.C., Canada. V6T 1Z3

The rhythmic, low-frequency events that characterize the EEG during various behavioral states reflect the coordinated activities of large groups of neurons. We propose that many neocortical neurons are follower rather than pacemaker cells, and have investigated the possibility that the intrinsic properties of the follower neurons predispose them to be driven at particular frequencies -- a feature that could contribute to intercellular synchronization. We examined, using whole-cell patch-clamp techniques, the subthreshold responses of neurons in neocortical slices to intracellular injections of oscillatory inputs in order to determine membrane impedance as a function of frequency. The neurons exhibited properties that bias their responses towards low frequencies (< 10Hz).

The magnitude of the impedance in some neurons declined steeply, and monotonically, with frequency (1/2 magnitude, 5-20 Hz). In other neurons, the impedance magnitude function near rest (-50 to -60 mV) showed a voltage-dependent resonant hump with a maximum between 1-3 Hz. The resonance shifted to higher frequencies (3-6 Hz) and gradually became less distinct with lower overall impedance as the cell was hyperpolarized by 10-50 mV. When voltage clamped near rest, and hyperpolarized, these neurons showed slow inward currents with a voltage dependence and time course which could be predicted from the resonance. These results suggest that the resonance is mainly due to the hyperpolarization-activated cation current, I_h. The resonance and the inward currents were reduced in extracellular Cs^+ -- also consistent with an involvement of I_h. The shape and position of the resonant hump did not depend on the time course of the input function, or whether current or voltage was used as input. Modeling of I_h in the absence of other slow voltage-gated currents such as I_T, showed that I_h by itself is capable of producing the resonance in neocortical neurons.

565.18

DIFFERENCES IN REPETITIVE FIRING BETWEEN ADULT AND IMMATURE RAT SENSORIMOTOR CORTICAL NEURONS MIMICKED BY CALCIUM CHELATION. Lorenzon, N. M*, and Foehring, R. C., Dept. of Anatomy and Neurobiology, Univ. of Tenn., Memphis, TN 38103-4901.

In adult cortical pyramidal cells, long current injections result in regular, repetitive firing followed by a slow afterhyperpolarization (sAHP). In immature (P6-10) neurons, the sAHP following repetitive firing is much larger and is greatly prolonged relative to adult cells, resulting in more prominent spike-frequency adaptation in the immature cells. Some immature neurons will not fire longer than 200 ms. Several potential mechanisms could explain these age-dependent differences. One possibility is that Ca^{2+}-handling differs at the two ages. A recent study in cat motor cortex (Schwindt *et al.* 1992. Neuroscience 47:571) reported increased sAHP amplitude and duration, and more phasic firing, when low doses of the Ca^{2+} chelator BAPTA were included in the intracellular electrode. We tested whether the immature firing pattern could be elicited in adult rat cortical neurons impaled with electrodes including 2mM BAPTA (in 2M K-methylsulphate), and whether higher doses of BAPTA (200 mM) resulted in loss of the sAHP and spike-frequency adaptation in young and mature neurons. Intracellular recordings were obtained from an in vitro slice preparation.

We found that 200 mM BAPTA greatly reduced AHPs and adaptation at both ages. 2 mM BAPTA caused adult neurons to have enlarged, prolonged AHPs and the immature firing pattern. The BAPTA-enhanced sAHP in adults was reduced by 100 µM isoproterenol and when extracellular Ca^{2+} was replaced with Mn^{2}.

Supported by NINDS grant #R29NS27180.

POSTSYNAPTIC MECHANISMS II

566.1

SIGNAL DELAY IN PASSIVE DENDRITIC TREES. I. Segev* and H. Agmon-Snir. *Dept. of Neurobiology, Hebrew University, Jerusalem, Israel.*

The signal delay of neurons is significantly affected by the cable properties and morphology of the dendrites. This dendritic delay plays an important role in information processing and in plastic processes at the neuron. A novel analytical approach for calculating the delay of electrical signals in any passive dendritic tree is introduced. The ***dendritic delay*** (D_Delay) is defined hereby as the difference between the *center of gravity (the centroid)* of the transient current input and the *center of gravity* of the resultant voltage transient, measured at any point in the tree. The D_Delay measured at the input point is non-zero and is called the ***local delay*** (L_Delay). ***Propagation delay*** (P_Delay) is then defined as D_Delay - L_Delay. Using the Laplace transform of the one-dimensional passive cable equation, and the mathematical relation between this Laplace transform and the centroid, an analytical expression for the D_Delay between any two points in the tree can be found.

With these definitions for delays, several general theorems are proven. For example: i) D_Delay between two points (x,y) in a given tree is independent of the properties (shape and duration) of the transient current input. ii) In a given tree, D_Delay(x,y) = D_Delay(y,x) iii) Both the P_Delay and the signal's speed of propagation are independent of the morphology of the tree "behind" the signal, and of the input location.

The delay in an isopotential, isolated, soma is τ, the time constant of the membrane. In an infinite cylinder with uniform properties, the local delay is $\tau/2$ and the speed of propagation is $2\lambda/\tau$, where λ is the space constant. In realistically complicated trees, the dendritic delay from distal dendritic input to the soma may range from 1-2τ. The delay between adjacent dendritic branches, however, may be as small as 0.3τ. The significance of the results for computation and learning at the neuronal level will be discussed.

566.2

SPACE-CLAMP ERRORS ASSOCIATED WITH MEASUREMENT OF ELECTROTONICALLY REMOTE SYNAPTIC EVENTS. *N. Spruston*, D. Jaffe, S.H. Williams & D. Johnston*, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

The frequency-dependent voltage attenuation and resultant space-clamp errors associated with the measurement of synaptic current are evaluated both in equivalent cylinder models and in a detailed compartmental model of a hippocampal CA3 pyramidal neuron. We show that in both models, as expected, higher-frequency voltage changes are attenuated to a much greater extent than steady-state or low-frequency voltage changes.

The effect of increases in membrane resistivity (R_m) were also examined and found to dramatically reduce steady-state voltage attenuation, while having only small effects on the non steady-state components of synaptic responses. The consequence of the frequency-dependent reduction in voltage attenuation by increases in R_m is that experimentally increasing R_m (e.g., with the use of Cs^+-containing patch-clamp electrodes) can result in improvements in the accuracy of measured reversal potentials (which are in error according to the steady-state electrotonic distance of the synapse) without substantial improvements in the accuracy of peak current, conductance, rise time, or decay time constant measurements (which are in error according to the electrotonic distance for the high-frequency components in fast synaptic responses).

Finally, we use simulations from a morphological model of a CA3 pyramidal neuron to demonstrate the distortion of mossy fiber and commissural/associational synaptic currents at the soma, and to estimate the electrotonic distances of mossy fiber and perforant path synapses. We conclude that the error associated with synaptic current measurement can be extremely large for synapses located at electrotonically remote sites in the dendritic tree. (Supported by MH44754, MH48431, the Keck Foundation, and the A. von Humboldt Foundation.)

566.3

INTEGRATIVE PROPERTIES OF HIPPOCAMPAL, CORTICAL AND THALAMIC NEURONS STUDIED BY WHITE NOISE ANALYSIS H. Jahnsen* and S. Karnup Institute of Neurophysiology, Blegdamsvej 3 c, DK-2200 Copenhagen N, Denmark.

Synaptic potentials in nerve cells activate intrinsic membrane properties and synaptic integration is therefore a result of complex interactions in space and time.

In an attempt to study these processes in mammalian central neurons intracellular recordings were obtained from hippocampal, cortical and thalamic neurons in slices or slice cultures. The cells were stimulated with a band-pass filtered white noise signal to simulate a synaptic barrage and the responses were analyzed in the frequency domain.

Our results show that at least four factors are important for the spectral characteristics of neurons below the threshold for firing of action potentials: the passive membrane properties, the average membrane potential, presence or absence of a Q-current and the presence or absence of a low-threshold Ca^{2+} spike. In the frequency domain these four factors are seen to give their own characteristic contribution to the integrative properties of central neurons.

566.4

BIOPHYSICAL MODEL OF HIPPOCAMPAL MOSSY FIBER SYNAPSES. M.Siegel[1], R.Gonzales[4], N.T.Carnevale*[1,3], B.Claiborne[4] and T.H.Brown[1,2,3]. Departments of Psychology[1] and Cellular and Molecular Physiology[2] and Center for Theoretical and Applied Neuroscience[3], Yale Univ., New Haven, CT 06520, and Division of Life Sciences[4], Univ. Texas, San Antonio, TX 78249.

The mossy fiber (MF) inputs to CA3 neurons, which are among the largest synapses in the mammalian brain, terminate on "thorny excrescences" (thorns) located on proximal apical dendrites. Because of their size and strategic location, these have been regarded as "detonator" synapses that might serve as "teacher signals" in the mnemonic operations of the hippocampus. We have probed their biophysical properties and functional implications using models based on quantitative morphometry and biophysical measurements.

Three-dimensional reconstructions, noting locations of thorns, were obtained from rat CA3 neurons that had been injected with horseradish peroxidase. Ultrastructure of thorns was based on 3-D reconstructions from electron micrographs (Chicurel & Harris, personal communication). Compartmental models (Claiborne et alia in: *Single Neuron Computation*, Academic Press, in press) were constructed from these data and whole cell patch clamp measurements (Xiang et al., *Soc. Neurosci. Abstr. 18*, in press). MF synapses represented by alpha functions were located on the heads of thorns (see Jaffe and Brown, *Soc. Neurosci. Abstr. 18*, in press).

Three principal conclusions emerged from these simulations. First, thorns have essentially no effect on transfer of potential, current or charge from spine head to dendritic shaft. Second, synaptic current rise time can be used to judge the "quality" of whole-cell voltage clamp recordings so that synaptic reversal potential, charge transfer, peak conductance and peak current can be determined accurately for many MF inputs. Third, MF synapses are not "detonators"—at least 10 must be activated to trigger a somatic spike. *Supported by ONR and NIH.*

566.5

MODELING DENTATE GRANULE CELLS WITH "OLD" AND "NEW" ELECTROTONIC PARAMETER VALUES. W.R. Holmes*, Neurobiology Program, Dept. of Biological Sciences, Ohio University, Athens, OH 45701.

Recent studies indicate that the typical values of the electrotonic parameters Rm and Ri used in models need to be revised. For dentate granule cells, studies with perforated-patch electrodes (Spruston and Johnston, 1992) suggest that Rm and Ri values of 40 kΩcm2 and 210 Ωcm are more appropriate than older estimates of 12 kΩcm2 and 70 Ωcm. Results are compared when these old and new sets of values are used in models of a dentate granule cell whose morphology is known from serial reconstructions.

Single action potentials and action potential trains due to current injection were modeled (conductances modified from Yuen and Durand 1991). The gbars in simulations with the old Rm and Ri values were nearly double those with the new values to get comparable action potentials. The DAP following a single action potential decayed much faster with the old values. With the new values, the action potential was more severely attenuated as it spread to the dendrites, but the dendrites were depolarized much longer because of the longer DAP. Spike train adaptation was greater with the old Rm and Ri values.

Synaptic activation in the mid-dendritic region was modeled. Synaptic conductance parameters were adjusted to give comparable peak EPSPs with both sets of Rm and Ri. When synapses were activated at 100 Hz, the peak potential was larger (due to action potential invasion), but average potential was smaller, and consequently, calcium influx through NMDA receptor channels was smaller with the old Rm and Ri values than with the new ones.

It might be possible to compensate for some of these differences by changing conductance kinetics, or by adding additional conductances. To limit the number of degrees of freedom, one must fix appropriately and accurately as many parameters as possible, beginning with Rm and Ri.

566.6

Voltage-Clamp and Current-Clamp Investigation of Ionic Conductances in Hippocampal CA1 Pyramidal Neurons using QX-314. J.Isaac, S.B.Colling, and H.V.Wheal(SPON: Brain Research Association) Dept. Physiology & Pharmacology, University of Southampton, Bassett Cres.East, Southampton, SO9 3TU, UK.

The excitatory afferents onto CA1 pyramidal cells in the hippocampus have recently attracted considerable attention in the investigation of long term potentiation (Malinow & Tsien, 1990 *Nature*,**346**,177-180). However by the use of QX-314, we have previously reported (Colling *et al.*, 1991, Neurosci.Abstr. **17**,605.8) that there is a Na^+ spike generating system in the apical dendrites of these cells which may effect Hebbian mechanisms. In order to clarify exactly which conductances QX-314 acts on, voltage- and current-clamp investigations were carried out on CA1 pyramidal cells in rat hippocampal slices maintained *in vitro*. Current-clamp data revealed that intracellular injection of QX-314 (35mM) blocks somatic and dendritic action potentials in a time dependent manner (30-56 minutes, n=5, at twice threshold stimulation), with no noticeable effect on the underlying EPSP's. When QX-314 (20mM) was introduced via whole-cell patch electrode the synaptically evoked Na^+ spike responsible for the action potential was blocked within 10 minutes (n=5, at twice threshold stimulation) and no adverse effects were observed on the underlying EPSC's. Observation of cellular voltage-gated conductances revealed that QX-314 blocked all the Na^+ currents leaving only inward Ca^{2+} currents. In comparison externally applied 0.5μM tetrodotoxin blocked the same voltage-gated currents but also blocked the EPSC. Thus QX-314, when used intracellularly, blocks the voltage-gated Na^+ conductances responsible for the action potential without adversely effecting either the EPSP/EPSC or voltage-gated Ca^{2+} currents making it ideal for the isolation and investigation of the synaptic mechanisms of these cells. (Supported by grants from the Wellcome Trust and MRC. QX-314 kindly donated by Astra Pharmaceuticals)

566.7

PREGNENOLONE SULFATE EFFECTS UPON CA1 HIPPOCAMPAL NEURONS IN A SLICE PREPARATION. J.H. Meyer * and D.L. Gruol, Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla CA, 92037.

Steroids have both genomic and non-genomic effects on neuronal function. For example, previous studies using cultured neurons under voltage-clamp conditions have demonstrated that neurosteroids such as pregnenolone can rapidly affect $GABA_A$-associated chloride conductances (e.g. Majewska et al., 1988; Neurosci Lett 90:279). To gain an understanding as to the range of steroid influences, we have initiated studies on the effects of neurosteroids on hippocampal CA1 pyramidal neurons in a slice preparation.

Hippocampal slices from female Sprague-Dawley rats were placed in an interface-type slice chamber and perfused with artificial CSF (ACSF). Current clamp recordings (electrodes: 3M potassium acetate) were used to assess membrane resistance and cell responses to hyperpolarizing or depolarizing pulses. Repeat recordings were made after a 1/2 hour time period during which slices were perfused with either ACSF (control group) or ACSF containing pregnenolone sulfate (PS; 50 µM; experimental group). Over the 1/2 hour monitoring period, PS exposure did not appear to affect cell membrane resistance. Similarly, the amplitudes of the after-hyperpolarization, occurring after a depolarizing pulse, and the off-response, seen after a hyperpolarizing pulse, were not affected by PS. There was, however, a tendency for increased excitability in PS-treated cells: Spike frequency associated with depolarizing current pulses increased in 6/9 PS-treated cells, while 2/6 control cells exhibited only slight increases in spike frequency. These preliminary findings suggest that neurosteroids may affect active cell responses as well as synaptic transmission. Supported by AA6420 and AA0756.

566.8

A SYSTEM FOR THE ELECTRICAL STIMULATION AND MEASUREMENT OF *c-fos* AND PPT GENE EXPRESSION IN RAT SUPERIOR CERVICAL GANGLION CELLS M.Hodaie[1], M.W.Salter[2] and A.Roach[1]* [1]S.Lunenfeld Research Institute and Dept. Mol. and Med. Gen.,Univ. of Toronto, [2]Div. Neurosci., Hosp. for Sick Children, and Dept. Physiol. Univ. of of Toronto, Toronto, Ontario, Canada

We are interested in the influence of neuronal activity on gene expression in the nervous system. Towards that end, we are developing complementary systems of electrical stimulation and measurement for neural-specific gene expression using intact explants of newborn rat superior cervical ganglia (SCG) as a model. The genes being studied are pre-protachykinin (PPT) and *c-fos*, both of which are regulated by neuronal activity. In order to assess the adequacy of the stimulation parameters, electrophysiological studies done using whole-cell patch recording on primary dissociated cultures showed that action potentials can be reliably generated and the stimulation parameters that elicited single action potentials were identified. In cells loaded with fura-2 AM, stimulation produced an increase in $[Ca^{2+}]_i$, dependant on the frequency of stimulation.

We have adapted a method for quantitating mRNA using competitive PCR. Internal controls corresponding to deletion fragments of the genes of interest were constructed, enabling the use the same set of primers for the RNA and its control. PCR reactions contain 0.5μCi [^{32}P]-CTP and the signals are quantitated using phosphorimager technology. To quantitate the mRNA levels, the PCR product from the mRNA sample is compared with that from serial dilutions of known amounts of control RNA. We found co-amplification of the *c-fos* and PPT RNA and their respective controls are parallel over the studied range of 2 ng to 0.2 pg of starting SP6 RNA. The accuracy of this technique was determined by comparing it to quantitation using RNA blots. These findings indicate that competitive PCR used in this way is a reliable method of quantitation of RNA and can accurately determine mRNA levels from 15 ng total SCG RNA.

These techniques will allow the study of defined patterns of electrical activity and gene expression in rat superior cervical ganglion neurons.

566.9

DENDRITIC LOCALIZATION AND SYNAPTIC REGULATION OF MAP KINASE IN BRAIN. R.S. Fiore*, T.H. Murphy, V. Bayer, S.L. Pelech, J.A. Cooper, and J.M. Baraban. Dept. of Neurosci. Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

To assess the role of mitogen-activated protein kinase (MAPK) in neuronal systems, we have examined its localization in brain immunohistochemically and its regulation by neurotransmitters in primary cortical cultures. Light-microscopic studies reveal prominent staining of neuronal cell bodies and dendrites particularly in the hippocampal CA3 region and dentate gyrus, pyramidal cells in the cortex, and Purkinje cells in the cerebellum. At the ultrastructural level, intense staining is localized to dendritic microtubules, as well as the Golgi apparatus.

In primary cortical cultures, picrotoxin, which enhances spontaneous excitatory synaptic activity, induces tyrosine phosphorylation and activation of MAPK. Activation by picrotoxin occurs within 10 min and persists for the duration of a 30 min stimulation. Activation by picrotoxin is blocked by preincubation with tetrodotoxin. Both ionotropic and PI-linked glutamate receptor agonists stimulate MAPK activity suggesting that multiple pathways may mediate synaptic activation of this enzyme. Our findings that MAPK is enriched in neuronal dendrites and cell bodies in the brain and responds rapidly to synaptic activity point to a key role of MAPK in neuronal signal transduction.

566.10

ASSEMBLY AND PKC-PHOSPHORYLATION OF RC3(NEUROGRANIN) IN RAT BRAIN SYNAPTOSOMES AND VOLTAGE-CLAMPED XENOPUS OOCYTES. P.M. Coulter II, R.W.Cohen, J.E. Margulies and J.B. Watson*. Mental Retardation Research Center and Dept. of Psychiatry and Biobehavioral Sciences, UCLA School of Medicine, Los Angeles, CA 90024.

RC3 (neurogranin) is a forebrain-enriched 78 amino acid protein containing overlapping sites for protein kinase C (PKC) phosphorylation and calmodulin binding that are shared with GAP-43. In contrast to GAP-43's presynaptic localization, RC3 accumulates primarily in dendritic spines of forebrain neurons. Here we use combined biochemical and immunological methods to examine RC3's state of assembly and PKC-phosphorylation in brain synaptosomal fractions. Concurrent electro-physiological studies of RC3 heterologously expressed in Xenopus oocytes test the hypothesis that phosphorylated RC3 has a function in PKC-activated signal transduction pathways. Western blot studies: brain synaptosomal RC3 monomers and dimers are resistant to most biochemical treatments but are solubilized by non-ionic detergents (triton X-100, sarcosine), guanidine hydrochloride, and alkaline conditions. We conclude that RC3 is tightly associated with synaptosomal membranes presumably through ionic interactions with postsynaptic membranes. Immunoprecipitation experiments (in progress) may reveal linkage between RC3 assembly and PKC-phosphorylation. Voltage-clamp experiments: folliculated Xenopus oocytes expressing RC3 show enhanced responses to acetylcholine (2-3 fold), measured as calcium-activated Cl^- currents. RC3 enhanced acetylcholine responses are dampened to control levels by the PKC-inhibitor H-7. Phorbol ester-treated oocytes expressing RC3 exhibit significant calcium/chloride currents that are not observed in control oocytes. The cummulative data suggest that PKC-phosphorylated RC3 modulates calcium ion levels in dendritic spines of forebrain neurons.

566.11

ELECTROPHYSIOLOGICAL AND ANATOMICAL IDENTIFICATION OF CHOLINERGIC CELLS IN THE MEDIAL SEPTUM/DIAGONAL BAND. L. Taylor*, H.T. Chang[1], M.C. Jasek and W.H. Griffith. Dept. of Medical Pharmacol. & Toxicol. College of Medicine, Texas A&M University, College Station, TX 77843, and [1]Dept of Anat. and Neurobiol, College of Medicine, The Univer. of Tennessee, Memphis, TN 38163.

We have electrophysiologically and anatomically identified cholinergic cells in the MS/nDB using a double labelling technique. These results extend earlier work on identified cholinergic cells using acetylcholinesterase histochemistry. Intracellular recordings were made from MS/nDB neurons from adult guinea pig in vitro. Cells were identified based on their electrophysiological properties and then filled with intracellular injection of biocytin (1%, 10-60 min). The biocytin injected neurons were visualized by reaction with avidin-Texas Red. Cholinergic neurons (immunoreactive for choline acetyltransferase, ChAT) in the same sections were revealed using FITC-linked secondary antibody. All cells that exhibited a slow-afterhyperpolarization (700ms - 2s; S-AHP) stained positively for ChAT (n=13). In contrast, rapidly firing cells did not stain for ChAT (n=10). Two cells characterized as burst-firing were not cholinergic. Both excitatory and inhibitory postsynaptic potentials were generated in both cholinergic and non-cholinergic cells. [Supported AG07805 (WHG) and AG05944 (HTC)]

566.12

D_1-D_2 INTERACTIONS ON cAMP PRODUCTION IN CULTURED RAT STRIATAL CELLS. S.Schinelli*, M.Paolillo, S. Preda and G.L.Corona. Institute of Pharmacology, University of Pavia, 27100 Pavia, ITALY.

The simultaneous dopamine stimulation of D_1 and D_2 receptors triggers some functional responses whose biochemical basis have not been explained yet. Cultured striatal neurons may represent a suitable model to investigate this interaction. Papain dissociated striatal cells from 16-17 days old rat embryos were seeded in 12 multiwell plates at a density of two million cells/well, grown in 10% fetal bovine serum and then incubated for 10 days in vitro. Intracellular cAMP was measured by prelabeling cells with tritiated adenine; cells were then stimulated for 15' with D_1 and D_2 selective agonists and antagonists. D_1 receptors, when stimulated with SKF38393 1μM increased cAMP levels while D_2 receptors, after stimulation with LY171555, counteracted the forskolin-induced cAMP production. These effects were respectively blocked by the selective antagonists SCH23390 and sulpiride. When striatal cells were simultaneously treated with D_1 and D_2 agonists or dopamine neither synergism nor antagonism on cAMP production was observed, compared to stimulation with D_1 alone. These data seem to suggest that the biochemical basis of the proposed synergism between D_1-D_2 dopamine receptors imply the involvement of other second messengers signalling systems.

566.13

RAPID COMMUNICATION BETWEEN NEURONS AND ASTROCYTE SYNCYTIA IN PRIMARY CORTICAL CULTURES. J.M. Baraban*, L.A. Blatter, W.G. Wier, and T.H. Murphy. Johns Hopkins University Sch. of Med. and University of MD Sch. of Med., Baltimore, MD.

Although the electrophysiological properties of isolated astrocytes have been well studied, much less is known about how astrocytes and neurons communicate *in situ*. To investigate the physiology of neuron-astrocyte signalling, we have applied both calcium imaging and whole-cell recording techniques to primary cortical cultures to identify physiological activity in astrocytes related to neuronal firing. Although a subset of astrocytes displayed slow calcium transients, these did not appear to be related to neuronal activity. In contrast, whole cell voltage clamp recordings of identified astrocytes revealed fast inward currents that coincide closely with neuronal activity. Inclusion of lucifer yellow within patch pipettes confirmed that astrocytes were extensively coupled to each other but not to adjacent neurons. Currents coincident with neuronal firing were observed in dye-coupled astrocytes several hundred microns away from neuronal clusters. In contrast to astrocytes, other glutamate responsive, presumed nonneuronal cells that are not dye-coupled did not show currents coincident with neuronal activity. These findings suggest that astrocytes respond rapidly to neuronal activity and these electrical signals are propagated effectively through astrocyte syncytia.

566.14

UNITARY CONDUCTANCE AND VOLTAGE DEPENDENCE OF GAP JUNCTION CHANNELS BETWEEN DISSOCIATED PAIRS OF RAT PINEALOCYTES EXPRESSING CONNEXIN26. A.P. Moreno,* J.C Sáez, and D.C. Spray. Dept. of Neuroscience, A. Einstein Coll of Med., Bronx, NY, 10461.

Pinealocytes are neurosecretory cells found in the pineal gland that are responsible for the synthesis and release of melatonin. They are postsynaptic to sympathetic neurons and respond to norepinephrine with a rise in intracellular Ca^{2+} (see abstract by Sáez, et al). Previous studies have shown that these cells are electrically coupled by gap junctions, and that connexin26 (Cx26) is the primary gap junction protein. Using a dual whole cell voltage clamp method with patch-type electrodes, we have characterized the physiological properties of pinealocyte gap junction channels after dissociation of cell pairs. 25% of the cell pairs studied showed electrical coupling, with junctional conductance values between 200 pS and 2 nS. The unitary conductances of the junctional channels were 40-50 pS; in some cell pairs simultaneous openings of clusters of channels were recorded, suggesting a novel type of cooperativity between the channels. Voltage dependence of macroscopic junctional conductance (g_j) was slightly asymmetric; whereas transjunctional voltages (V_j) of both signs decreased g_j, V_0 (the V_j value at which the voltage sensitive component of g_j is reduced by 50%) was about ± 40 mV, and the equivalent gating charge was ~ 2.5, the proportion of g_j remaining at high voltages was about 0.3 for depolarization and about 0.5 for depolarization. These channel characteristics differ from those seen for other connexins expressed in other tissues, therefore providing additional evidence that the junctional protein may be connexin26.

566.15

RAT PINEALOCYTES: NOREPINEPHRINE INDUCES DESENSITIZING Ca^{2+} RELEASE FROM INTRACELLULAR STORES AND NONDESENSITIZING Ca^{2+} INFLUX FROM THE MEDIUM. J.C. Sáez, A.P. Moreno, L.C. Barrio*[1] and D.C. Spray. Dept. of Neurosc., Albert Einstein Coll. of Med., Bronx, NY 10461 & [1]Depto. de Invest., Hospital Ramón y Cajal, Madrid, Spain.

Synthesis and secretion of melatonin by mammalian *epiphysis cerebri* is elicited by norepinephrine (NE) secreted by sympathetic neurons. Changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) induced by NE were studied in rat pinealocytes using the Ca^{2+} indicator fura-2. Pineal glands were obtained between 4-6 PM from adult female or male Sprague-Dawley rats (150-200 g) maintained on a 12:12 light dark cycle. Glands were enzymatically dissociated, cells were plated on glass coverslips coated with polylysine and cultured in RPMI/F12 supplemented with 10% FCS as described (Brain Res. 1991;568:265-275). Cells were loaded with the Ca^{2+} indicator fura-2 by incubating them with fura-2AM (10 μM) for 30 min. Twenty four hour old cultures were used in all experiments and while recording, cells were perfused with medium at 30-34°C. Application of NE (10^{-6}M) induced a rapid and transient increase in $[Ca^{2+}]_i$ from ~100 nM to ~700 nM, followed by a decrease to a plateau level of around ~350 nM. In Ca^{2+}-free saline, the initial phase of the NE-induced $[Ca^{2+}]_i$ response was as large and rapid as in Ca^{2+}-containing saline but $[Ca^{2+}]_i$ returned to basal levels, suggesting that the initial phase involved Ca^{2+} release from intracellular stores. Upon repetitive application of NE the transient $[Ca^{2+}]_i$ response became progressively smaller until it was undetectable, indicating desensitization. Then, readdition of Ca^{2+} to the bath induced a rapid increase in $[Ca^{2+}]_i$ to ~350 nM, the plateau level, suggesting that nondesensitizing Ca^{2+} influx was involved in the plateau phase of the response. NE thus appears to increase $[Ca^{2+}]_i$ in pinealocytes by inducing 1) phasic Ca^{2+} release from intracellular stores, and 2) sustained Ca^{2+} influx from the extracellular medium.

567.1

THE VARIABILITY OF SYNAPTIC TRANSMISSION. A. Shirke, N. Otmakhov, A. Kay* & R. Malinow. Depts. of Physiology & Biophysics and Biology, Univ. of Iowa & Inst. of Theoret. and Experim. Biophysics, Russian Academy of Sciences.

The impact of probabilistic transmission on the input-output characteristics of individual neurons has received some theoretical, but little experimental evaluation. If action potential generation requires the summed input of a large percentage of the 1000+ synapses on an average CNS neuron, the total response will show little inter-trial variation even though individual synaptic events are variable.

In this study we investigate the trial-to-trial variability of postsynaptic responses in hippocampal neurons given a constant afferent stimulus that recruits sufficient presynaptic fibers to produce a postsynaptic action potential. We note a significant inter-trial variability that is of synaptic origin and can be modulated by physiological manipulations. Two identified cell populations have different variability at threshold.

Theoretically, pre- or postsynaptic modifications will have different effects on trial-to-trial variability. We find a decrease in inter-trial variability associated with LTP. If postsynaptic responses are returned to the threshold level by recruiting fewer fibers, the trial-to-trial variability returns to that observed before potentiation. We thus observe maintained variability of the input-output relations despite the large gain changes observed with LTP.

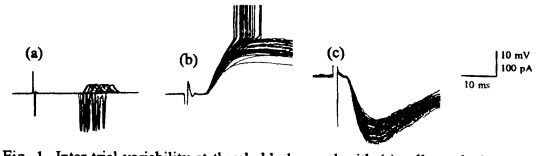


Fig. 1. Inter-trial variability at threshold observed with (a) cell-attached patch, and whole-cell recordings in (b) current-clamp and (c) voltage-clamp.

567.2

GLUTAMATE INDUCED LTP IN THE ABSENCE OF AFFERENT STIMULATION AND ENDOGENOUS NEUROTRANSMITTER RELEASE. R.J. Cormier*, M. D. Mauk and P. T. Kelly. Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77225.

Previous studies have shown that the induction of many forms of long-term potentiation (LTP) require presynaptic afferent stimulation. Using *in vitro* hippocampal slices we have tested the hypothesis that the induction of LTP requires evoked presynaptic activity. Repeated iontophoresis (5 pulses in 5 min) of glutamate in the stratum radiatum of CA1 produced LTP in the absence of afferent stimulation ("ionto-LTP"). Glutamate iontophoresis reliably induced LTP (56% increase of EPSP slopes, n = 5) which persisted at least an hour. Ionto-LTP was also induced in the absence of afferent stimulation in medium containing adenosine (200 μM). These adenosine conditions severely inhibit several neuronal processes, including transmitter release. Preliminary results indicate that the induction of ionto-LTP can also be achieved in the presence of TTX, under conditions in which EPSPs and presynaptic-fiber volleys were undetectable. Ionto-LTP was blocked when extracellular Ca^{2+} was chelated with EGTA, or when slices were pre-treated with MK-801. Thus, like LTP induced by high-frequency stimulation, ionto-LTP requires Ca^{2+} and activation of NMDA receptors. These results demonstrate that the induction of LTP by glutamate iontophoresis occurs in the absence of certain types of presynaptic activity and support the notion that presynaptic mechanisms are not required for the induction of ionto-LTP. Moreover, these results place constraints on the roles of presynaptic mechanisms and putative retrograde messengers in the persistent expression of LTP in that their involvement must be effective in the absence of certain forms of presynaptic activity. Indeed, these data suggest that application of a putative retrograde messenger should induce potentiation in the absence of presynaptic activity.

567.3

CAN HEBBIAN RISES IN CALCIUM PRODUCE LTP IN THE ABSENCE OF SYNAPTIC TRANSMISSION ? J.I. Gold and I. Mody*. Dept. of Neurology & Neurological Sciences, Stanford Univ. Sch. of Med., Stanford, CA.

The simultaneous activation of pre- and postsynaptic elements, and an accompanying rise in intracellular Ca^{2+} concentration, is a necessary condition for LTP induction. We have tested the hypothesis that simultaneous (Hebbian) rises of pre- and postsynaptic Ca^{2+} are sufficient to induce LTP in the absence of fast glutamatergic and GABAergic neurotransmission.

Extracellular recordings were done in the str. radiatum of the CA1 region of rat hippocampal slices maintained at 34-35°C. Schaffer collateral-commissural fibers were stimulated once every 30 s in the presence of 100 μM picrotoxin and the rate of rise of field EPSPs was recorded for a control period ranging between 15 and 30 min. The non-specific glutamate antagonist kynurenic acid (10 mM) was perfused for 5 min, resulting in the complete abolishment of synaptic field potentials. Once all synaptic activity was blocked, an orthodromic tetanus (100 Hz/0.5 s) was given, followed 200 ms later by an identical tetanus delivered via an antidromic stimulating electrode positioned in the str. oriens. Following washout of kynurenic acid, this stimulation paradigm resulted in a lasting and significant potentiation (126.3 ± 2.1% of control; 45-50 min following the tetani) of the EPSP's rate of rise. Either an antidromic or an orthodromic tetanus delivered alone did not potentiate the orthodromic EPSP. The potentiation was also blocked by raising the extracellular Mg^{2+} concentration from 2 mM to 4 mM, presumably as a consequence of reduced Ca^{2+} entry through voltage-gated Ca^{2+} channels. Our results are consistent with the hypothesis that a simultaneous rise in pre- and postsynaptic Ca^{2+} levels even in the absence of NMDA and non-NMDA receptor-mediated synaptic transmission is sufficient to induce long-term potentiation. Supported by NINDS grant NS 27528.

567.4

NMDA RECEPTOR ACTIVATION AT SYNAPSES ON CA1 NEURONS IS NOT NECESSARY FOR THE INDUCTION OF LTP. A. C. Field, S. J. Redman and C. Stricker*. Division of Neuroscience, JCSMR, Australian National University, Canberra, ACT 2601, Australia.

The induction of LTP at synapses on hippocampal CA1 neurons is believed to occur as a result of calcium influx through NMDA channels, since it is prevented by the NMDA receptor antagonist 2-amino-5-phosphono-pentanoic acid (AP5). However, an AP5-resistant component of LTP has been observed when the rate of tetanic stimulation of presynaptic fibres was increased to 200 Hz (1).

We have investigated the AP5-sensitivity of LTP induced by pairing low frequency (2 Hz) activation of small numbers of afferents with depolarization of the postsynaptic neuron to 0 mV, under whole-cell voltage clamp conditions and with caesium-based intracellular solutions, in CA1 pyramidal cells in 400 μm rat hippocampal slices. In the absence of AP5, an enhancement of EPSC amplitude (mean increase 80%) lasting at least 15 minutes was observed in 14/26 cells following pairing. When the bath contained 20 μM AP5, the same conditioning protocol produced a similar enhancement of EPSC amplitude (mean increase 65%) in 16/46 cells, and this potentiation could be maintained for at least 1 hr. Preliminary results indicate that AP5-resistant potentiation is specific to the synapses that were active during postsynaptic depolarization. Thus, NMDA receptor activation is not necessary for the induction of LTP in CA1 pyramidal cells by pairing when using the whole-cell recording technique.

1. Grover, L.M. & Teyler, T.J. (1990) *Nature* **347**: 477-479

567.5

Synaptic plasticity in the rat prefrontal-accumbens pathway studied in vitro. C.M.A. Pennartz*, R.F. Ameerun and F.H. Lopes da Silva, Dept. of Experimental Zoology, University of Amsterdam, Kruislaan 320, 1098 SM, Amsterdam, Netherlands.

In this study we examined what types of plastic changes can be found in the projection from prefrontal cortex to nucleus accumbens (Acb), a structure implicated in reward-dependent learning. Intracellular recordings were made in parasagittal slices (400 μm thick) from male Wistar rats. Stimulus electrodes were positioned at the border between infralimbic cortex and Acb. Picrotoxin (10 μM) was present in the bathing medium. Resting membrane potential, input resistance and action potential amplitude of the recorded neurons (N=75) were -78 ± 2 mV, 42 ± MΩ and 93 ± 1 mV. Tetanization (2 sec., 50 Hz) induced 3 types of changes in the AMPA/kainate receptor-mediated EPSP: long-term potentiation (21 cells), decremental potentiation (8 cells) and long-term depression (8 cells). Expression of LTP and LTD was not accompanied by changes in passive membrane properties. No correlation was found between the occurrence of potentiation/depression and various parameters of the tetanic depolarization (e.g. peak voltage, integral under curve). The NMDA receptor antagonist D-AP5 (50 μM) prevented LTP induction in 7 out of 9 slices that proved to be capable of producing LTP following D-AP5 washout. No difference in LTP was found between dopamine-treated slices (10 μM; N=15) and controls (N=14). Likewise, slices treated with the D1 receptor antagonist Sch 23390 (1 μM) and S(-)-sulpiride (1 μM; N=6) generated a similar amount of LTP as controls (N=10). In conclusion, both LTP and LTD can be induced in the prefrontal-Acb pathway. LTP strongly depends on NMDA receptor activity, but is not significantly affected by 10 μM dopamine.

567.6

LONG-TERM POTENTIATION OF PERFORANT-PATH INPUT TO CA3 PYRAMIDAL NEURONS IN HIPPOCAMPAL SLICES. *S.-F. Chen* & D. Johnston*, Dept. of Molecular Physiol. and Biophys. & Div. of Neuroscience, Baylor Col. of Med., Houston, Tx 77030.

Direct projections of perforant path (PP) fibers from entorhinal cortex to the hippocampal area CA3 have been demonstrated anatomically but their physiological properties have only recently begun to be investigated (Yeckel and Berger, 1990, *Proc. Natl. Acad. Sci. USA* 87:5832-5836). As a prelude to further study of the cellular events associated with distal dendritic inputs, we examined by field recordings and lesion methods whether direct PP input to CA3 can be selectively activated and whether action potentials and LTP can be evoked by this input in the rat hippocampal slice preparation.

In slices with part of CA1 and DG removed, paired-pulse facilitation could be recorded in S. lacunosome-moleculare (SLM) and S. pyramidale of CA3 by stimulating remnant SLM of CA1 or residual SM of dentate gyrus (DG). The latencies of the pEPSPs were ≈3 ms, which is consistent with a monosynaptic response. Lesion of the PP termination zone between stimulating and recording electrodes completely blocked the pEPSPs as did application of DNQX (10μM) plus D-APV (50μM). These results suggested that the PP has functional monosynaptic input to CA3 pyramidal neurons via SM of both CA1 and DG in the slice preparation.

Tetanizing stimuli of 400Hz (0.1s, ×10) delivered to the outer third of SM of DG could induce a 122±6% (S.E.) increase in the slope of the pEPSP in intact slices (n=3), but only a 56±16% (S.E.) increase in slices with a cut between CA3 and DG (n=5). The induction of the LTP could be blocked in D-APV (50μM) solution. These results suggest that LTP of the PP inputs to CA3 can be observed in the slice preparation and at least a component of the LTP can be blocked by D-APV. (MH44754,MH48431)

567.7

L-TYPE CALCIUM CHANNELS ARE INVOLVED IN THE INDUCTION OF NMDA RECEPTOR-INDEPENDENT LONG-TERM POTENTIATION (LTP) IN ADULT RAT VISUAL CORTEX. V.A. Aroniadou*, A. Maillis, E. Koutsoukos, E. Angelopoulos, T.J. Teyler and C. Stefanis. Univ. of Athens, Dept. of Psychiatry, Eginition Hospital, 11528 Athens, Greece, and Northeastern Ohio Coll. of Med., Rootstown, OH 44272 U.S.A.

In visual cortical slices from Wistar rats (age 60-80 days) the field potential in layer III elicited by white matter stimulation consisted of a negative component (N1) with peak latency 4-8 msec, which was unaffected by bath applied APV. In a few slices a second component was present (peak 12-19 msec) which was insensitive to APV in most of these slices. Bath applied DNQX blocked N1 and revealed the presence of an APV-sensitive component (peak 9-20 msec). Tetanic stimulation in normal medium induced LTP (138-385%) of N1 in 72% of the slices. In the presence of APV, LTP of N1 (145-279%) was induced in 75% of the slices. Potentiated responses were unaffected by APV. Bath application of the dihydropyridine antagonist nifedipine did not affect control responses. Following tetanic stimulation in the presence of nifedipine no change or long-term depression was observed in 80% of the slices, while a small magnitude LTP (130-150%) was induced in the remaining slices. The known reduction of NMDA receptor activity with age is accompanied by an increased importance of voltage-gated Ca++ channels in maintaining synaptic plasticity.

567.8

DEXAMETHASONE BLOCKS THE INDUCTION OF HIPPOCAMPAL PRIMED BURST POTENTIATION. David M. Diamond*, Berrilyn Branch, M. Catherine Bennett, Monika Fleshner and Gregory M. Rose. Department of Pharmacology, UCHSC and VAMC, Denver, CO 80262

There is a negative correlation between corticosterone (CORT) levels and PB potentiation, a form of long lasting hippocampal plasticity induced by physiologically patterned stimulation (*Psychobiol.*, 19:301, 1991). In the present study, we investigated the possibility that this effect was mediated by the Type II glucocorticoid receptor.

We stimulated the hippocampal commissure and recorded CA1 population spikes in urethane-anesthetized adrenalectomized rats. PB stimulation (5 pulses: a priming pulses followed 170 ms later by a burst of 4 pulses at 200 Hz) was delivered 3-4 hours after administration of dexamethasone phosphate (DEX; 300-900 μg, i.p.), a selective Type II agonist. A dose-dependent inhibition of the incidence and magnitude of PB potentiation by DEX was observed. PB depression, a lasting decrease in the amplitude of the population spike, occurred at the 900 μg dose. PB depression was originally identified in animals with high levels (> 60 μg/dl) of CORT. Therefore, DEX mimicked the effects of elevated levels of CORT.

These findings suggest that occupation of the glucocorticoid Type II receptor results in inhibition of hippocampal plasticity. Our observations imply a role for CORT in mediating the effects of stress on hippocampus-dependent learning.

567.9

EFFECT OF TRIMIPRAMINE, AN ANTIDEPRESSANT, ON HIPPOCAMPAL SYNAPTIC PLASTICITY. G. MASSICOTTE*, M. OHAYON AND J. BERNARD. Lab. of Neurobiology, Université du Québec à Trois-Rivières, Québec, Canada, G9A 5H7

The effect of trimipramine (TRIM), an antidepressant agent, on both the induction and the maintenance of long-term potentiation (LTP) was investigated in field CA_1 of hippocampal slice preparation. Chronic administration (7-9 days) of TRIM in rat caused a large reduction in the magnitude of LTP induced by a theta burst stimulation (TBS) paradigm. Trimipramine had no significant effect on either the degree of facilitation in postsynaptic responses occurring during TBS or the amount of paired-pulse facilitation. Furthermore, the facilitation of postsynaptic responses occurring in the first two minutes following the high frequency stimulation was not reduced by trimipramine administration. These results indicate that TRIM interfere with the formation of LTP; an effect that is not due to alteration of physiological event that triggers LTP. The data suggest that the loss of LTP maintenance is more likely the result of the disruption by trimipramine of cellular processes that follow LTP induction. In addition, the present results provide evidence for a possible correlation between reduction in LTP expression and learning deficit produced by chronic administration of trimipramine.

This work was supported by NSERC of Canada.

567.10

PATHWAY SPECIFICITY OF METOPROLOL-INDUCED PLASTICITY IN THE DENTATE GYRUS. *R. D. Kirkby*, M. R. Pelletier, and M. E. Corcoran. Dept. of Psychology, U. of Victoria, BC, Canada, V8W 3P5.*

Previous research has demonstrated that application of β-noradrenergic agonists in vitro can produce long-lasting potentiation (LLP) of field responses evoked in the dentate gyrus (DG) by stimulation of the medial perforant path (PP) and long-lasting depression (LLD) of responses evoked by stimulation of the lateral PP [Dahl & Sarvey, PNAS, 1989; Pelletier, Kirkby & Corcoran, Soc. Neurosci. Abstr., 1991]. We report here that β-noradrenergic blockade produces LLD of responses evoked by stimulation of the medial PP and LLP of responses evoked by stimulation of the lateral PP.

Transverse hippocampal slices from male hooded rats were placed in an interface chamber, and field EPSPs were recorded from the outer or middle molecular layer. After stable baseline responses were obtained, the β,-adrenergic antagonist (±)-metoprolol (+)-tartrate (MET; 20 μM) was bath applied for 30 min, followed either by a 30 min wash and tetanization (TET) or by a 60 min wash with no TET.

MET produced LLD and LLP of responses evoked by stimulation of the medial PP and the lateral PP, respectively, that persisted for the 60 min wash. TET of the medial PP potentiated evoked responses back to baseline levels, above the depression produced by MET. TET of the lateral PP produced additional potentiation above that produced by MET.

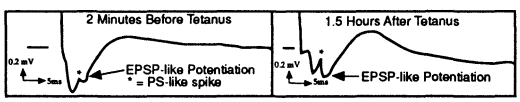
These results provide further evidence for the pathway specificity of β-noradrenergic plasticity of responses evoked in the DG, and they also suggest that antagonism of β-adrenoceptors has long-lasting effects on synaptic transmission in the DG.

567.11

WIDELY DIVERGENT LONG TERM POTENTIATION OF POPULATION SPIKE AND EPSP IN VISUAL CORTEX OF THE ADULT RAT IN VIVO. X. Wang* and W.T. Greenough, Neurosci. Prog., Beckman Inst., Univ. of Illin., Urbana, IL 61801.

Previous studies have shown that LTP in layer II/III of visual cortex is difficult to induce and of a limited size (in terms of percent change in amplitude of field potentials) in adult rats (Perkins & Teyler, 1988; Kato et al., 1991). Moreover, seemingly identical stimulation regimes may induce either long term depression (LTD) or LTP (Berry et al., 1989). Here, we present a new form of LTP in adult rat visual cortex. In our experiments, extracellular recording electrodes were placed in layer II/III of rat visual cortex, while stimulating electrodes were located in the white matter lateral to the recording electrode. Evoked responses were examined only if population spike-like spikes (PS-like spike,onset latency from 3 ms to 7.5 ms), EPSP-like potentials (peak before 12 ms) and long-lasting inhibitory responses following EPSP-like potentials all were present. We find that PS-like spike amplitudes were increased to about 200% (some times more) of control levels after tetanus in most cases. In many cases the onset latency of PS-like spikes was shortened after tetanus. In contrast, the sizes of EPSP-like potentials showed decreases (7 cases), no clear changes (4 cases) or an increase (1 case). Thus, there is a mismatch between EPSP-like potentials and PS-like spikes during cortical LTP. Many cases could be viewed as indicating LTD if only the EPSP-like potentials were measured. However, if only the amplitude of the PS-like spike is considered, we find that there is still a substantial apparent induction of LTP. Because of this mismatch, the measurement of PS-like spikes may be a more sensitive measurement of LTP in visual cortex.

Supported by MH35321 and NSF BNS-88-21219



567.12

EFFECTS OF BURST STIMULATION ON NEIGHBORING SINGLE CA1 NEURONS IN RAT HIPPOCAMPUS. P.D. Martin, N. Lake*, M.L. Shapiro. Depts. of Psychology & Physiology, McGill University, Montreal, Quebec, Canada, H3A 1B1.

The effects of burst stimulation (ten 25 ms bursts of 400 Hz pulses at 200 ms intervals) on single CA1 neuron activity were recorded with stereotrodes placed in the pyramidal layer of CA1 and stimulating electrodes implanted in the contralateral CA3 of urethane anesthetized rats. Individual units were distinguished by waveform parameters, and the firing pattern of each unit was analyzed separately. The latency of evoked unit firing was commensurate with the latency of evoked field potentials. Four of nine discriminated cells fired spontaneously. Five cells fired upon stimulation, and no cells fired both spontaneously and upon stimulation. Burst stimulation increased field EPSP amplitude (>30 min) and decreased spontaneous firing in three of four spontaneously firing cells. Of the five cells that displayed evoked firing, burst stimulation increased the firing probability in one cell, reduced the firing probability in one cell, and had no effect on three cells. Thus, neighboring cells recorded simultaneously were affected differentially by burst stimulation. Changes in both feed forward excitatory and inhibitory connections may contribute to the pattern of altered neural responses produced by burst stimulation and LTP induction.

567.13

LONG-TERM POTENTIATION OF THE IPSILATERAL-ASSOCIATIONAL PATHWAY IN THE RAT DENTATE GYRUS. P.A. Hetherington*, K.B. Austin, and M.L. Shapiro. Dept. of Psychology, McGill Univ., Montreal, Quebec, Canada, H3A 1B1.

Mossy fibers from granule cells in the dentate gyrus of the rat hippocampus project to mossy cells in the hilar region, which in turn make excitatory synapses in the inner one-third of the molecular layer of the dentate gyrus. To relate physiological and anatomical properties of this excitatory feedback system, multiple electrodes (100μ, tungsten rod) were located along the longitudinal axis of the dorsal leaf of the dentate gyrus in the urethane-anesthetized rat (2.0 g/Kg, Long Evans). Single pulses delivered to the hilar region evoked negative-going, mono-synaptic electrical field potentials (EFP s) in the inner one-third of the molecular layer. These EFP s were recorded simultaneously at four locations, the peak amplitude of which varied linearly with distance from point of stimulation, and could be elicited either rostral or caudal to the stimulating electrode. A stimulus train (ten 25ms bursts of 400 Hz pulses at 200ms intervals) was delivered to the hilar region, and the initial slopes of the recorded EFP s were potentiated (21%). Potentiation lasted at least two hours and was specific to responses from the tetanized stimulating electrode: the responses to a second stimulating electrode in the hilus and a third in the angular bundle of the perforant path did not change. The results suggest that the ipsilateral association system of the dentate gyrus supports long-lasting and synapse-specific changes in electrical response.

567.14

FURTHER EVIDENCE THAT MOSSY FIBER POTENTIATION IS EXPRESSED BY A CHANGE IN RELEASE VARIABLES. U.Staubli*[1], J.Larson[2] and G.Lynch[2]. [1]Dept. Psychology, McGill University, Montreal, H3A 1B1; [2]Center for Neurobiology of Learning & Memory, Univ. California, Irvine, Ca 92717.

Previous studies have shown that paired-pulse facilitation, a presynaptic effect, is markedly reduced by mossy fiber potentiation (MFP) but is unaffected by LTP. Conversely, the nootropic drug aniracetam which selectively increases currents mediated by the AMPA subclass of glutamate receptors, has smaller effects on the amplitude of synaptic responses following induction of long-term potentiation (LTP) in field CA1 but not after induction of MFP. This pattern of results indicates that expression of MFP involves distinctly different changes than LTP.

Subsequent studies have found that aniracetam acts by prolonging the open time of the AMPA receptor channel (Tang et al., 1991) and thereby changes the waveform of synaptic responses, an effect that is altered in a characteristic fashion after induction of LTP: the drug i) produces a smaller increase in the amplitude of potentiated responses, ii) shifts the initial slope of the potentiated response to the right and iii) is delayed in its facilitatory effect in the rising phase of the potentiated EPSP. These results are consistent with the hypothesis that LTP is due to modified glutamate receptors.

Here we report data from intracellular recordings of CA3 neurons to afferent stimulation of mossy fibers and show that the effects of aniracetam on the peak amplitude, displacement of initial slope and onset of response facilitation were not detectably different in seven control slices and 4 slices with mossy fiber potentiation. These results are consistent with the hypothesis that mossy fiber potentiation reflects a change in release variables.

567.15

LTP CHANGES THE WAVEFORM OF SYNAPTIC RESPONSES: PROXIMAL VERSUS DISTAL APICAL DENDRITIC SYNAPSES. A. Kolta,* J. Larson, P. Xiao, & G. Lynch. CNLM, Univ. of Calif., Irvine, CA 92717

In a previous study, we demonstrated that long-term potentiation (LTP) was accompanied by a decrease in the decay time constant of excitatory synaptic field potentials in field CA1 of hippocampus, suggesting that potentiation alters the mean open time of AMPA-type glutamate receptor channels. However, the degree to which synaptic location affects measured time constants or correlates with channel kinetics is not known. In the present experiments, we compared field potentials evoked by stimulation of axons at proximal and distal levels of the apical dendritic tree, and measured waveform parameters of responses recorded at corresponding locations during paired pulse facilitation (PPF) and LTP.

Excitatory responses in minislices of field CA1 were isolated by blocking GABAergic responses with picrotoxin and 2-hydroxysaclofen. Decay time constants (DTCs) were measured by exponential fitting. LTP was induced by theta burst stimulation and PPF was measured at inter-pulse intervals of 85 msec.

Under control conditions, responses were larger when recorded locally (at the same proximal-distal position as the stimulation), indicating that the stimulation electrodes activated spatially distinct synapses. DTCs were longer for responses recorded proximally, regardless of stimulation locus. PPF had no effect on DTCs; however, LTP significantly reduced the DTC for all responses. LTP was larger when recorded proximally, regardless of stimulation position; PPF was not affected by either stimulation position or recording position.

Comparison of PPF with LTP provides further support for the hypothesis that LTP involves changes in postsynaptic receptors. PPF did not change response DTCs whereas LTP did. Differences between proximal and distal synapses suggest spatial variations in LTP induction and/or expression mechanisms.
(Supported by ONR #N00014-89-J-1255 and FRSQ and NSERC of Canada.)

567.16

REVERSAL OF LTP BY STIMULATION AT THE THETA FREQUENCY. J. Larson,* P. Xiao, & G. Lynch. CNLM, Univ. of Calif., Irvine, CA 92717

Long-term potentiation (LTP) is optimally elicited by brief high frequency bursts repeated at the frequency of the endogenous limbic theta EEG rhythm (5 Hz). The present experiments tested the effects of single pulses repeated at the theta frequency on control and potentiated synapses; the results indicate that theta frequency stimulation shortly after LTP induction causes a reversal of LTP.

Stimulation electrodes were placed in the CA1 field of hippocampal slices to activate two independent sets of Schaffer/commissural fibers; field EPSPs were recorded in the apical dendritic region. LTP was induced by theta burst stimulation (4-pulse, 100 Hz bursts repeated at 5 Hz). Theta pulse stimulation (30-60 sec. of 5 Hz stimulation) was used to reverse LTP.

In control medium, a 1-min. episode of 5 Hz stimulation beginning 1-3 min. after LTP induction had no effect on the degree of LTP measured 30 min. later. However, in the presence of norepinephrine (200 μM), 5 Hz stimulation reduced LTP by 29% ($\pm$7). Theta pulse stimulation was only effective when administered within 10 min. of LTP induction and had no lasting effects on control synapses. Stimulation at 1 Hz did not reverse LTP and stimulation at 10 Hz was no more effective than 5 Hz stimulation. LTP could be nearly completely reversed (86% $\pm$15) by theta pulse stimulation when potentiation was induced by milder and more naturalistic stimulation patterns (burst pairs) . Under these conditions, LTP reversal was blocked (only 18% $\pm$8) by an adenosine A_1 receptor antagonist (8-cyclopentyl-1,3-dipropylxanthine, 200-350 nM).

These results suggest that the hippocampal theta rhythm promotes both the induction of LTP and its subsequent reversal with the latter process being facilitated by norepinephrine and involving adenosine receptors. LTP reversal may function to refine or sharpen recently encoded representations.
(Supported by ONR #N00014-89-J-1255.)

567.17

FACTORS REGULATING THE MAGNITUDE OF LONG-TERM POTENTIATION INDUCED BY THETA PATTERN STIMULATION. A.Arai* and G.Lynch. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717-3800.

Electrical stimulation patterned after the hippocampal theta rhythm produces robust and stable long-term potentiation (LTP). The present studies sought to relate response parameters occurring during theta stimulation to the degree of LTP following it. Comparisons were made using 5 or 10 burst stimulation which respectively induce sub-maximal or near maximal degree of LTP in field CA1. An adenosine A1 receptor antagonist (DPCPX) was used to enhance the depolarization produced by individual theta bursts; this markedly increased the amount of stable LTP induced by 5 theta bursts but did not affect that resulting from 10 bursts. Similar effects on the size of the burst response and on the degree of LTP were obtained with aniracetam, a nootropic drug acting on AMPA receptors. Forskolin, an activator of adenylate cyclase, blocked the hyperpolarization present between theta bursts. This drug also augmented the degree of LTP resulting from 5 theta bursts, however, in contrast to DPCPX and aniracetam, forskolin nearly doubled that obtained with 10 bursts. These results raise the possibility that the magnitude of NMDA receptor mediated currents affects the degree of potentiation produced by individual theta bursts while the duration of the currents sets a limit on the maximum LTP induced by a series of bursts. (Supported by Grant #N00014-89-J-1255 from the Office of Naval Research).

567.18

PRIMING OF ASSOCIATIVE LTD IN THE DENTATE GYRUS BY THETA-FREQUENCY SYNAPTIC ACTIVITY.
B. R. CHRISTIE * AND W. C. ABRAHAM
Department of Psychology and the Neuroscience Research Centre, University of Otago, Dunedin, New Zealand

The present study evaluated associative (ASS) and non-associative (N-ASS) LTD in the lateral (LPP) perforant path projections to the dentate gyrus in barbiturate anaesthetized rats. ASS synaptic interactions were investigated using short 100 Hz trains delivered at 5 Hz to the MPP in association with single pulses to the LPP, interleaved between the MPP trains. N-ASS interactions were studied using the MPP conditioning trains alone. Paired-pulses (50 ms interpulse interval) were administered prior to and 30 minutes following the application of the conditioning stimuli.

Normally only non-associative, NMDA-dependent (N-methyl-D-aspartate), LTD is elicited in naive LPP pathways with either protocol (Christie and Abraham, Synapse 10: 1-6, 1992). LTD is significantly increased in the LPP by the ASS protocol (ASS: -40$\pm$3%, N=5; N-ASS: -13$\pm$4%, N=13; $t_{(16)}$=3.8, p<0.05), however, after being "primed" by a brief period of synaptic activity at a theta rhythm frequency (5 Hz). 5 Hz priming of ASS LTD lasts at least 2 hrs, and significantly enhanced LTD is not seen following priming activity at 1 Hz (-24$\pm$9%, N=7) or 15 Hz (-13$\pm$4%, N=5), nor when the N-ASS protocol is administered following 5 Hz priming (-9$\pm$9 %, N=5). NMDA-receptor activation is critical for establishing the priming effect, but not for the subsequent induction of ASS LTD. The ASS, but not N-ASS, LTD is also accompanied by an increase in paired-pulse facilitation following its induction (ASS:15$\pm$4%, $t_{(4)}$=3.6, p<0.05). This suggests that ASS and N-ASS LTD involve different expression mechanisms, and that ASS LTD may involve a decrease in presynaptic transmitter release.

567.19

INPUT ASYNCHRONY PROLONGS THE RISING PHASE OF MOSSY FIBER-EVOKED EPSCS IN RAT HIPPOCAMPAL CA3 PYRAMIDAL CELLS. R. B. Langdon*, J. W. Johnson, and G. Barrionuevo, Depts. of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260

Investigation of LTP at mossy fiber (MF) synapses depends upon discrimination of monosynaptic, MF-evoked EPSCs (MF EPSCs) from higher order EPSCs, based upon expectations for MF EPSC kinetics. Using whole-cell recording, we have examined EPSCs evoked by stimuli applied to the *s. granulosum*. Rise-time durations ranged from 0.7 to 5.0 msec (mean = 2.5 msec; N = 27). Most rising phases were inflected, implying asynchronous arrival of inputs. Hypothetically, this could be due to "contamination" via activation of local axon collaterals. If so, then generalized suppression of neurotransmission should alter not only the amplitude but also the shape of waveforms. However, exposing slices to CNQX + APV, or low $[Ca^{++}]$, high $[Mg^{++}]$ medium (using a submersion-type slice chamber) did not shorten long rise-times or remove inflections (N = 5; amplitudes were reduced 64 to 88%).

We examined the kinetics of MF impulse activity by recording (from slices in medium that blocked neurotransmission) the extracellular potential generated by action currents in MFs (the MFV). Typical MFVs lasted 3 to 4 msec, with long and complex negative phases, implying impulse asynchrony (N = 15). This temporal dispersion could be the result of (*hypoth.1*) dispersion of MF conduction velocities, (*hypoth.2*) dispersion of conduction distances, or (*hypoth.3*) unconventional MF physiology. We dismiss hypoth.1 because antidromically activated granule cell population spikes broaden only slightly as a function of distance between stimulating and recording sites. Hypoth.2 would contribute to asynchrony if impulses travelled antidromically in hilar MF collaterals, then orthodromically in MFs. However, lesions that would interrupt such impulses did not produce brief, simple (triphasic) MFV waveforms. Therefore, long and inflected MF EPSC rising phases may be due to intrinsic physiology of MF axons or their boutons. Support: NS24288 and MH45156.

567.20

QUANTAL ANALYSIS IN THE CA3 REGION OF THE HIPPOCAMPUS BY MAXIMUM LIKELIHOOD ESTIMATION. S. Smerin*, R.B Langdon, D. Henze, G. Barrionuevo, and T.R. Chay. *Departments of Behavioral Neuroscience and Biology, University of Pittsburgh, Pittsburgh, PA 15260.*

Quantal analysis by the method of maximum likelihood estimation was applied to the mossy fiber synapse and the fimbrial synapse, both of which are on the CA3 pyramidal neuron of the hippocampus. The hippocampus of the rat was sliced and maintained *in vitro*. Stimulating electrodes were placed in the fimbria and in the *s. granulosum* of the dentate gyrus. Two hundred to 500 EPSCs evoked from each stimulation site were collected in whole-cell voltage clamp configuration. The probability of observing the peak EPSC amplitudes was formulated into a likelihood function under the hypothesis that the probability of release (**p**) of the quantum follows a simple binomial distribution and the amplitude (**a**) of the quantum is normally distributed. We obtained the values of **p** and **a** by maximizing the likelihood function using an algorithm on the Pittsburgh Supercomputer Center's Cray-YMP.

At the mossy fiber synapse we found that **p** = 0.7-0.8 and **a** = 9-12 pA, and at the fimbrial synapse **p** = 0.3-0.9 and **a** = 9-10 pA. In this initial study all estimations assumed that the number of release sites was ten. Higher numbers of release sites (up to 600 for the mossy fiber synapse, and up 10,000 for the fimbrial synapse) will be assumed for future estimations. Quantal analysis by maximum likelihood estimation is now being applied before and during the induction of LTP at these synapses. Supported by NS24288.

567.21

QUANTAL ANALYSIS OF PLASTICITY IN RAT HIPPOCAMPAL MOSSY-FIBER SYNAPSES. A.C. Greenwood*[1], Z.Xiang[2], E.M. Landaw[3], E.W. Kairiss[2], and T.H. Brown[1,2]. Depts. of Cell. & Mol. Phys.[1] and Psych.[2], Yale Univ., New Haven, CT 06520 and Dept. of Biomath.[3], Univ. of Calif., Los Angeles, CA 90024.

We have developed a maximum likelihood (ML) approach to quantify uncertainty within the context of several testable models. Systematic Monte Carlo simulations amplify the method's power and justify the application of ML theory to each specific data set.

These methods of quantal analysis were applied to rat hippocampal mossy-fiber (mf) synapses, which offer a number of advantages for studies of synaptic microphysiology (Siegel et al, *Soc. Neurosci. Abstr. 18*, in press; Yu and Brown, *Soc. Neurosci. Abstr. 18*, in press; Jaffe and Brown, *Soc. Neurosci. Abstr. 18*, in press; Xiang et al, *Soc. Neurosci. Abstr. 18*, in press). Whole-cell voltage-clamp recordings were made of evoked currents that satisfied appropriate criteria for mf origin (Xiang et al, *Soc. Neurosci. Abstr. 18*, in press).

In a study of mf paired-pulse facilitation, a mf input was stimulated twice in rapid succession for several hundred trials. For various measures, the first and second response amplitude distributions could be well-fit by a Poisson or binomial probability function for quantal release and a gamma or Gaussian probability density function for quantal size. The mean quantal size did not increase with facilitation. Facilitation resulted, instead, from an increase in the mean quantal content. We are currently investigating the quantal basis of long-term potentiation in mf synapses. *Supported by NIH and ONR.*

567.22

MEASUREMENT AND ANALYSIS OF HIPPOCAMPAL MOSSY-FIBER SYNAPSES. Z. Xiang*[1], A.C. Greenwood[2], and T.H. Brown[1,2]. Depts. of Psych.[1] and Cell. & Mol. Physio.[2], Yale Univ., New Haven, CT 06520

The hippocampal mossy-fiber (mf) synapse is attractive for neurophysiological and optical studies of plasticity because of its large size (Yu and Brown, *Soc. Neurosci. Abstr. 18*, in press; Jaffe and Brown, *Soc. Neurosci. Abstr. 18*, in press) and electrotonic proximity to the soma (Siegel et al, *Soc. Neurosci. Abstr. 18*, in press). The complex circuitry of the CA3 region of the hippocampus, however, makes it difficult to excite the mf synapses selectively when using extracellular microstimulation.

We have devised a set of procedures and criteria for isolating and identifying mf excitatory postsynaptic currents (EPSCs) (Xiang et al, *Soc. Neurosci. Abstr. 17*, 1991; Claiborne et al, submitted). Here we report results of whole-cell recordings of mf EPSCs from rat hippocampal slices that satisfy these criteria and procedures. Slices were cut on a vibratome (450 µm thick) in the plane of the mf projection system (Yu and Brown, *ibid*). An attempt was made to activate unitary mf inputs by delivering small stimulating currents through optimally positioned 25 µm bipolar stimulating electrodes. The patch pipette access resistance was less than 15 MΩ.

The mf peak conductance averaged about 1.2 - 4.7 nS and the net charge transfer from a -80 mV holding potential averaged about 1.3 - 3.4 pC. For quantal analysis (Greenwood et al, *Soc. Neurosci. Abstr. 18*, in press) we explored several response measures, including the peak current, slope of the rising phase, total charge, and partial charge. The fit of quantal models and the estimated quantal parameters depended on the response measure. *Supported by NIH and ONR.*

567.23

HEBBIAN LEARNING IS JOINTLY CONTROLLED BY ELECTROTONIC AND INPUT STRUCTURE. B.A. Pearlmutter*[1] and T.H. Brown[1,2]. Departments of Psychology[1] and Cellular & Molecular Physiology[2], Yale Univ., New Haven, CT 06520.

Hebbian learning in a linear isopotential neuron with instantaneous response has been shown to cause the synaptic weights to tune to the principal eigenvector of the instantaneous input correlation matrix Q, $Q_{ij} = \langle \xi_i \xi_j \rangle$ (Amari 1977 *Bio. Cyb.*; Oja et al 1985 *J. Math. Anal.& Appl.*) Here, we show that in the nonisopotential case, the important matrix is $\hat{Q}$, $\hat{Q}_{ij} = \int Q_{ij}(s) A_{ij}(s) ds$, where $Q_{ij}(s) = \langle \xi_i(t) \xi_j(t-s) \rangle$ is the cross-temporal correlation of the input and A the matrix of Green's (electrotonic transfer) functions of the neuron. That is, if a unit charge is injected at synapse j, $A_{ij}(s)$ is the voltage change recorded s seconds later at synapse i.

If $\hat{Q}$ has a principal eigenvector then the weights tune to it. This is qualitatively similar to the isopotential situation, but with the correlation matrix smeared and skewed in both space and time. But because $\hat{Q}$ is asymmetric, it need not have a principal eigenvector. In that case, $\hat{Q}$ must perform a rotation on the 2D principal eigenspace, and the weights tune to this space and rotate within it.

The theory can be applied to a cable in order to derive a characteristic length scale of cluster formation when confronted with unpatterned input, thereby predicting a relationship between lamination scale and the dendritic length constant at the input's temporal frequency. *Supported by ONR and DARPA.*

567.24

HETEROSYNAPTIC POST-TETANIC DEPRESSION (PTD) IN AREA CA1 OF ADULT RAT HIPPOCAMPUS: RESTRICTION TO SPECIFIC DENDRITIC DOMAINS. L.M. Grover and T.J. Teyler. Neurobiology Dept., N.E. Ohio Univ. Col. Med., Rootstown, OH 44272.

Tetanic stimulation of afferents to area CA1 can induce heterosynaptic PTD. Tetanic stimulation of one input pathway (25 Hz, 15 sec) reduced EPSPs evoked by test stimulation of a second input pathway 60-80%, lasting 4-5 min, when both input pathways converged on the same dendritic domain (apical or basilar dendrites). When inputs did not converge on the same dendritic domain, PTD averaged only 20-25%. This pattern of results was seen for both K/APMA and NMDA receptor mediated EPSPs. Tetanic stimulation was followed by a post-tetanic hyperpolarization (PTH) of 3-4 mV, lasting 1-2 min. PTD was not prevented by 20 µM DNQX, 50 µM AP5, 500 µM AP3, 500 µM CGP 35348, 3 mM Cs^+, or internal BAPTA and internal Cs^+. PTH was blocked by DNQX, and external and internal Cs^+, but not by by AP5, AP3, CGP 35348, or internal BAPTA. PTD may be presynaptic in origin and induced by limited diffusion of an external messenger.

567.25

SHORT & LONG-TERM SYNAPTIC DEPRESSION IN THE NEOSTRIATUM. E. C. Tyler*, D. M. Lovinger and A. Merritt. Dept. of Mol. Physiol. Biophys., Vanderbilt Med. Sch., Nashville TN 37232.

The neostriatum, a part of the basal ganglia involved in motor control and cognitive function, receives a large glutamatergic input from the cortex. Synaptic plasticity at corticostriate synapses has not been well characterized. Using patch/slice and field potential recordings in slices of 2-4 wk old and adult rat neostriatum, we have found that high frequency stimulation of glutamatergic afferents leads to both short- and long-term depression of synaptic transmission. Studies were done using sustained trains (4, 100Hz trains 1s duration, 1/10s) and bursts (5 pulses at 100Hz given 5/s for 5s). The burst paradigm was included because cortical neuron firing probably more closely resembles bursting than sustained trains. Field potential studies showed that following the train paradigm there was a decrease to 45.1±6.2% (n=23) of control in the population spike (PS) evoked by single stimuli while the burst paradigm showed a decrease to 49.7±13.3% (n=7) of control. The mean time to recovery was 7.4±2.3 min in the train paradigm and 4.4±2.4 min in the burst paradigm. Overall 30% of slices showed depression that lasted >20 min. Patch/slice studies showed a decrease to 55.4±9.5% (n=7) of control in the EPSP following sustained trains and to 68.75±14.72% (n=4) of control after bursts. For all slice/patch cells that showed depression the mean recovery was to 80.6±6.9% of control and this occured within 5-15 min. For cells that recovered fully the mean time to recovery was 2.7±.25 min. 47% of cells showed depression lasting >10 min. It was also observed that phorbol diacetate (PDAc) inhibits the decrease in the PS after trains. Without PDAc there was a reduction to 53±5.6% of control but with PDAc a reduction to 95±9% of control was seen (n=5).

567.26

PRIOR SYNAPTIC ACTIVITY ENHANCES THE INDUCTION OF LONG-TERM DEPRESSION (LTD) IN HIPPOCAMPUS E.M. Wexler* and P.K. Stanton. Dept. Neuroscience, Albert Einstein Coll. Med., Bronx, NY 10461

Long-term potentiation (LTP) is an extensively studied form of synaptic plasticity and putative memory mechanism. While constraints on LTP amplitude are often assumed, it is still unclear whether LTD could play a role in either memory processing or regulating dynamic system stability. Associative LTD can be elicited at Schaffer Collateral-CA1 synapses when a low-frequency input is negatively correlated in time with a separate high-frequency train of bursts. In contrast to LTP, associative LTD is not blocked by antagonists of N-Methyl-D-Aspartate (NMDA) receptors. Instead, the induction of this form of LTD is blocked by 2-amino-3-phosphonopropionic acid (AP3; 25μM), a relatively selective inhibitor of glutamate-stimulated phosphoinositide turnover. We report here that low-frequency Schaffer collateral stimulation (1-5Hz/15min) in area CA1 of *in vitro* hippocampal slices induces a non-associative homosynaptic LTD (LF-LTD) of extracellular epsp's, but only at previously potentiated synapses (100hz/1sec; 30min before LF stimulation). Unlike associative LTD, induction of LF-LTD was blocked by either the metabotropic antagonist AP3 or the NMDA blocker D-2-amino-5-phosphonovalerate (AP5; 10μM). Furthermore, ionotophoretic application of NMDA to CA1 apical dendrites was insufficient to prime synapses for LF-LTD. However, induction of a series of short-term potentiations (6x 30Hz/0.3sec) did prime synapses for subsequent LF-LTD These results suggest that the threshold for the induction of LTD can be lowered by the previous history of synaptic activity. *(Supported by NIMH Grant #45752 and the Office of Naval Research)*

567.27

NMDA RECEPTOR BLOCKADE UNMASKS LONG-TERM DEPRESSION (LTD) OF HIPPOCAMPAL SYNAPTIC TRANSMISSION IN THE DEVELOPING RAT. L. Velíšek, S.L. Moshé* and P.K. Stanton. Depts. of Neurology, Neuroscience and Pediatrics, Albert Einstein Coll. Med., Bronx, NY 10461, U.S.A.

High-frequency stimulation activates postsynaptic N-methyl-D-aspartate (NMDA) receptors and causes influx of calcium into neurons, leading to long-term potentiation (LTP) of synaptic transmission. We hypothesized that a) when NMDA receptors are blocked during high-frequency stimulation, homosynaptic LTD would be unmasked and b) this effect may be age dependent. We stimulated Schaffer collateral-CA1 synapses in hippocampal slices from 15, 30 and 60 day old rats. Two stimulating electrodes were placed in Schaffer collateral axons on opposite sides of extracellular recording electrodes in CA1 pyramidal cell and apical dendritic layers. After a stable baseline period, we tetanized one input (50 Hz, 2 s, 50 μs pulses, 6 trains) while the other served as control. After a brief both homo- and heterosynaptic reduction in responses, we observed either LTP or LTD (30 min post-stimulus) of population spike and e.p.s.p.s in the tetanized input. LTP was observed in 60% of slices from 60 day old rats (138±5% of baseline; n=30), in 57 % of slices from 30 day old rats (128±6%; n=16) and in 65% of slices from 15 day old rats (156±14%; n=23), whereas LTD was seen in only 13%, 6% and 13% in slices from 60, 30 and 15 day old rats, respectively. In contrast, when the NMDA antagonist D-2-amino-5-phosphonovaleric acid (AP5; 25 μM) was added, LTD occurred in 42% of slices (80±3% of baseline; n=19) from 60 day olds, in only one of 14 slices from 30 day olds, and in 47% of slices from 15 day olds (27±6%; n=17), whereas the incidence of LTP decreased to 15%, 14% and 29% in slices from 60, 30 and 15 day old rats, respectively. These studies demonstrate: a) that LTP is largely NMDA dependent in 30 and 60 day old, but not in 15 day old, rats; b) an unmasking of LTD by blockade of NMDA receptors in slices from 60 and 15 day old rats. Developmental differences in the amplitude of LTD, as well as the amount of NMDA versus non-NMDA LTP, may contribute to age-dependent differences in both physiologic plasticity and seizure susceptibility. *(Supported by American Epilepsy Society and Klingenstein Foundation.)*

567.28

LONG TERM DEPRESSION IN RAT CEREBELLAR PURKINJE CELL IN VITRO, IS INDUCED BY COACTIVATION OF IONOTROPIC (AMPA) OR METABOTROPIC GLUTAMATE RECEPTORS AND VOLTAGE-GATED CALCIUM CHANNELS.

H. Daniel, N. Hemart, D. Jaillard and F. Crepel *,
Laboratoire de Neurobiologie, Université PARIS XI, URA CNRS 1121, ORSAY, FRANCE.

Long Term Depression (LTD) of synaptic transmission at Parallel-Fiber (PF)-Purkinje Cell (PC) synapses following coactivation of PCs by PFs and by climbing fibers (CFs) has been proposed as the cellular basis of motor learning in the cerebellum. According to the scheme initially proposed by ITO, LTD is due to a desensitization of AMPA receptors of PCs at PF-PC synapses, following their activation by glutamate released by PFs and the transient increase in internal calcium (Ca^{2+}) due to activation of CFs.

In an in vitro slice preparation, using intracellular and whole-cell patch-clamp techniques, a LTD at PF-PC synapses is induced by coactivation of AMPA receptors and voltage-gated calcium channels by stimulation of PFs and direct depolarisation of the PC respectively. The cascade of events leading to this AMPA-dependent form of LTD involves the production of NO, thus confirming earliers observations (Crepel and Jaillard, Neuroreport 1:133).

On the other hand, coactivation of mGLU receptors and voltage gated Ca^{2+} channels seems sufficient to induce an AMPA-independent LTD, whereas activation of mGLU receptors alone induces fully reversible depression of PF-mediated EPSPs.

567.29

LONG-TERM DEPRESSION (LTD) OF SHAFFER-COLLATERAL TRANSMISSION TO CA1 NEURON INDUCED BY REPEATED APPLICATIONS OF GABA X.-D. Yang* & D. S. Faber Neurobiology Lab, SUNY at Buffalo, Buffalo, NY 14214

While repeated strong stimulation of glutamatergic pathway results in LTP, we previously reported that pairing postsynaptic inhibition with weak tetanization of the excitatory pathway led instead to LTD (PNAS vol 88:4299-4303, 1991). The present study shows that repeated application of the inhibitory transmitter GABA alone can also cause LTD of glutamatergic transmission. Intracellular recordings were obtained from CA1 neurons in hippocampal slices. The Shaffer collateral pathway was stimulated at 0.1 Hz to produce the test EPSP. Repeated pressure applications (150 ms) of 10 mM GABA (10 at 40 sec) to the vicinity of the postsynaptic neuron resulted in a 50% depression of the EPSP that lasted up to 50 minutes. The duration of the depression tended to be shorter when less GABA was applied (n=12). The depression was not due to a decreased input resistance of the postsynaptic neuron, and it could be reversed by high frequency stimulation, the paradigm typically used to induce LTP. This depression could be induced in the presence of 50 μM of APV (n=6). Furthermore, 10 mM of baclofen (n=5) could induce a depression similar to that induced by GABA. The above results suggest that repeated action of the inhibitory transmitter GABA causes prolonged depression of glutamatergic transmission, which is most likely mediated through the activation of $GABA_B$ receptors. Together with LTP, this inhibition-induced LTD may play an important role in activity-dependent, long-term synaptic plasticity in mammalian brain.

567.30

A RISE OF $[Ca^{++}]_i$ IN THE POSTSYNAPTIC CELL IS NECESSARY AND SUFFICIENT FOR THE INDUCTION OF LONG-TERM DEPRESSION (LTD) IN NEOCORTEX. A. Artola*, T.Hensch and W.Singer. Max Planck Institute for Brain Research, Frankfurt/M, FRG.

In slices of the visual cortex of adult rats synaptic responses were recorded intracellularly from layer III pyramidal cells (Vmr from -96 to -67 mV) after electrical stimulation of white matter (w.m.) and of tangential intracortical (i.c.) pathways. In all cells whose Vmr was less negative than -79 mV (n=8), raising extracellular $[Ca^{++}]_0$ transiently from 2 to 4 mM for 10 min produced, after return to 2 mM $[Ca^{++}]_0$, a marked (>-10% and up to -35%:-17.2 ± 7.5%, ± S.D.) and lasting (>90 min) depression of w.m. and i.c. EPSPs. This Ca^{++}-induced LTD affected both amplitude and rising slope of the EPSPs and was equally pronounced for both w.m. and i.c. responses. In more polarized cells (Vmr above -79 mV) transiently raising $[Ca^{++}]_0$ had no lasting effect on synaptic transmission (+1.4 ± 8.6%, n=5). However, in these hyperpolarized cells, low intensity tetanic stimuli, which under control conditions did not induce synaptic modifications, produced LTD (-27.4 ± 2.4%, n=5) when applied during the Ca^{++} pulse. This depression was selective for the tetanized pathway and could be blocked by loading the cell with BAPTA before stimulation (n=3). In cells with Vmr below -79 mV, in which already high Ca^{++} alone induced LTD, tetanic stimuli applied in conjunction with the Ca^{++} pulse caused no further enhancement of LTD: Depression was of similar amplitude for the tetanized and non-tetanized input. These results suggest that both Ca^{++}- and tetanus-induced LTD depend for their induction on the same postsynaptic process. The trigger signal in both cases appears to be a surge of intracellular Ca^{++} which results at least in part from Ca^{++}-entry through voltage-gated Ca^{++} conductances.

567.31

A POSTSYNAPTIC ACTION OF SODIUM IONS IS REQUIRED FOR THE INDUCTION OF CEREBELLAR LONG-TERM DEPRESSION IN CULTURE
D.J. Linden*, M. Smeyne, and J.A. Connor, Department of Neurosciences, Roche Institute of Molecular Biology, Nutley, NJ 07110.

Long-term depression (LTD) of responses to AMPA test pulses in the tissue cultured mouse Purkinje neuron (PN) is induced when iontophoretic quisqualate pulses and PN depolarization are given together. Both AMPA and metabotropic receptor activation are necessary for LTD induction in a voltage-clamp paradigm: in PNs clamped to -80mV in TTX saline, LTD may not be induced by depolarization together with pulses of *t*-ACPD or (quisqualate+CNQX). The AMPA receptor in these PNs does not appear to exert its effect by directly gating Ca influx (P_{Ca}/P_{Na} <0.2 for AMPA, >5.0 for NMDA). Replacement of external Na during quis/depolarization conjunction with either the impermeant ions NMG or TEA or the permeant ion Li, caused a blockade of LTD induction, suggesting that Na influx through the AMPA associated channel is necessary for this process. To determine whether activation of voltage-gated Na channels could substitute for AMPA receptor activation, responses to AMPA pulses were measured in current clamp mode following ACPD/depolarization conjunction in TTX-free saline. LTD was induced 3/16 times in normal medium and 7/16 times in veratridine (2 μM) indicating that while Na influx via voltage-gated channels may suffice to induce LTD infrequently, activation of AMPA receptors is more effective. Na influx might exert its effects through Na_i/Ca_o exchange, a process not stimulated by Li_i. Antiserum to a bovine cardiac Na/Ca exchanger shows strong immunoreactivity in our cultured PNs. We are currently employing microfluorometric imaging of Na and Ca to evaluate the potential contribution of this process to LTD induction.

567.32

LONG-LASTING DEPRESSION OF LATERAL PERFORANT PATH SYNAPTIC TRANSMISSION IN THE RAT HIPPOCAMPAL DENTATE GYRUS IS NOT MEDIATED BY ADENYLATE CYCLASE ACTIVATION.
A.T. Gage and P.K. Stanton*. Depts. of Neuroscience & Neurology, Albert Einstein Coll. Med., Bronx, NY 10461.

Norepinephrine (NE) elicits long-lasting potentiation (LLP) of perforant path synaptic transmission in the hippocampal dentate gyrus. NE LLP is mimicked by the β agonist isoproterenol (ISO), and by forskolin, a direct activator of adenylate cyclase. Both compounds presumably act by increasing intracellular cyclicAMP. The perforant path can be separated anatomically and physiologically into distinct medial (med) and lateral (lat) components. While ISO causes LLP of med responses, it elicits long-lasting depression (LLD) of lat synaptic transmission. Since it is not known if lat LLD is also mediated by cAMP, we tested the effects of forskolin on med and lat synaptic evoked potentials in hippocampal slices *in vitro*.

Separate stimulating electrodes alternately activated axons of med and lat every 60s. Potentials were recorded from glass microelectrodes placed in the proximal (med) or distal (lat) half of stratum molecularé. After a baseline period, forskolin (100μM) was perfused for 30min. As reported previously, NE (50μM) elicited LLP of med and LLD of lat perforant path responses. In contrast, forskolin persistently potentiated both med and lat evoked potentials. These results suggest that only med LLP is mediated by an increase in intracellular cAMP, whereas lat LLD is independent of cAMP. Since both NE LLP and LLD are blocked by NMDA receptor antagonists, LLD may represent a heterosynaptic depression secondary to enhancement of med NMDA receptor activation by NE, similar to heterosynaptic LTD after high-frequency stimulation.

(Supported by NIMH Grant #45752 and the Klingenstein Foundation)

567.33

DIFFERENTIAL EFFECTS OF GANGLIOSIDES ON TETANUS-INDUCED SYNAPSE POTENTIATION AND KAINATE-INDUCED SYNAPSE SUPPRESSION.
H.-M. Hwang*, J.-L. Teng and T.-H. Chiu. Dept. of Anatomy, Chang Gung Med. College, Taoyuan, Taiwan 33333 and Dept. of Physiology, National Yang Ming Med. College, Taipei, Taiwan 11221, R.O.C.

Gangliosides are found to be enriched in the synaptic membranes of the mammalian CNS. Among members of gangliosides, monosialogangliosides such as GM1, and disialoganglioside, GD1a, are most abundant in the CNS. Treatment with enzyme neuraminidase (NA) was found to increase mono- and di-sialoganglioside contents. To elucidate extent of their influence, two modes of activity changes in CA1 areas of rat hippocampal slices were used, i.e. synapse potentiation by 3 trains of 100 Hz stimuli on Schaffer's collaterals and synapse suppression by infusing 5 uM kainate for 20 min. While GM1 enhanced synapse potentiation, GD1a and NA did not have obvious effects. NA prevented 80% of synaptic suppression, but GM1 and GD1a only prevented 40%. NA raised up input resistance of CA1 pyramidal cell and reduced depolarization potential. No change of resting membrane potential was found after NA treatment. However, NA prevented change of input resistance during kainate perfusion. (supported by CMRP324)

SYNAPTIC PHARMACOLOGY I

568.1

INHIBITION OF NEUROTRANSMITTER RELEASE FROM PC12 CELLS BY THE CA^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II INHIBITOR, KN-62.
Erik S. Schweitzer*[1] & Claude Wasterlain[2]. [1]Department of Anatomy & Cell Biology, and [2]VA Medical Center Department of Neurology, UCLA Medical School, Los Angeles, CA 9002.

PC12 cells secrete catecholamines in a depolarization and Ca^{2+} dependent manner. We have examined the effects of a variety of pharmacological agents on regulated secretion from PC12 cells by monitoring the rate of release of ^{3}H-norepinephrine from PC12 cells previously loaded with this neurotransmitter. Treatment of these cells with the selective Ca^{2+}/calmodulin-dependent protein kinase II inhibitor, KN-62, results in an inhibition of the regulated secretion of neurotransmitter. KN-62 inhibits norepinephrine release in a manner that is rapid, reversible, and dose-dependent. Half-maximal inhibition is obtained at 3 μM KN-62, and the extent of regulated secretion returns halfway to control values approximately 6 minutes after washout. These results, together with the effects of other agents that affect calmodulin-dependent cellular processes, suggest the involvement of both calmodulin and protein kinase II in the regulated release of catecholamine neurotransmitters.

568.2

FACILITATION BY DOPAMINERGIC MECHANISM OF ATP-ACTIVATED CURRENTS AND DOPAMINE RELEASE IN PC12 CELLS.
K. Inoue*, K. Nakazawa, T.Watano, K.Fujimori and A. Takanaka, Div. Pharmacology, NIHS, 1-18-1 Kamiyoga, Setagaya, Tokyo 158, Japan

We have previously reported that ATP stimulates P2y-like purinoceptor, activates Ca-permeable cation channels, stimulates Ca influx and dopamine (DA) release in PC12 cells (a revew in NIPS, April, 1992). Though DA doesn't evoke currents nor release from these cells, it is presumably that released-DA modifies the ATP-evoked responses. We report here that both DA receptor agonists and antagonists facilitate ATP-activated currents and DA release from PC12 cells.

DA(10μM) enhanced ATP(100μM)-activated inward current by about 50%. Similar enhancement of the ATP-evoked current was observed with apomorphine(10μM), a non-selective DA receptor agonist, SKF-38393(10μM), a selective D1 receptor agonist, and quinpirole(10μM), a selective D2 receptor agonist. Moreover, SCH-23390(30μM) and sulpiride (30μM), selective D1 and D2 antagonist, respectively, enhanced the current. These agonists and antagonists stimulated ATP(100μM)-induced DA release from PC12 cells at the same concentration. These data suggest that the ATP-evoked responses are facilitated by dopaminergic mechanisms through a kind of DA-receptor subclass which is not characterized yet.

568.3

SYNAPTIC PHARMACOLOGY OF RAT SYMPATHETIC NEURON AND CARDIAC MYOCYTE CO-CULTURE. P.T. Toth*, V.P. Bindokas, and R.J. Miller. Dept. Pharm/Phys. Sci., U. of Chicago, Chicago, IL 60637.

We are studying the pharmacology of synapses formed between neurons isolated from neonatal rat superior cervical ganglia (SCG) and atrial myocytes in co-cultures maintained for up to 2 weeks with the goal of determining the types of calcium channels (VSCCs) involved in transmitter release. We have found multiple types of VSCCs in these neurons including α_{1A}, α_{1C}, α_{1D} and α_{1x} (see also Marubio et al., this vol.). SCG neurons co-cultured with myocytes are able to release multiple transmitters (Matsumoto et al., J.Neurosci., 7:380, 1987). Evoked synaptic activity was monitored by fura-2 microfluorimetry of spontaneous calcium oscillations in myocytes. Neurons were stimulated by whole-cell patch clamp method, holding cells at -75mV and stimulating at 4 or 10 Hz. Stimulation of 51 pairs examined resulted in increased myocyte oscillation frequency or basal $[Ca^{2+}]_i$ (57%), decrease of basal $[Ca^{2+}]_i$ or inhibition of oscillation frequency (31%), and no apparent effect (12%). Frequency increases are mimicked by noradrenaline (NA) and blocked by phentolamine. Frequency decreases are mimicked by carbachol (CCh) or by NA and blocked by atropine and atenolol, respectively. CCh decreased, and NA increased, basal $[Ca^{2+}]_i$. In addition, some synapses appear to be purinergic and/or peptidergic. The pharmacology of the VSCCs involved in transmitter release is being studied by application of toxins. ω-Conotoxin GVIA (CgTx; 5μM) was focally applied by pressure ejection. Excitatory and inhibitory effects of stimulation were largely blocked by CgTx. These results indicate, that ω-CgTx sensitive VSCCs (N-channels) play an important role in the regulation of release of a variety of transmitters from sympathetic nerves.

PTT is supported by a Fogarty International Fellowship.

568.4

DIFFERENTIAL EFFECTS OF NOREPINEPHRINE ON FIELD POTENTIALS IN LAYERS 1A AND 1B OF RAT OLFACTORY CORTEX. M.C.Vanier and J.M.Bower*, California Institute of Technology, Pasadena, CA 91125.

In rat olfactory (piriform) cortex, layer 1a contains afferents from the olfactory bulb while layer 1b contains intrinsic pyramidal cell associational fibers. We have previously demonstrated (Hasselmo and Bower, J. Neurophysiol. 1992) that the cholinergic agonist carbachol, when bath-applied in a brain slice preparation, causes a large suppression of synaptic transmission in layer 1b of rat olfactory (piriform) cortex while having essentially no effect on layer 1a. These effects were shown to be computationally significant when incorporated into a computer model of olfactory cortex (Hasselmo, Anderson, and Bower, J. Neurophysiol. 1992). In the present study, we examined the effects of bath-applied norepinephrine in a brain slice preparation on extracellular field potentials in layers 1a and 1b of piriform cortex. We found that 25 mM NE decreased field potential height in layer 1b to 33.2 ± 11.5% of control, while NE increased field potential height in layer 1a to 139.6 ± 15.1% of control. Both effects were reversible after washout of NE. Thus NE has an effect very similar to ACh in layer 1b, but, unlike ACh, NE increases field potential height in layer 1a. Based on previous modeling efforts, these results are consistent with a role for this neuromodulator as a "state switch" for associative learning in piriform cortex.

568.5

PHARMACOLOGICAL DISTINCTIONS BETWEEN PRE- AND POST-SYNAPTIC DOPAMINE RECEPTORS IN INTERMEDIATE PITUITARY. P.J.WILLIAMS,* T.V. DUNWIDDIE, & G.A.GERHARDT, Dept. Pharmacology, University of Colorado Health Sciences Center, 4200 E. Ninth Ave, Denver, Colorado, U.S.A. 80262

The intermediate lobe of the pituitary (IL) receives a monosynaptic DA projection from the arcuate nucleus which synapses onto a single class of postsynaptic cells, the melanotrophs, which bear D2 receptors. DA release from the neurons innervating the IL is modulated by presynaptic autoreceptors which are inhibited by D2 antagonists. The IL thus contains both pre- and post-synaptic D2 receptors with different physiological functions. Both pre- and post-synaptic D2 receptor function were assessed simultaneously in a single IL by measuring electrically stimulated (SS) DA release using carbon fiber electrochemical electrodes. (to assess autoreceptor status) and simultaneous intracellular recording from the melanotrophs (to assess postsynaptic D2 receptor status). Haloperidol, a D2 antagonist, increased DA release while simultaneously abolishing the postsynaptic response to SS. Sulpiride, another D2 antagonist, increased presynaptic DA release by 100% at 500nM, however, sulpiride did not block the postsynaptic hyperpolarization due to SS. Domperidone, (1μM) another D2 antagonist, blocked the postsynaptic response to SS without increasing DA release. S(-)PPP, thought to effect autoreceptors selectively, increased DA release but had no effect on postsynaptic response while R(+)PPP caused a postsynaptic hyperpolarization but had no effect on DA release. The ability of these agents to distinguish between these two sets of D2 receptors suggests that they may represent subtypes of the D2 receptor. Supported by USPHS 0634 and VA Med Research Service

568.6

ESTROGEN ATTENUATES α_2-ADRENERGIC INHIBITION OF NOREPINEPHRINE RELEASE FROM HYPOTHALAMIC SLICES. G.B. KARKANIAS* AND A.M. ETGEN. Depts. of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY, 10461

The purpose of this study was to determine whether norepinephrine (NE) release in the hypothalamus is controlled by α_2-adrenergic inhibition and whether this mechanism is regulated by estrogen. Slices were prepared from female Sprague-Dawley rats that were bilaterally ovariectomized and treated with oil (OVX) or 2 μg of estradiol benzoate (EB) 24 and 48 hrs. prior to sacrifice. Slices were preincubated for 45 min. with .1 μM ^{3}H-NE and washed for 30 min. in the chambers of a superfusion apparatus at a flow rate of 1ml/3min. Slices were stimulated twice for 3 min. with 10 mM KCl (S1 and S2). S1 and S2 were separated by 24 min. The α_2 antagonists idazoxan (IDA) or RX821002 (RX) at 10 μM were applied 15 min. prior to S2 and were present until the end of the experiment. Basal ^{3}H-NE release was not affected by α_2 antagonists or hormone pretreatment. KCl-evoked release of ^{3}H-NE was Ca^{2+} dependent. The amount of ^{3}H-NE released during S1 was 20% greater in slices from EB-treated animals than in slices from OVX animals. When IDA or RX were infused prior to S2 in slices from OVX animals, ^{3}H-NE release was increased 50-80% relative to S1. In contrast, IDA and RX had little or no influence on ^{3}H-NE released during S2 in slices from EB-treated animals. Similar effects of estrogen administration were observed in slices from the preoptic area. These results suggest that α_2-adrenergic inhibitory mechanisms are active in the hypothalamus of OVX animals and that these mechanisms are attenuated by estrogen.

568.7

SLOW EPSPS AND RESPONSES TO SUBSTANCE P OF DORSAL HORN NEURONS IN THE ADULT RAT SPINAL CORD SLICES. M. Yoshimura*, T. Shimizu and S. Nishi. Dept. Physiol., Kurume Univ. Sch. Med., Kurume, 830 Japan

To study the effect of substance P (SP) on nociceptive transmission in the dorsal horn of the spinal cord, intracellular recordings were made from substantia gelatinosa (SG, lamina II) and lamina IV-V neurons of the adult rat spinal cord slices.

Substance P applied by perfusion produced no changes in membrane potential or conductance in 18 of 18 SG neurons but it increased spontaneous EPSPs in amplitude and frequency in 30 % of the SG neurons examined. In 50 % of lamina IV-V neurons, SP produced a concentration-dependent depolarization associated with a decreased membrane conductance. The depolarization decreased in amplitude with membrane hyperpolarization and was nullified at about -90 mV. The SP-induced depolarization was not affected by tetrodotoxin, while it was blocked by Co^{++}.

Repetitive dorsal root stimulation with intensity sufficient to activate C afferent fibers evoked slow EPSPs which were associated with a decreased membrane conductance in 20 % of lamina IV-V neurons. The slow EPSPs decreased in amplitude with membrane hyperpolarization and was nullified at about -90 mV. These neurons were also depolarized by SP. The slow EPSPs and SP-induced depolarization were attenuated by SP receptor antagonist spantide.

These observations suggest that SP mediates the slow EPSPs at the synapse between primary afferent and lamina IV-V neurons in the SG.

568.8

SYNAPTIC MODULATION OF RAT MEDIAL VESTIBULAR PACE-MAKER DISCHARGE. Y. Lin* and D. Q. Carpenter. Wadsworth Labs, NYS Dept. Health and School of Public Health, Albany, NY 12201.

Medial vestibular neurons in rat brain slices show an endogenous pacemaker discharge. Synaptic modulation of this activity was evaluated by combining recording of spontaneously active neuronal activity, and iontophoretic application and bath application of various neurotransmitter receptor agonists and antagonists.

Iontophoretic application of the muscarinic receptor agonist, oxotremorine-M, increased the firing rate of most neurons and decreased the firing rate in a few. The non-selective muscarinic receptor antagonist, atropine (50 μM), depressed the firing rate (10-27%) in most neurons. Application of the muscarinic M_2 receptor antagonist, gallamine (50 μM), initially increased the firing rate (35-65%) followed by a decrease (10-15%). Little effect was observed with the muscarinic M_1 receptor agonist pirenzepine (50 μM) in most neurons.

2-Amino-4-phosphonobutyrate (AP4 100 μM), an agent known to block presynaptic neurotransmitter release, decreased firing rate (10-33%). The $GABA_A$ receptor antagonist, bicuculline (50 μM), increased the firing rate (21-60%) whereas GABA decreased it. Application of bicuculline during the presence of AP4 failed to induce excitation.

Conclusion: The endogenous pacemaker activity of medial vestibular neurons is modulated by various neurotransmitters. Even in isolated brain slice preparations, release of transmitters from presynaptic terminals appears to influence spontaneous neuronal activity, since application of antagonists alters firing rate. Supported by the Aaron Diamond Foundation & NS23807.

568.9

ROLE OF SYNAPTIC TRANSMISSION AND CHLORIDE CONDUCTANCE IN THE ELECTROPHYSIOLOGICAL EFFECTS OF LINOPIRDINE ON CA1 HIPPOCAMPAL NEURONS *IN VITRO*. P. A. Murphy and B. S. Brown*. CNS Diseases Research, The DuPont Merck Pharmaceutical Co., Wilmington, DE 19880-0400

Because linopirdine (DuP 996) is known to enhance neurotransmitter release *in vitro*, it was of interest to determine whether its previously reported electrophysiological effects on CA1 neurons in the hippocampal slice (reduced spike frequency adaptation, prolonged spike duration and increased spontaneous firing) represented direct somatic effects and, if so, to assess the possible role of chloride conductance in mediating these responses. Standard intracellular recording techniques were used to study the effects of 10 μM linopirdine under conditions of synaptic transmission blockade (omega-conotoxin, CnTX) and an altered chloride equilibrium potential (KCl electrodes). The presence of 0.3 μM CnTX had no influence on the effects of linopirdine recorded with K acetate electrodes. Conversely, the use of KCl electrodes completely blocked the electrophysiolgical effects of linopirdine but had no effect on those of muscarine (thought to act through K channel blockade). These results suggest that linopirdine exerts direct effects on the CA1 hippocampal soma which may be mediated by the block of a chloride conductance.

568.10

INTERNEURONAL ELECTROTONIC COUPLING IN A SYSTEM MODEL USING WHITE NOISE ANALYSIS: ETHANOL UNCOUPLES NEURONS. P.Fu, N. Wright, B.L. Bardakjian and P.L. Carlen*. Playfair Neuroscience Unit, The Toronto Hospital, 399 Bathurst St., and the Addiction Research Foundation, Toronto, Ont., Canada, M5T 2S8.

A system model of dentate granule neurons (Fu et.al.,IEEE Trans Biomed Eng, 36,1:55-64) using white noise analysis is used to study the electrotonic properties. The model is based on a multi-compartmental circuit including interneuronal coupling, and comprises a combination of resistors and capacitors. Analytical expression of the input resistance is written using the Z-transform. The model parameters are estimated using an optimization procedure from in vitro voltage recording of white noise current injected into dentate granule neurons of the rat. White noise analysis allows extraction of the linear component (the first Weiner kernel) of the voltage response. The most appropriate fit to the experimental data (input impedance) required interneuronal electrotonic coupling. Also, the membrane capacitance obtained was close to 1 $\mu F/cm^2$. Acute ethanol exposure (50mM) caused a large increase in the junctional resistance resulting in uncoupling of neurons, as has been shown with higher order alcohols in other cellular systems.

Supported by the MRC and ABMRF

568.11

EFFECTS OF NEUROTRANSMITTERS ON PYRAMIDAL AND NON-PYRAMIDAL NEURONS IN HIPPOCAMPAL SLICES. N. Otmakhov, R.Malinow & R.E .Fellows*. Dept. of Physiology and Biophysics, Univ. of Iowa & Instit. of Theoret. and Experim. Biophysics, Russian Academy of Sciences.

Whereas there are many studies on phenomenology and underlying mechanisms of neurotransmitter actions on principal cell types of CNS, only a few reports investigate the pharmacological sensitivity of local-circuit interneurons. We investigated effects of acetylcholine (ACh), norepinephrine (NE), 5-hydroxytryptamine (5-HT) and gamma-aminobutyric acid (GABA) on extracellular recorded single cell spontaneous firing activity in the CA3 field of guinea pig hippocampal slices. Three groups of neurons were investigated: nonpyramidal neurons of stratum radiatum-moleculare (N), neurons with single spike discharges of stratum pyramidale (SS) and neurons with complex spike discharges (CS) in the same stratum. Effects of ACh and NE were also tested on p-_sumed interneurons of str. oriens-pyramidale (I). Similarity between N, I and SS units and their difference from CS units may be suggested on the basis of drug action. Activity of CS units was suppressed by NE, 5-HT and GABA, while in half of these units ACh had biphasic (inhibitory, then excitatory) effects. In contrast, N, I and SS units were activated by NE and ACh. Though 5-HT and GABA suppressed the activity in some N and SS units, many of them were activated. Excitatory influence of NE, ACh and 5-HT on N was preserved with the blockade of synaptic transmission (Fig.1). The data suggest direct excitatory influence of NE-, ACh- and 5-HT-ergic afferents on nonpyramidal cells and therefore an indirect inhibitory influence on hippocampal pyramidal cells.

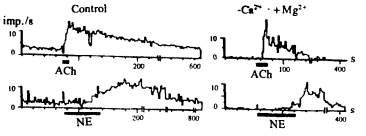


Fig.1. Preservation of excitatory actions of ACh and NE on hippocampal nonpyramidal neurons during synaptic blockade.

568.12

SYNAPTIC RESPONSES RECORDED FROM BIOCYTIN LABELLED NEURONS IN MOUSE CINGULATE CORTEX IN VITRO. M. Kiraly* and P.I. Magistretti. Institut de Physiologie, Université de Lausanne, CH-1005 Lausanne, Switzerland.

In a submerged slice (300 μm thick) preparation, intracellular recordings were combined with biocytin injections to characterize the membrane properties, synaptic responses and morphology of 3-4 week old mouse anterior cingulate cortex neurons.

On the basis of their firing pattern in response to depolarizing current pulses, neurons were classified as regular spiking (36/42) and bursting (6/42). The resting membrane potential (-75 mV vs -73 mV), the input resistance (51 MΩ vs 56 MΩ), the time constant (11 ms vs 7 ms), the action potential amplitude (84 mV vs 79 mV) and duration (1.8 ms vs 1.7 ms) of these two populations were quite similar. Biocytin labelling of regular spiking and bursting neurons revealed pyramidal shaped cells located mainly in layer V and, in a few cases, layers II/III. Stimulation of corpus callosum elicited four synaptic potentials in both cell types : (i) early, monosynaptic EPSP (time-to-peak 6.6 ± 2.4 ms; amplitude 8.7 ± 3.7 mV) blocked by the non-NMDA receptor antagonist CNQX 20 μM. (ii) early monosynaptic IPSP (time-to-peak 11.0 ± 6.4 ms; amplitude 7.0 ± 0.8 mV) blocked by the $GABA_A$ antagonist bicuculline 20 μM. (iii) late EPSP (time-to-peak 32.2 ± 6.9 ms; amplitude 10.5 ± 5.9 mV) blocked by the NMDA receptor antagonist AP5 50 μM. This late potential could be enhanced by removal of extracellular Mg^{2+} and by tonic depolarization of the neuronal membrane. (iv) late IPSP (time-to-peak 101 ± 44 ms; amplitude 3.6 ± 0.5 mV) abolished by the $GABA_B$ antagonist saclofen 300 μM and showing a reversal potential of ≈ -90 mV, suggesting a K^+-channel involvement.

568.13

AMPHETAMINE-INDUCED INCREASE IN [^{3}H]TCP INTACT CELL BINDING OF PRIMARY CULTURED NEURON. H.Yamamoto[1], T.Yamamoto[1], N.Sagi[1], K.Yang[1], A.Baba[1], T.Moroji[1] and K.Yoshikawa*2 [1]Dept. of Psychopharmacology, Tokyo Inst. of Psychiatry, Tokyo 156, [2]Dept. of Mol. Biol., Tokyo Metropolitan Inst. for Neurosci., Tokyo 183, Japan.

To investigate factors regulating [^{3}H]TCP binding sites under the physiological condition, we conducted intact cell binding studies in primary cultured neuronal cells derived from fetal rat telencephalon. In modified Locke medium (with 95% O_2/5% CO_2, 25°C), [^{3}H]TCP(4nM) binding to the intact cells was saturable, reversible, and displaced by non-competitive NMDA receptor antagonist or σ ligand. Displacement studies of [^{3}H]TCP intact cell binding by σ/PCP ligands (haloperidol, desipramine, PCP, DTG and GBR12909) revealed the potency of the inhibition with IC_{50} values of 0.448, 1.649, 7.02, 12.71 and 1.296 μM, respectively. The [^{3}H]TCP binding of cells cultured with serum was more sensitive to APV than that of the cells cultured without serum. However, the potency of inhibition by σ ligand was not influenced by the culture condition (serum or serum-free medium).

Subchronic exposure to amphetamine induced increases in the amount of [^{3}H]TCP intact cell binding in a dose-dependent manner. The inhibition of [^{3}H]TCP intact cell binding by σ ligands was not influenced by amphetamine treatment. These results suggest that amphetamine-induced change in neuronal cells is involeved in a cellular excitability and formation of reverse tolerance. Furthermore, σ ligands may modulate the amphetamine-induced changes through NMDA receptor coupled ion channels.

568.14

INHIBITORY EFFECT OF LITHIUM ON PROTEIN PHOSPHORYLATION SYSTEM ASSOCIATED TO NEURONAL CYTOSKELETON. J. Perez, R. Zanardi §, S. Mori, C. Cagnoli, L. Formica, D. Tinelli, N. Brunello* and G. Racagni. Center of Neuropharmacology, § San Raffaele Hospital, University of Milan, Italy.

Lithium is the most effective drug in the treatment of manic-depressive illness. Its therapeutic action includes antimanic and antidepressant effects, as well as prevention by reducing frequency or intensity of bipolar episodes. Likewise, lithium could be used in the prophylaxis of recurrent unipolar depressive illness. Although the molecular basis of its therapeutic efficacy is still unclear, the biochemical action of lithium is mainly based on signal transduction processes beyond the receptor level. Protein phosphorylation-dephosphorylation represents a fundamental step for signal transduction in the CNS, and recently evidence obtained in our laboratory suggests that it could be involved in the therapeutic effect of antidepressant drugs. We have now extended these studies to determine whether protein phosphorylation system associated to rat cerebrocortical microtubule fraction may also be a neurochemical target for the action of lithium. In vitro addition of lithium (0.5 to 2 mM) was able to inhibit of 20% the basal ^{32}P incorporation in microtubule fraction after 30 minutes of incubation. Moreover, the presence of high lithium concentration (5 mM) induced decrease of 30% and 40% in the basal phosphorylation of microtubule fraction after 5 and 30 minutes of incubation respectively. Since, cAMP protein kinase type II is known to be associated with microtubules, we have investigated the cAMP dependent endogenous phosphorylation. In this experimental condition 5 μM of cAMP caused a 3 fold increase in the initial rate of ^{32}P incorporation into MAP_2, a phosphoprotein associated to neuronal microtubules which is itself a substrate for cAMP dependent protein kinase. Lithium 1 and 5 mM decreased the initial rate of ^{32}P incorporation into MAP_2 from 3 to 2.4 and 1.8 respectively. By contrast, Rubidium 5 mM was unable to affect the rate of ^{32}P incorporation into MAP_2. These studies support the hypothesis that protein phosphorylation system associated with microtubules might be a neurochemical target for the action of lithium.

569.1

SYNAPSES IN THE MEDIAL AND LATERAL PERFORANT PATHWAYS EXAMINED WITH THE WHOLE-CELL VOLTAGE CLAMP IN THE RAT HIPPOCAMPAL DENTATE GRANULE NEURONS. S. Wang, A. Baskys and J.M. Wojtowicz*. MRC group, Department of Physiology, University of Toronto, Toronto, ONT, M5S 1A8.

Lateral (LP) and medial (MP) perforant pathways terminate on distal and medial portions of dendrites of the dentate granule neurons. Several differences between synapses of LP and MP have been described previously. We examined the properties of transmission at these synapses using the whole-cell voltage clamp technique. Experiments were performed on granule neurons in the hippocampal slices taken from 15-30 day old Wistar rats. MP and LP were activated selectively by means of stimulating electrodes placed in the molecular layer. A putative glutamate receptor blocker L-AP4 (20 μM) selectively antagonized synaptic transmission in LP (55% inhibition, S.E.=9.4%, n=5). With the use of quantal recordings we have shown that its action was presynaptic. Similar presynaptic actions on LP were obtained with application of an NMDA blocker D,L-APV (50 μM). However, in MP the effect of D,L-APV was mainly postsynaptic. The results confirm different locations and characteristics of glutamate receptors in the two pathways. Possible roles for these receptors during high-frequency activity are under investigation. Supported by MRC of Canada.

569.2

SPERMINE DEPRESSES POPULATION EPSPs AND MONOSYNAPTIC POPULATION IPSPs IN THE RAT HIPPOCAMPAL SLICE PREPARATION. P.G. DiScenna*, P.A. Ferchmin, E. Rivera, V. Eterovic & T.J. Teyler. Neurobiology Department, NEOUCOM, Rootstown, OH 44272 and Department of Biochemistry, School of Medicine, Universidad Central del Caribe, Bayamon, P.R. 00960.

Spermine, a polyamine, is present in the brain in significant concentrations and is known to affect several receptors, protein kinases and other neural regulatory processes. High concentrations of extracellular spermine are observed after strong depolarization and ischemia. It has been proposed that spermine released during ischemia is partially responsible for its pathogenesis. We examined the effects of spermine on extracellular evoked responses in area CA1 of the rat hippocampal slice preparation. Spermine depressed both excitatory and inhibitory synaptic potentials. An effect of spermine was detectable at 50uM. At 2mM (10-20min @ 0.5ml/min), spermine reversibly depressed the population EPSP and spike by 75-100%, population NMDA responses (DNQX/0mM Mg) by 65-75% and monosynaptic population IPSPs (DNQX/APV) by 50-65%.

Given the known effects of spermine, the most likely explanation is that presynaptic calcium currents are depressed or mitochondrial uptake of calcium from the presynaptic terminal is enhanced. Therefore we are presently comparing the effects of spermine with low calcium ACSF.

Rather than contribute to ischemic damage, perhaps spermine could reduce damage by depressing synaptic transmission.

Supported by NIH RCMI RR03035 (PAF) and NIH NS28698 (TJT).

569.3

INHIBITION OF NMDA RECEPTOR-MEDIATED SYNAPTIC CURRENTS BY A MU OPIOID AGONIST IN DISINHIBITED DENTATE GRANULE CELLS. C.W.Xie* and D.V.Lewis. Pediatric Neurology, Duke Univ. Med. Ctr., Durham, NC 27710.

We have previously reported that the mu opioid agonist PL017 suppressed GABAergic inhibition and thus indirectly enhanced NMDA receptor-mediated responses in hippocampal dentate gyrus. The present study examined the direct effect of PL017 on NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) of granule cells in the absence of $GABA_A$ inhibition. Isolated NMDA EPSCs were evoked by outer molecular layer stimulation and recorded from granule cells using whole cell voltage clamp techniques in the presence of DNQX (AMPA antagonist, 10 μM) and bicuculline methiodide ($GABA_A$ antagonist, 10-50 μM). The current was enhanced by removing magnesium from the perfusion medium, and completely blocked by D-APV. Bath application of PL017 (3-10 μM) significantly reduced the amplitude of isolated NMDA EPSCs by 27-43%. This effect could be reversed by opiate antagonist naloxone (10 μM). These results indicate a direct inhibition of NMDA currents by mu receptor activation in the dentate, which may serve as a protective mechanism to limit the hyperexcitability of NMDA channels during opioid disinhibition.

569.4

METABOTROPIC RECEPTOR-INDUCED SUPPRESSION OF SYNAPTIC TRANSMISSION IS NOT BLOCKED BY L-2-AMINO-3-PHOSPHONO-PROPIONIC ACID (L-AP3). J. W. Goh* and M. A. Musgrave, Department of Pharmacology & Toxicology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Activation of a metabotropic glutamate receptor (mGluR), found in various brain regions, produces an increase in phosphoinositide (PI) metabolism. 1-Aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD), a specific agonist of a mGluR, stimulates PI turnover that is antagonized by AP3. However, some electrophysiological actions of *trans*-ACPD, including slow depolarization and inhibition of the late after-spike hyperpolarization, are not blocked by AP3. We report here that inhibition of hippocampal excitatory postsynaptic potentials (EPSPs) by the mGluR agonist is also resistant to blockade by AP3. Experiments were conducted on rat hippocampal slices, where EPSPs were evoked by stimulation of stratum radiatum and recorded in the CA_1 dendritic region. 1S,3R-ACPD and L-AP3, the active isomers of *trans*-ACPD and AP3, respectively, are used in the present studies. 1S,3R-ACPD (100 μM) produces a suppression of EPSPs (24 ± 3% (SEM) of control, n = 4), an action that is not antagonized by L-AP3 (500 μM) (21 ± 3% of control, n = 8). L-AP3 (500 μM) application alone does not result in any significant change in the synaptic response (86 ± 6% of control, n = 8). A washout period of 20 minutes reverses inhibition of the EPSP by 1S,3R-ACPD application alone (73 ± 11% of control, n = 4) or in combination with L-AP3 (74 ± 12% of control, n = 6). Since AP3 blocks PI metabolism induced by trans-ACPD but not many of its electrophysiological actions, we suggest that i) an alternate effector system and/or ii) a different subtype of mGluR is involved in the resistant responses.

Supported by MRC (Canada). JWG is an MRC Scholar.

569.5

L-AP4 AND *trans*-ACPD REDUCE PAIRED-PULSE DEPRESSION RECORDED FROM THE RAT DENTATE GYRUS MOLECULAR LAYER. J.S. Kahle* and C.W. Cotman Department of Psychobiology, University of California, Irvine, CA 92717.

The glutamate analogue, L-2-amino-4-phosphonobutanoic acid (L-AP4), has been shown to be a ligand at several glutamate binding sites including: glutamate autoreceptors or AP4 receptors, chloride-dependent transport sites, metabotropic receptors, and NMDA receptors. At low concentrations (10μM), L-AP4 activates the glutamate autoreceptor and reduces field potentials recorded from select pathways. We have recently observed that applications of low concentrations of L-AP4 (10-20μM) also reduce paired-pulse depression recorded from the medial perforant path, without a reduction in field potential amplitude. The possibility that L-AP4 may be producing this effect by binding to transport sites, the metabotropic receptors, or NMDA receptors was examined. Paired-pulse depression of field potentials (40-800 ms interstimulus intervals) was recorded from the middle third of the dentate gyrus molecular layer of rat hippocampal slices *in vitro*. The transport inhibitors L-*trans*-pyrrolidine-2,4-dicarboxylic acid (250μM) and L-α-aminoadipate (200μM) did not alter or mimic the effect of L-AP4 on paired-pulse depression. The NMDA antagonist 2-amino-5-phosphonopentanoic acid (20μM) did not block the action of L-AP4. These results suggest that L-AP4 is not acting at the NMDA receptor or glutamate transport site to reduce paired-pulse depression. However, applications of the metabotropic receptor agonist 1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD; 50μM) reduced paired-pulse depression similarly to L-AP4. It has been shown that L-AP4 has a low affinity for the metabotropic receptor and *trans*-ACPD may also activate AP4 receptors (Trombley and Westbrook, *J. Neurosci.*, in press). One hypothesis consistent with these results is that *trans*-ACPD and L-AP4 activate the glutamate autoreceptor to reduce paired-pulse depression.

569.6

ACUTE AND CHRONIC EFFECTS OF GLUTAMATE RECEPTOR ANTAGONISTS ON EPILEPTIFORM ACTIVITY IN CULTURE. W.J. Koroshetz, and E.J. Furshpan*, Neurology Dept., Mass. General Hospital and Dept. of Neurobiology, Harvard Medical School, Boston Ma. 02215.

Hippocampal neurons grown chronicaly with blocking agents (e.g. 1mM kynurenate and 11.3 mM Mg^{2+}) show intense epileptiform activity when the blockers are removed (Furshpan and Potter [19189] Neuron, 3:199). Prominent components of this activity are synchronous events resembling paroxysmal depolarization shifts (PDSs); they are abolished by the combination of NMDA (APV) and non- NMDA (CNQX) antagonists. We report that PDSs occurred when either APV or CNQX was present alone. The currents (PDCs) underlying these PDSs were recorded with two-electrode voltage clamp. In the absence of antagonists, the initial phase of current (duration, 30-75 ms) was often followed by a tail of variable amplitude and duration. In APV this tail was reduced and the PDCs occurred at higher frequency, in clusters. In CNQX the PDCs had much slower rising phases, were more prolonged and less frequent. As the holding potential was made more positive, PDC amplitude increased sharply before decreasing and reversing sign near 0 mV. However, NMDA currents were adequate to synchronize the activity of the neuronal population even when most neurons were initially at resting potential.

Epileptiform activity is seen in unblocked cultures prior to a period of neuronal death. Long term neuronal survival and PDS activity is markedly enhanced in the blocked cultures. Many lucifer yellow injected neurons in months old blocked cultures had complex dendritic arbors; neurons in unblocked cultures had relatively simple dendritic arbors. The opportunities for neurons to make recurrent excitatory synapses are enhanced in blocked cultures and may contribute to intense seizure-like activity.

569.7

NMDA AND AMPA RECEPTOR COMPONENTS OF EPSCS FROM RAT DENTATE HILAR INTERNEURONS. T.A. Brown* and R. Dingledine. Dept. Pharmacology, Univ. North Carolina-Chapel Hill, 27599.

Spontaneous excitatory postsynaptic currents (EPSCs) from interneurons located in the hilus of the dentate gyrus of neonatal rat hippocampal slices were isolated at both positive and negative membrane potentials in 1 μM tetrodotoxin and 10 μM bicuculline. Cells were morphologically identified by a biocytin staining protocol. Marked heterogeneity exists in cellular morphology and degree of NMDA and non-NMDA contribution to EPSC kinetics. At -70 mV EPSCs rose within 2 ms and decayed with a time constant of less than 15 ms which was well fit by a single exponential. The NMDA receptor antagonist D-APV (50 μM) had little or no effect on EPSC kinetics or amplitude at -70 mV while CNQX, a non-NMDA receptor antagonist, nearly abolished all activity. At +50 mV a variety of EPSC kinetics and amplitudes were observed between neurons. Some neurons had decay phases best fit with 2 exponentials while others were best fit with one. D-APV always completely blocked the EPSC component best fit with the longer decay time constant. Interestingly, in 5 interneurons, 2 tentatively identified as dentate pyramidal basket neurons and 1 as aspiny fusiform, EPSCs at +50 mV decayed with a single rapid time constant which was unaffected by D-APV. Diversity in NMDA and non-NMDA receptor contribution to EPSCs suggests different roles for subpopulations of these neurons and may help explain differential sensitivity to insult. (Supported by NS17771 and Bristol-Myers Squibb Company)

569.8

REDOX MODULATION OF THE NMDA-MEDIATED COMPONENT OF SYNAPTIC FIELD POTENTIALS IN HIPPOCAMPAL AREA CA1. D. L. Tauck*, J. E. Tullis, and R. L. Sasich, Department of Biology, Santa Clara University, Santa Clara, California, 95053.

Accumulated evidence suggests a role for redox modulation of NMDA currents in a variety of neuronal preparations. The present study shows that the sulfhydryl redox agents dithiothreitol (DTT) and 5-5-dithio-bis-2-nitrobenzoic acid (DTNB) reversibly antagonize each other's effects on synaptic transmission in rat hippocampus. To reveal the NMDA-mediated component of the synaptic responses, the field potentials evoked by single stimuli were compared to those generated by the fifth of 5 stimuli at 10 Hz. All experiments were performed in saline containing 5 mM K^+ to increase the magnitude of NMDA-mediated responses. The conditioned response was 62.5 ± 7.4% (mean ± S.E.M.) larger than the response to a single stimulus (n=12). The NMDA antagonist D,L-2-amino-5-phosphonovaleric acid (250 μM) blocked the potentiation induced by conditioning stimuli. DTT (1 mM) and DTNB (1 mM) were repeatedly applied in the bath. DTT reversibly potentiated the NMDA component of the synaptic potentials (79.7 ± 7.0%, n=17) while DTNB had the opposite effect (40.0 ± 3.8%, n=17). DTNB did not reverse the potentiation induced by DTT in slices exposed to 300 μM N-ethylmaleimide, an alkylating agent (n=7).

569.9

DIFFERENTIAL DEPRESSION OF NMDA RECEPTOR-MEDIATED SYNAPTIC RESPONSES BY MK-801 AND CPP. B. Esplin* and R. Čapek. Dept. of Pharmacology and Therapeutics, McGill Univ., Montreal, Quebec, H3G 1Y6, Canada.

The influence of repetitive synaptic activation on depression of the NMDA receptor-mediated synaptic responses by a non-competitive antagonist, MK-801, and a competitive antagonist, CPP, was studied in the hippocampal slice preparation of the rat. These responses, evoked by stimulation of the stratum radiatum and recorded in the stratum pyramidale of the CA1 region in slices superfused by a Mg^{2+}-free medium, consisted of a burst of population spikes (PSs) which followed the primary PS. Both, MK-801 (2 to 100 μM) and CPP (0.2 to 10 μM) applied by superfusion, depressed the number and the amplitudes of the secondary PSs in a concentration-dependent manner. The primary PS remained unaffected. In the absence of a drug, repetitive stimulation at 0.2 Hz for 5 min was without any consistent lasting influence on the PS burst. Such stimulation also failed to alter a partially diminished burst during superfusion with the competitive antagonist CPP. In contrast, during superfusion with MK-801, the secondary PSs were greatly diminished immediately after the repetitive stimulation even with low concentrations of the drug, which produced no or only minimal depression without stimulation. The responses depressed by CPP fully recovered within 30 min, whereas those depressed by MK-801 did not, even after 60 min of superfusion with the drug-free medium. These results demonstrate that, similarly to responses evoked by the exogenous NMDA-receptor agonist in cultured cells (MacDonald and Nowak, TIPS 11:167,1990), the NMDA receptor-mediated synaptic responses evoked by the endogenous neurotransmitter in the slice preparation are depressed by MK-801 in a use-dependent manner.

(Supported by the MRC of Canada.)

569.10

A NON-GENOMIC ACTION OF ESTROGEN ON SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS. M. Wong* and R.L. Moss. Dept. Physiology, UT Southwestern Medical Center, Dallas, TX 75235.

Steroids exert rapid neurophysiological effects that are independent of the classical genomic mechanism mediated by intracellular steroid receptors and most likely involve direct interactions with specific membrane receptors. Estrogen can induce a short-term potentiation of the extracellular CA1 field potential in the hippocampus (Teyler et al. 1980, Landgren 1992) and of responses to exogenous glutamate in the cerebellum (Smith et al. 1988). The present study investigated the rapid effects of estrogen on responses of individual CA1 neurons to synaptic stimulation and local application of glutamate agonists. Intracellular recordings were made from CA1 neurons in rat hippocampal slices. Synaptic responses were elicited by Schaffer collateral stimulation with a metal electrode and glutamate agonists were applied to the CA1 neurons through an iontophoretic electrode.

Superfusion of 10^{-8} M 17β-estradiol, but not 17α-estradiol, significantly increased the amplitude of the Schaffer collateral-induced EPSP, causing a previously subthreshold EPSP to reach threshold. This facilitation of the EPSP by 17β-estradiol usually occurred within minutes and was reversible shortly after washout, suggesting a direct membrane action. The synaptic facilitation still occurred in the presence of the NMDA antagonist, AP5, but was blocked by the non-NMDA antagonist, CNQX. 17β-estradiol also potentiated depolarizing responses to iontophoretic pulses of glutamate and non-NMDA agonists, but not to NMDA. These results suggest that estrogen can induce a rapid potentiation of excitatory synaptic transmission in the hippocampus that involves postsynaptic non-NMDA receptors. (MH47418)

569.11

INWARDLY RECTIFYING SYNAPTIC CURRENTS ON HIPPOCAMPAL INTERNEURONES. C.J.McBain* and R.Dingledine. Dept. of Pharmacology, Univ. of North Carolina, Chapel Hill, NC 27599-7365.

Spontaneous miniature EPSCs (mEPSCs) and kainate-evoked currents were recorded from interneurones of CA3 st. radiatum of neonate rat hippocampal slices in the presence of TTX (1μM) and bicuculline (5μM). Two distinct populations of interneurone (Type I and Type II) were identified. The I-V relation of kainate in Type I interneurones was linear (Erev = ~0mV). The kainate I-V relation in Type II interneurones was strongly inwardly rectifying with little or no outward current at potentials up to +50mV. At -70mV mEPSCs received by Type I interneurones had fast rise times (~1msec) and decay time constants (~5msec) and were mediated by AMPA receptors. mEPSCs on Type I interneurones reversed polarity at ~0mV. At +50mV the kinetics of the mEPSCs on Type I interneurones were slowed and were comprised of both AMPA and NMDA receptor mediated components. The kinetics of mEPSCs on Type II interneurones were markedly slower than their Type I counterparts at -70mV. mEPSCs received by Type II interneurones showed extreme inward rectification with no interneurones possessing fast events at +50mV. In a few cells slowly rising and slowly falling mEPSCs were observed at +50mV. These events were abolished by D-APV and were therefore mediated solely by NMDA receptor activation. On both Type I and II interneurones the rise times of individual mEPSCs were correlated with their halfwidth and decay time constant, suggesting that the shape of the mEPSC is in part determined by the dendritic origin of the synaptic input. The inward or outward rectification observed in the two interneurone types is likely to be due to expression of different glutamate receptor subunits. Supported by NS17771 and Bristol-Myers Squibb.

569.12

KETAMINE DEPRESSES NMDA TRANSMISSION AND DOES NOT ENHANCE $GABA_A$ INHIBITION IN ISOLATED NEONATAL RAT SPINAL CORD. D. Brockmeyer and J. J. Kendig*. Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305-5123

Proposed anesthetic mechanisms include depression of excitatory neurotransmission and enhancement of inhibition. We investigated the anesthetic ketamine, which blocks NMDA receptors, in isolated superfused spinal cords from 1-6 day old rats. Lumbar dorsal root stimulation elicits a fast monosynaptic reflex and a slow ventral root potential (slow VRP) in the corresponding ipsilateral ventral root, and a dorsal root potential (DRP) in the adjacent dorsal root. The monosynaptic reflex is CNQX, but not APV, sensitive. The slow VRP is depressed by APV. The DRP reflects $GABA_A$-mediated presynaptic inhibition, and can also be elicited by direct focal application of the $GABA_A$ agonist muscimol.

Ketamine (1-50 μM) depressed the NMDA-mediated component of the slow VRP with an approximate ED_{50} of 25 μM; its actions resembled those of APV. The monosynaptic reflex was not affected. Ketamine depressed both the dorsal root-evoked DRP (1-20 μM) and the muscimol-evoked DRP (10 μM). Ketamine's serum anesthetic concentration is ~3 μM. Ketamine's effects on the spinal cord can be explained by block of NMDA receptors. Unlike some other anesthetics, ketamine does not enhance $GABA_A$ inhibition at concentrations in its clinically effective range.

569.13

ISOFLURANE DEPRESSES A $GABA_A$ INHIBITORY PATHWAY IN ISOLATED NEONATAL RAT SPINAL CORD. L. M. Gibbs* and J. J. Kendig. Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305-5123

Volatile general anesthetic agents have been suggested both to depress glutamate excitation and enhance $GABA_A$ inhibition. The volatile anesthetic isoflurane (0.2-1.2 vol %) was applied in the gas phase to the isolated spinal cords of 1-6 day old Sprague-Dawley rat pups (adult rat anesthetic partial pressure = 1.4 vol %). The dorsal root potential (DRP) is a population-evoked response which reflects $GABA_A$-mediated depolarization of primary afferent nerve terminals. DRP's were recorded from a lumbar dorsal root in response to stimulation of an adjacent dorsal root. Isoflurane reversibly depressed the DRP at all partial pressures. DRP's were also sensitive to both APV (10 μM) and CNQX (10 μM), suggesting that the GABA-ergic interneurons which mediate the DRP are activated by glutamate acting on both NMDA and non-NMDA receptors. We have previously shown that isoflurane (0.2-1.2 vol %) depresses both NMDA and non-NMDA receptor-mediated ventral root potentials in this preparation. Isoflurane may act directly on $GABA_A$ receptors to increase inhibition. However, in the circuit which generates the DRP, isoflurane's depressant effect on glutamatergic excitation of the inhibitory interneurons is overriding, and inhibition is depressed. Isoflurane thus differs from anesthetic barbiturates, which enhance the DRP.

569.14

EVIDENCE FOR $GABA_A$ RECEPTOR MEDIATED SLOW INHIBITORY POSTSYNAPTIC POTENTIAL (sIPSP) IN THE RAT LATERAL PARABRACHIAL NUCLEUS (LPBN). J.A. Zidichouski and J.H. Jhamandas. Dept. of Medicine (Neurology & Div. of Neuroscience), Univ. of Alberta, Edmonton, Alberta, Canada T6G 2E1.

The LPBN is the recipient of a diverse array of autonomic inputs from more caudal levels of the brainstem. Our prior data demonstrated that both N-methyl-d-aspartate (NMDA) and non-NMDA receptors mediate evoked fast excitatory post-synaptic potentials *in vitro* (Neurosci Abs 17: 246, 1991). In addition, we have recorded sIPSPs from LPBN neurons and this study examined the role of gamma-aminobutyric acid (GABA) receptors in mediating inhibitory synaptic transmission within this pontine nucleus.

LPBN neurons were recorded from coronal (400 μM) slices using whole-cell patch recording. IPSPs were evoked using platinum bipolar electrodes (10-70V @ 0.2 Hz) placed at the dorsomedial pole of the LPBN. Exogenous application of GABA (1 mM), the selective $GABA_A$ receptor agonist muscimol (1-10 μM) and the $GABA_B$ agonist baclofen (10 μM) all produced a membrane hyperpolarization (or outward current) that was accompanied by a decrease in the input resistance. Stimulus-evoked sIPSPs or slow inhibitory post-synaptic currents (sIPSCs) reversed at or about the reversal potential of chloride (E_{Cl}). sIPSPs or sIPSCs were attenuated by $GABA_A$ receptor antagonists bicuculline (10-30 μM) or picrotoxin (100 μM) but were unaffected by $GABA_B$ antagonists saclofen or CGP-035348.

These results suggest that evoked sIPSPs and sIPSCs observed in the LPBN are mediated by an increase in membrane conductance to chloride ions and support a role for $GABA_A$ receptors in mediating inhibitory neurotransmission within this nucleus.

Supported by Medical Research Council of Canada.

569.15

MULTIPLE FACTORS MODULATE THE $GABA_B$ IPSP IN GUINEA PIG VENTRAL TEGMENTAL AREA *IN VITRO*. D.L.Cameron* and J.T.Williams. Vollum Institute, Oregon Health Sciences University, Portland OR 97201.

The ventral tegmental area (VTA) has been implicated by a number of studies in the mediation of addictive behavior. Intracellular recordings were made from VTA cells in a horizontal slice preparation. The majority of cells (35/52) were hyperpolarized by dopamine (100µM) while a smaller proportion (14/50) were hyperpolarized by [met^5]-enkephalin (ME, 10µM). Approximately half (13/24) of the cells tested were depolarized by 5-HT (30µM). When bipolar electrical stimulation was applied to the preparation, most (29/38) cells exhibited a slow IPSP that was blocked by the $GABA_B$ receptor antagonist, 2-hydroxysaclofen (100µM). The magnitude of this IPSP was reduced by ME, the κ-agonist U69593 and 5-HT. While naloxone (1µM) reversed the actions of the opioids, the action of 5-HT was not affected by either the $5\text{-}HT_{1A}$ receptor antagonist NAN-190 (100nM) or the $5\text{-}HT_{1B}$ receptor antagonist cyanopindolol (100nM). These results indicate that GABA mediated synaptic potentials in the VTA can be modulated through both µ and κ opioid receptors and by 5-HT, possibly via $5\text{-}HT_{1D}$ receptors. These findings have important ramifications for understanding the actions of drugs of abuse such as morphine and cocaine in the VTA. Supported by an NH&MRC C.J.Martin Fellowship to D.L.C. and by NIH grants DA04523 and MH45003 to J.T.W.

569.16

INHIBITORY SYNAPTIC CURRENTS IN THE IMMATURE ZEBRA FISH BRAIN. P. Legendre* and H. Korn. Institut Pasteur 75015 Paris, France.

Synapses on the Mauthner (M-) cell of the Zebrafish (Brachydario Rerio) are morphologically differentiated after hatching (50 hours of gestation). Patch recordings were performed under direct visualization on the isolated brain of newly hatched larvae with pipettes containing (mM) CsCl 165 (or KCl 165), MgCl2 2, NaATP 4, EGTA 10 and HEPES 10. Brains were quickly removed, mounted on coverslips using plasma-thrombin embedding and stored for 1/2h prior to experiments, in an oxygenated recording solution containing (mM), NaCl 145, KCl 5, CaCl2 2, MgCl2 1, NaH2PO4 1.25, NaHCO3 26, glucose 10. During whole cell recording (165mM [KCl]i) the input resistance (Ri) of the M-cell was close to 35.9MΩ (± 6.1 SD; n=5) with a Vm of -56.2mV (±2.8 SD; n=5). Ri was increased to 66.1MΩ (±10.8 SD; n=13) after substitution of [KCl]i by [CsCl]i. Ongoing electrical activity consisted of fast Cl-dependent synaptic currents of various amplitudes blocked by 0.5 µM strychnine but not by bicuculline (50µM). TTX (1µM) reduced the mean amplitude and the frequency of these currents with the time to peak and decay time of the remaining ones being close to 500µs and 6ms respectively. Amplitude Histograms of TTX-resistant IPSCs from cells held at a membrane potential of -50 mV had an initial and prominent peak of 50-60 pA. Adding 10mM [MgCl2]o and decreasing [CaCl2]o to 0.1mM did not modify this distribution. In outside-out patches, glycine-activated single channels fell into three conductance states : 34.2 pS (±2.1SD; n=6), 42.7pS (±2.3SD ; n=8) and 82.1pS (±6.4SD; n=6), the 43pS one being dominant. The open time histogram of the latter was well fitted with two exponentials with time constants of 0.81 ms (±0.3,SD; n=6)and 4.8 ms (±1.0,SD; n=6). Therefore, glycinergic quanta correspond to the opening of 20 to 30 Chloride channels. Channel Open-state probability was remarkably voltage-dependent, nPo increasing 7-fold from -50mV to +50mV, a property also reported for glycinergic currents in the adult M-cell.

569.17

CELLULAR EFFECTS OF CHOLINERGIC INPUT IN SEPTO-HIPPOCAMPAL SLICES. R. Bianchi[1,2] and R.K.S. Wong*,[1]. [1]Dept. of Pharmacology, SUNY-HSC, Brooklyn, NY 11203 and [2]Dept. of Physiology and Biochemistry, Pisa (Italy) 36100.

In the mammalian CNS the Medial Septum/Diagonal Band complex provides a strong cholinergic projection to the Hippocampal Formation (HF). We prepared Septo-Hippocampal (SH) slices acutely isolated from adult guinea pigs, in order to study the cholinergic input to the CA3 hippocampal pyramidal cells (HPCs). In such preparation the gyrus dentate, CA1 and CA3 areas of the HF, the septal complex and the fimbria connecting the septum and the hippocampus were readily identifiable. Intracellular recording from CA3 HPCs showed that these cells retained passive and active membrane properties comparable to those described in transversal hippocampal slices. Occasionally, electrical tetanic stimulation of the septal complex or, more often, of the fimbria induced depolarization associated with increase in input resistance and firing in the CA3 HPCs. These effects were mimicked by "bolus" application of carbachol (CCh), increased by bath application of eserine (1uM) and blocked by bath-applied atropine (1uM). Addition of excitatory amino acid blockers (CNQX and CPP 10-20 uM each) did not affect the above described effects. We also observed that electrical tetanic stimulation (septal area or fimbria) and CCh application elicited phasic depolarizations when the cells were held more hyperpolarized than -70mV. Such depolarizations were associated with a decrease of input resistance and blocked by atropine (1uM).

569.18

EFFECT OF Cd++ AND FUNNEL WEB SPIDER TOXIN (FTX) ON TRANSMITTER RELEASE AT THE MOUSE NEUROMUSCULAR JUNCTION D.A.Protti, V.Sanchez, B.D.Cherskey, M.Sugimori, R.R.Llinas and O.D.Uchitel* Inst. Biol Cel. Faculty of Medicine Univ. Buenos Aires and Dept. Physiol Biophys. New York Univ Med Ctr.

FTX is a potent blocker of presynaptic Ca++ currentes and transmitter release at the mammalian neuromuscular junction (Uchitel et al PNAS 1992). We further studied synaptic transmission in a curarized nerve muscle preparation in order to analyzed the effect of Cd++ and FTX on transmitter release at low and high frequency nerve stimulation . As expected quantal content was diminished by both agents .In the absence of the blockers high frequency stimulation (40Hz) produces a decline in endplate potential amplitude due to a decrease in quantal content. In contrast in the presence of Cd++ or FTX and early facilitation and subsequent less pronounced decline in quantal content was observed. similar to the small facilitation seen in mammalian neuromuscular junctions of animals treated with IgG from Eaton Lambert human patients

569.19

ACTIONS OF ANABASEINE AND DMAB-ANABASEINE UPON NEURONAL $\alpha_4\beta_2$ AND PC12 CELL NICOTINIC RECEPTORS. W.R. Kem*[1] and R.L. Papke[2], [1]Dept. of Pharmacol. Ther., Univ. of Fla. Coll. Med., Gainesville, FL 32610-0267 and [2]Mol. Neurobiol. Lab, Salk Institute, San Diego, CA 92138.

The marine worm toxin anabaseine (2-(3-pyridyl)-3,4,5,6-tetrahydropyridine) structurally resembles nicotine, but is even more potent at the frog neuromuscular junction. Previous ligand binding experiments with rat brain synaptosomes demonstrated that anabaseine and its 3-(4-dimethylaminobenzylidene) adduct interfered with (^{3}H)methylcarbamyl choline binding at 100–200 nM concentrations. In contrast with muscle nicotinic receptors, neuronal receptors (rat $\alpha_4\beta_2$ subtype expressed on *Xenopus* oocytes and PC12 cell subtypes) displayed less physiological response to anabaseine relative to ACh ($\alpha_4\beta_2$) or nicotine (PC12). On the $\alpha_4\beta_2$ subtype anabaseine acted only as a partial agonist (EC_{50} ~ 30 μM, relative to ACh EC_{50} ~ 2 μM); the peak current was less than half of the ACh maximum inward current. On PC12 cells, anabaseine acted as a full agonist on $^{86}RB^+$ efflux, but was approximately 5x less potent. In contrast, DMAB-anabaseine acted primarily as a nicotinic antagonist on $\alpha_4\beta_2$ and PC12 neuronal nicotinic receptors as well as upon neuromuscular receptors. However, at 100 μM, DMAB-anabaseine produced a small inward current (2% of the maximal ACh-induced current); thus it may possess an extremely small partial agonist activity, at least on the $\alpha_4\beta_2$ neuronal subtype. (Partially supported by Taiho Pharmaceutical Co., Ltd.)

569.20

KINETIC AND EQUILIBRIUM CHARACTERIZATION OF HIGH-AFFINITY ANALOGS OF VESAMICOL. G.A. Rogers*, W.D. Kornreich, K. Hand, S.M. Parsons. Dept. of Chemistry, Univ. of California, Santa Barbara, CA 93106.

Twelve derivatives of vesamicol have been synthesized that exhibit from three to one thousand times higher affinity for the vesamicol receptor (VR) in Torpedo synaptic vesicles. Kinetic data for the dissociation of analogs from the VR were obtained using a 'back-titration' assay (Rogers and Parsons, Neuroreport 1, 22-25, 1990). Equilibrium data were obtained by determining IC_{50} values for displacement of [^{3}H]vesamicol from synaptic vesicles at very low concentrations (0.2 μg protein/ml) and for long incubation periods (24 hr). Three analogs were resolved and the individual enantiomers were characterized. The VR exhibited an enantiomeric selectivity ratio of 250 for cis-4-fluoromethylvesamicol. One derivative, 4-aminobenzovesamicol (ABV), was radiolabelled with tritium which allowed a direct determination of the equilibrium binding isotherm as well as rates of association and dissociation. (-)-ABV binds to the VR with a K_D value of 6.5 ± 0.5 pM. The rate constants for association and dissociation are (1.0 ± 0.1) X 10^8 $M^{-1}min^{-1}$ and (8.4 ± 0.05) X 10^{-4} min^{-1} ($t_{1/2}$ = 14 hr), respectively. ABV represents a valuable new tool for studies of the cholinergic presynapse.

ION CHANNELS: CELL FUNCTION

570.1

MEMBRANE PROPERTIES OF HUMAN FETAL BRAINSTEM NEURONS *IN VITRO* S. Chung, P.J. Kontur, L.L. Kaczmarek, R. Robbins, B.S. Bunney* Departments of Pharmacology and Psychiatry, and Neuroendocrinology, Yale Univ. Sch. of Med., New Haven, CT 06510

The whole-cell patch clamp method was used to characterize membrane potentials and currents in human fetal brainstem neurons. Cultures were prepared from fetuses of 9-12 weeks gestation and maintained in culture for 8-13 days prior to recordings. Some cells showed spontaneous membrane depolarizations. In most cells, depolarizing currents triggered short (< 10 ms) action potential that appeared to be mediated by calcium and potassium currents. The majority of inward current was not blocked by TTX. In contrast, 1 mM Co^{2+} blocked the current completely indicating that the inward current was probably carried largely by calcium channels. Two major components of outward potassium current were observed. The fast transient component was blocked by 4-AP but not by TEA. The slow transient component was blocked by TEA but not by 4-AP.

In summary, we have identified both potassium and calcium currents in developing human neurons. These cultured brainstem neurons may provide a valuable means to characterize ion channels in human neural membrane.

570.2

IONIC MECHANISMS OF OSCILLATORY FIRING ACTIVITY OF RAT CEREBELLAR PURKINJE CELLS. W. Chang*, H. K. Strahlendorf and J. C. Strahlendorf, Departments of Physiology and Neurology, Texas Tech Health Sciences Center, Lubbock, TX 79430.

Rhythmic firing patterns of neurons have been shown to be mediated by the sequential activation of a set of conductances. However, mechanisms of intrinsic rhythmicity vary considerably among neurons. This study is investigating the contribution of sodium current (I_{Na}), inward rectifying cationic current (I_h), transient outward K^+ current (I_A) and Ca^{2+}-activated K^+ current ($I_{K(Ca)}$) to oscillatory firing of Purkinje cells. We have confirmed that oscillatory firing activity can be initiated and sustained by membrane ionic conductances in the absence of effects of action potential-induced release of extrinsic neurotransmitters. Adding TTX to the superfusion solution produced a typical pattern of repetitive burst firing consisting of Ca^{2+} dependent action potentials and long periods of hyperpolarization. We used the I_h blocker, cesium, and found that this current probably is not involved with initiation of oscillation, but plays an important role in maintenance of rhythmicity. Cs^+ produced long duration hyperpolarization after bursting to very negative membrane potentials (-130 mv) which exceeded the K^+ equilibrium potential. Low doses of 4-aminopyridine (4-AP, I_h blocker) induced oscillatory firing whereas, high doses changed the pattern and frequency of firing, especially the duration and amplitude of action potentials. Apamin ($I_{K(Ca)}$ blocker) reduced part of the afterhyperpolarization during and after bursting and terminated oscillatory activity. These data indicate an important contribution of I_{Na}, I_h, I_A and $I_{K(Ca)}$ to rhythmic pacemaker firing of Purkinje neurons.

570.3

DUAL PATCH-CLAMPING OF MAMMALIAN PURKINJE CELLS IN CEREBELLAR SLICES. M. Sugimori* and R. Llinás, Dept. Physiology/Biophysics, NYU Medical Center, 550 First Avenue, NY, NY 10016.

Double somatic patch recording (P_1 and P_2) was implemented to study the electrophysiology of Purkinje cells under patch-clamp conditions. One electrode (P_1) was utilized to patch-clamp the neuron while the second (P_2) recorded voltage in a current-clamp configuration. The access resistance for electrodes was on the order of 1 mega-ohm and was monitored continuously for P_1, by computing the difference in voltage between the two electrodes (P_1–P_2). Voltage-clamp depolarization steps resulted in either a fully controlled sodium current or, more often, in the generation of action potentials. The latter event occurred most often in cells with less than optimal viability where the leakage resistance was high.

The voltage electrode demonstrated action potential generation at the somatic level, even in conditions where the Purkinje cells were properly patched and the access resistance of electrodes was low (1–2 mΩ).

In order to test whether non-somatic sodium spikes could be generated, dendritic depolarization was obtained with glutamate iontophoresis at the distal dendrites. The firing level for the sodium spike in Purkinje cells that ensued was unmodified by voltage-clamp conditions which held the somatic potential at –90 mv and was generated by the initial activation of a low-threshold noninactivating sodium current, even in the absence of voltage-dependent calcium conductances. This finding indicates that only one site for sodium spike generation is present in Purkinje cells.

The results indicated that sodium spikes and Purkinje cells arise at or near the somatic membrane and that dendrites are unlikely to support sodium spikes, in accordance with previous results. (Supported by NS13742 and AG09480)

570.4

INTRINSIC MEMBRANE POTENTIAL OSCILLATIONS IN RAT PIRIFORM CORTEX PYRAMIDAL CELLS.

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The rat piriform cortex displays oscillatory field potential and EEG dynamics with peaks in the power spectra at 3-10 Hz and 40-60 Hz. These oscillatory properties may provide the optimal dynamics for associative memory function in this region (Hasselmo et al., *J. Neurophys.* 67:1230-1246). The oscillations have been linked to the time constant of inhibition within the cortex (Wilson and Bower, *J. Neurophys.* 67:981-995). However, recent evidence from this laboratory suggests that piriform cortex pyramidal cells may show intrinsic oscillatory membrane dynamics as well.

In brain slice preparations of piriform cortex, we performed intracellular recording in layer II from a population of pyramidal cells (n=32, Vm = -74.3 ±5.2 mV, spike height = 102 ±7.5 mV, Rm = 23.2 ±7.1 MΩ, firing threshold = 17.1 ±4.8 mV). A large number of these cells (n=15) show low amplitude (up to 6mV) oscillations of membrane potential during a 1 sec. current injection which depolarizes the cell membrane above -60mV. These oscillations in membrane potential also appear during a sustained depolarizing current injection. Hyperpolarizing current injections did not induce membrane potential oscillations. The oscillations show a predominant peak to peak interval of between 140 and 50 msec. (7 to 20 Hz), with some change in frequency with increased depolarization. Frequently, spiking activity was synchronized with the peak of oscillatory activity, such that the spike intervals during the later portions of a 1 second current injection were strongly correlated with the wave length of the subthreshold membrane oscillations. The membrane potential oscillations were still present during perfusion with a low Ca/high Mg solution or with zero Ca and cadmium chloride (100μM). In addition, perfusion with the cholinergic agonist carbachol (100μM) did not appear to strongly alter the oscillatory properties of these neurons. These oscillations do not fully match the predominant frequencies in field potential data, but may interact with circuit characteristics to contribute to the oscillatory dynamics of piriform cortex activity. *Supported by The French Foundation for Alzheimer Research.*

570.5

TONIC ACTIVATION OF I_h IN CA1 PYRAMIDAL NEURONS

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The whole cell variation of the patch clamp technique was used to investigate the role of the hyperpolarization activated inward current (I_h) in CA1 pyramidal cells from hippocampal slices maintained *in vitro*. Under voltage clamp, I_h was activated at potentials between -50 and -150 mV and displayed no time-dependent decay. Manipulations of extracellular K^+ and Na^+ concentrations both affected current amplitude due to its mixed ionic nature. External Cs^+ ions (2-10 mM) reversibly blocked the current. In current clamp experiments, 2 mM Cs^+ elicited a small (around 4 mV) hyperpolarization at cell resting potential (-56 to -65 mV); this hyperpolarizing shift was enhanced by cell hyperpolarization by negative tonic current injection. In addition, the relatively small Cs^+-induced hyperpolarization was sufficient to prevent neuronal firing evoked by depolarizing current steps (20 pA for 2 seconds) from cell resting potential. Taken together, our results suggest that I_h may play a role in the regulation of resting membrane potential in hippocampal pyramidal cells, thus contributing to the regulation of cellular excitability.

570.6

CALCIUM CURRENTS AND CALCIUM INFLUX IN CULTURED RAT MYENTERIC NEURONS: A FUNCTIONAL ANALYSIS.

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We have been studying the effects of chronic depolarization on the development of rat myenteric neurons in cell culture. Previously, we found that growth in medium containing elevated potassium (25 mM), which depolarizes these neurons to a mean resting membrane potential of -40 mV, decreases the density of voltage-dependent Ca currents and causes persistent elevation of intracellular Ca ($[Ca]_i$) (Franklin et al., J. Neurosci., 1992). Here we report a test of the hypothesis that the decreased density of Ca currents leads to decreased Ca influx in response to neuronal action potential activity.

Myenteric neurons dissociated from intestines of 2-3 d old rat pups were grown in cell culture for 3-5 d in medium containing control (5 mM) or elevated (25 mM) KCl. $[Ca]_i$ was monitored in fura-2-loaded cells, using video-based image analysis. Calcium current density was determined by whole cell tight-seal recordings in solutions designed to isolate Ca currents from other voltage-gated currents. Neurons were stimulated with a pair of extracellular Pt wire electrodes. Antagonists of Ca currents and other drugs were applied from extracellular pipets.

Brief (300 msec, 25 Hz) trains of pulses (2.5 ms) elicited rapid (< 3 sec to peak) increases in $[Ca]_i$ that decayed back to baseline within 30 sec. The absolute height of the peak varied from cell to cell, but typically, chronically depolarized cells had peaks that were 50-70% of those observed in control cells. In both types of cell, conotoxin- and dihydropyridine-sensitive components were observed. We conclude that the effects of chronic depolarization on Ca current density are functionally significant, resulting in significantly reduced Ca influx during neuronal electrical activity. Supported by NIH grants to ALW.

570.7

LUNG-SPECIFIC VISCERAL C-FIBER NEURONS IN THE GUINEA PIG NODOSE GANGLION EXPRESS A CHARYBDOTOXIN-SENSITIVE K^+ CONDUCTANCE THAT CONTRIBUTES TO ACTION POTENTIAL REPOLARIZATION.

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Afferent neurons in the nodose ganglion innervate organs in the cardiovascular, respiratory, and gastrointestinal systems. These neurons are also heterogeneous with regard to several ionic conductances. Experiments were thus conducted to determine whether nodose neurons innervating the lung show any distinctive electrophysiological features, relative to neurons that were sampled without regard to end organ innervation. Rhodamine or fast blue fluorescent dye was instilled into the airways via an endotracheal catheter in anesthetized guinea pigs. After 5-8 days nodose neurons were acutely dissociated. From 2-5% of the dissociated neurons were retrogradely labeled, and thus taken to be lung-specific. Single microelectrode current- and voltage-clamp techniques revealed physiologically acceptable passive and active membrane parameters in dye-labeled neurons. Lung-specific C-fiber neurons as a population did not differ from non-labeled neurons with respect to several electrophysiological and pharmacological parameters. The most notable exception was a concentration-dependent broadening of the action potential duration in 24/26 lung-specific C-fiber neurons in response to either charybdotoxin or iberiatoxin (1-100 nM). In contrast, the action potential in only 13/23 non-labeled neurons was affected by these peptide toxins. Thus lung-specific C-fiber afferent neurons may be enriched in the expression of a Ca^{2+}-activated K^+ channel that is known to be blocked selectively by these peptide toxins.

570.8

GAP JUNCTIONS BETWEEN CHICK PINEALOCYTES: ULTRASTRUCTURE, IMMUNOCYTOCHEMISTRY AND POSSIBLE INVOLVEMENT IN MELATONIN SECRETION.

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The chick pineal gland secretes the hormone melatonin with a circadian rhythm regulated by light. Chick pinealocytes in primary cell culture retain their ability to secrete melatonin rhythmically; the rhythm is light-entrainable, and secretion is maximal in the middle of the night. Synchronization of function between cells might be facilitated by intercellular communication through gap junction (GJ) channels which are permeable to ions and small molecules. The protein monomers of these channels are termed connexins (Cx), which are encoded by a gene family. Pineal glands were dissected from White Leghorn chicks one day after hatching, and fixed for morphological studies or dissociated enzymatically for primary cell cultures that were maintained in a light-dark cycle. The functional state of GJs was evaluated by dye coupling (Lucifer yellow). Secretion of [^{14}C]-melatonin (derived from [^{14}C]-tryptophan) into the culture medium was measured. In thin sections, septilaminar GJs were seen between pinealocytes. In freeze-fracture replicas, GJs of the crystalline-arrayed type were rare and integrated within tight junctional networks. Immunofluorescence of pineal cells in culture revealed Cx43 and Cx45 immunoreactivity at appositional membranes. Dye coupling between pineal cells in culture was extensive. Octanol inhibited dye coupling and nocturnal melatonin secretion in a dose-dependent manner and more potently than hexanol. It is suggested that GJs between chick pinealocytes might favor melatonin secretion.

570.9

REAL-TIME VOLUME MEASUREMENTS IN PRIMARY HIPPOCAMPAL CULTURES BY CONFOCAL MICROSCOPY.

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Activation of non-NMDA channels depolarizes cells by sodium influx and is believed to partially account for cell swelling after anoxia or exposure to excitatory agents. Simultaneous measurement of ion fluxes and cell volume may expand the understanding of the cellular regulation of these parameters. Observation of both cell volume and membrane potential can be achieved by simultaneous application of physiologic and microscopic methods. Due to superior depth resolution, confocal microscopy is particularly well suited for real-time imaging of fluorescence labeled live cells. The use of an inverted microscope permits to simultaneously record electrophysiologically while imaging in the confocal mode.

DiI is known to label cell membranes with little fading even after repeated exposure to the laser light source. Primary hippocampal cultures were incubated for one hour in medium containing 1 μM of DiI and pluronic F-127 (0.02%) to facilitate the incorporation of the dye in the cell membrane. Depolarization after application of kainic acid (40 μM) was accompanied by an increase in cell volume after 30 minutes. Cell processes retracted during this time.

We conclude that cell swelling after application of kainic acid succeeds depolarization with a time lag of several minutes.

Supported by grants from the Deutsche Forschungsgemeinschaft, NIH RR06904, NSF DIR9108492 and NS 23807.

570.10

SIMULATION OF SPACE-CLAMP ERRORS.

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Recent voltage-clamp studies have used poorly space-clamped cells, despite the methodological flaws involved. We investigated space-clamp errors using computer simulations of monopolar model cells with a single primary neurite and a single voltage-dependent channel type having various spatial distributions. Peak I(V) curves were fitted with an equation of the form:

$$I = \bar{g}(V-V_{rev})/(1+\exp((V_o-V)/v)).$$

Uniform distribution of outward current channels: We find that non-soma current is reduced by negative feed-back from the outward sign of the current, the steep voltage-dependence of the channels, and foreshortened length constants. In a typical case, when $\bar{g}$ density in the neurite is increased 100x, only a *ca.* 6x increase in contamination of the soma clamp-current results. For moderate conductance densities (e.g. 10x rest), parameters most affected by the poorly space-clamped membrane are $\bar{g}$ (reduced) and V_{rev} (positive shift). With higher $\bar{g}$ densities, fitted $\bar{g}$ errors are larger and other errors tend to be smaller.

Outward current in neurite only: When the same number of channels are confined to the neurite farther from the soma, $\bar{g}$ and V_{rev} errors continue to increase, V_o shows a positive shift and v increases. An electrically compact cell with 10x rest conductance concentrated at the neurite tips can show errors of 20x in fitted $\bar{g}$, 2x in v, over 30 mV in V_{rev}, and over 80 mV in V_o.

Inward current: Most errors are in the same direction as with outward current but are of greater magnitude. Errors in v can be somewhat smaller. Even without overt evidence of regenerative escape at distant sites, severe distortion of quantitative parameters can occur.

In all cases, significant distortions of measured current kinetics can occur as well. Support: NSF BNS-8920698.

570.11

ROLES OF Ca^{+2} AND K^+ IN NERVOUS SYSTEM TUMOR CELL GROWTH. Y.S. Lee, M. Weber, and R.D. Wurster*. Dept. of Physiology, Loyola Univ. of Chicago Sch. of Med., Maywood, IL 60153.

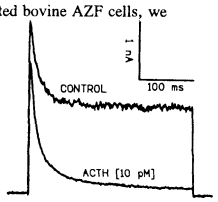
The roles of Ca^{+2} and K^+ fluxes in the tumor cell growth were evaluated. A human neuroblastoma cell line (SK-N-MC) and a human astrocytoma cell line (U-373 MG) were grown for 2 days with or without various ion-channel-related compounds after 1 day culture for cell attachment. The number of cells was measured using a hemocytometer. K^+-channel blockers (tetraethylammonium-TEA and 4-aminopyridine-4-AP) and Ca^{+2} channel blockers (verapamil, Ni^{+2}, and Co^{+2}) inhibited the growth of both tumor cells in a concentration-dependent manner. Both cell lines showed a tremendous decreased cell number when grown in media depleted of free calcium ions by titration with relatively high concentrations (2-3mM) of EGTA. High extracellular K^+ or Ca^{+2} induced a significant decreased cell growth. The responses of the neuroblastoma cell line were more sensitive to all manipulations used than those of the astrocytoma cell line. Because moderate concentrations of EGTA (0.1-1.0mM) were ineffective, neoplastic cells may have the capacity to continue to proliferate at low Ca^{+2} levels. Increased intracellular Ca^{+2} seems to have dual effects: growth-promoting and growth-inhibiting effects. The very steep response-curves of the cells to all the drugs except TEA suggest that these tumor cells have very precise regulatory mechanisms of ion fluxes relating to cell proliferation. K^+-channel activity and high $[K^+]_o$-induced depolarization may influence Ca^{+2} influx through activation of Ca^{+2} channels or changes in the electrochemical gradient for Ca^{+2} ions. Taken together, the results further suggest that although the mechanisms of Ca^{+2} influx and existence of Ca^{+2} channels in both cell lines remain to be established, Ca^{+2}-regulating mechanisms may play an important role in tumor-cell growth and that ion channels may be potential targets for the management of tumor development.

(Supported by the Mr. and Mrs. Barney Kahn Fund)

570.12

A NOVEL POTASSIUM CURRENT IN ADRENAL ZONA FASCICULATA (AZF) CELLS IS INHIBITED BY ADRENOCORTICOTROPIC HORMONE (ACTH) AND ANGIOTENSIN II (AII). B. Mlinar@, B.A. Biagi#,* and J.J. Enyeart@ . Depts. of Physiology#, Pharmacology and Neuroscience Program@, The Ohio State University, Columbus, OH 43210-1239.

Adrenal corticosteroid secretion is regulated by peptide hormones including ACTH and AII. The ionic conductance changes associated with peptide-stimulated cortisol secretion have not been identified. In whole cell patch clamp recordings from enzymatically dissociated bovine AZF cells, we have identified two separate K^+ currents, including a rapidly inactivating A-type current and a novel rapidly activating, non-inactivating, high unitary conductance channel whose expression is abolished by including 200 μM GTP-γS in the pipette.



This novel K^+ current was potently and completely blocked by ACTH (IC_{50} = 10 pM, n=18) by a mechanism which required several minutes to develop (see figure). AII also reduced this current (IC_{50} = 118 pM, n=14) but inhibition was incomplete (mean = 83%). ACTH and AII also depolarized adrenal cells by a maximum of 53 mV and 48 mV respectively with half maximal effects observed at 10 pM and 253 pM. These results indicate that this new K^+ channel may set the resting potential of AZF cells. Further, two different peptide hormones known to work through different second messengers might trigger depolarization-dependent cortisol secretion by selective inhibition of this channel.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY VI

571.1

SOURCE-SPECIFICITY OF Ca^{2+} NEUROTOXICITY IN CULTURED NEURONS. C.H. Tator, M. Tymianski, R. Robitaille', M.P. Charlton. U. of Toronto, and Playfair Neuroscience Unit, Toronto, Ont. M5T 2S8.

The Ca^{2+} neurotoxicity hypothesis dictates that neurotoxic phenomena are triggered when free cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) rises too high, for too long. We asked whether depolarization-induced and excitatory-amino acid (EAA)-mediated neuronal injury was due solely to a sustained elevation in $[Ca^{2+}]_i$. Cultured murine spinal neurons were exposed to 50 min challenges with EAA's or high-K^+. $[Ca^{2+}]_i$ was imaged with the Ca^{2+} indicator fura-2. Neuronal death was gauged with trypan blue and ethydium homodimer staining. In 1mM Ca^{2+}, challenges with glutamate (GLU; 250μM) or K^+ (50mM) evoked a transient peak rise in $[Ca^{2+}]_i$ followed by a decay to a lower "plateau". 100% of neurons which died underwent a second, irreversible sustained rise in $[Ca^{2+}]_i$, unaffected by switching perfusions to Ca^{2+}-free solutions- indicating that decompensation of neuronal Ca^{2+} homeostasis precedes a loss of plasma membrane integrity. GLU neurotoxicity was not manifest in zero Ca^{2+}. GLU and high-K^+ evoked equivalent peak- and mean increases in $[Ca^{2+}]_i$, but the toxicity of GLU was 5 times greater than that of high-K^+. The relationship between Ca^{2+} influx and neurotoxity was dissected further with EAA agonists and antagonists. In the absence of NMDA blockade, GLU (250μM) and NMDA (100μM) challenges were lethal within 1.5 hrs of the EAA challenge (85% neuronal death). APV (50μM) attenuated GLU neurotoxicity to 16$\pm$6% ($p<0.0001$), but NOT the rise in $[Ca^{2+}]_i$, which was only blocked by adding the AMPA/Kainate antagonist CNQX (50μM). Kainate (KAIN; 100μM) produced a marked rise in $[Ca^{2+}]_i$ which was NOT neurotoxic. The lack of KAIN neurotoxicity was not a consequence of reduced Ca^{2+}-load, because pre-treatment of neurons with Concanavalin-A increased KAIN-and GLU-induced increases in $[Ca^{2+}]_i$, but had NO effect on neurotoxicity ($p=0.34$). We conclude that the trigger for Ca^{2+} neurotoxicity is source-specific, and depends not only on a sustained, elevated Ca^{2+}-load, but on the route of Ca^{2+} influx.

571.2

GLUTAMATE-INDUCED $[Ca^{2+}]_i$ CHANGES IN RAT CORTICAL NEURONS: CORRELATION WITH NEURONAL DEATH. S.Rajdev* and L.J.Reynolds. Dept. Pharmacology, Univ. Pittsburgh, Pittsburgh,PA 15261.

It is widely believed that glutamate released during conditions such as ischemia kills neurons by increasing $[Ca^{2+}]_i$. However, the precise mechanism responsible for increased $[Ca^{2+}]_i$, especially during ischemia is not clear. We have previously shown (Soc. Neuro. abstract # 314.14, 1991) that addition of KCN in glucose-free buffer (to simulate *in vitro* chemical ischemia) significantly increased the $[Ca^{2+}]_i$ changes induced by glutamate, NMDA, kainate and high K^+ in single rat forebrain neurons. We are further studying whether KCN also increases the neurotoxicity of these agonists.

By using a dual detection system we have been able to simultaneously study the $[Ca^{2+}]_i$ changes and neurotoxicity in cultured neurons. Cells are loaded with fluo-3AM (5μM, 60min), and recordings are made with propidium iodide (4μM) in the buffer during the experiment. Fluorescence from PI is observed as cells die as this probe is excluded from healthy cells.

Almost all the neurons exposed for 5 min. to glutamate 3μM and glycine 1μM, or kainate show an initial $[Ca^{2+}]_i$ increase and most recover from this Ca^{2+} load. However, many of those treated with glutamate show a latent rise in $[Ca^{2+}]_i$ with a variable time delay (2-3 hrs). KCN (5mM) pretreatment appears to enhance the initial $[Ca^{2+}]_i$ rise and also shorten the latency period for the delayed irreversible $[Ca^{2+}]_i$ rise. However, most neurons treated with 100 μM kainate or KCN and kainate did not show a delayed $[Ca^{2+}]_i$ rise within 3.5 hrs of experiment duration. Cell death, measured by an increase in PI fluorescence, usually occurs following the late $[Ca^{2+}]_i$ rise.

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571.3

Ca^{2+} BINDING PROTEINS IN CHICK RETINA AND SENSITIVITY TO EXCITOTOXICITY. G.D. Zeevalk* and D.M. Jacobowitz. Neurology Dept.,UMDNJ-RWJ Med.Sch., Piscataway, N.J. 08854 and Lab.of Clin.Sci.NIMH, Bethesda, MD. 20892.

Uncontrolled Ca^{2+} flux is thought to orchestrate cell death due to excitatory amino acids. Studies suggest a relationship between Ca^{2+} binding proteins (CaBPs) and resistance to excitotoxicity. It is not clear, however, if neurons survive due to an absolute resistance to the excitotoxin. For example, in chick retina, NMDA kills most amacrine and some ganglion cells, whereas other retinal populations are unaffected. The resistance of neurons in the outer layers of retina is due to the absence of NMDA receptors on these cells. Amacrine neurons in retina are ideal to study the relationship between CaBPs and excitotoxicity because most amacrines are sensitive to NMDA in a dose dependent manner and the CaBPs, parvalbumin,(PV); calretinin,(CR); and calbindin, (CB) are found in this layer. Exposure of embryonic day 19 chick retina for 60 min to either 25, 100, 250 or 500μM NMDA caused a dose dependent increase in LDH release measured after 24hr of recovery. At 24hr, retina was fixed and processed for PV, CR and CB immunoreactivity. The number of amacrine cells positive for the CaBPs were counted and correlated to LDH release. Statistical analysis showed a negative correlation between NMDA mediated LDH release and loss of PV + amacrines and no correlation with loss of CR or CB + amacrines. Thus, as LDH increased, the number of PV cells, but not CB or CR cells declined. Exposure to 500 μM NMDA for 24hr resulted in a near total loss of PV and CB and 66% of CR + amacrines. These data suggest that although CB and CR + amacrines show a relative resistance to NMDA, the presence of CaBP, per se, is not sufficient to confer resistance.

571.4

INTRA-NEURONAL Ca^{2+}-BUFFERING WITH BAPTA ENHANCES GLUTAMATE EXCITOTOXICITY IN VITRO AND ISCHEMIC DAMAGE IN VIVO. K.G. Baimbridge* and K.M. Abdel-Hamid. Dept. of Physiology, University of British Columbia, Vancouver, B.C., Canada, V6T 1Z3.

Primary cultures of rat hippocamapal pyramidal neurons were exposed for 30 min at room temperature to various levels of glutamate. Survival was assessed 48 h later using the fluorescent vital stains, calcein/am and ethidium homodimer. Compared to controls, prior loading of the neurons for 60 min in a 10 μM solution of the fast Ca^{2+}-buffer BAPTA-AM greatly increased the excitotoxic effect of glutamate. BAPTA-AM loading alone had no observable toxicity although it was demonstrated using fura-2 that the rate of rise and maximum amplitude of glutamate induced transients $[Ca^{2+}]_i$ were reduced whereas their decay times were considerably extended; consistent with the action of an enhanced Ca^{2+}-buffering capacity.

An in vivo hemorrhagic hypotensive model of ischemia in the rat was also used to test the effects of prolonged microinjection of BAPTA-AM, or the vehicle DMSO, in the region of the dorsal CA1 pyramidal cell layer. No sparing of CA1 neurons occurred in the BAPTA loaded regions. On the contrary, when the injection site included parts of the subiculum (usually spared form ischemia-induced cell death), the damage was considerably enhanced. In addition, parvalbumin containing interneurons in the CA1 region were also lost in regions exposed to BAPTA-AM whereas they are spared in controls or DMSO injected rats.

These results demonstrate that enhanced Ca^{2+}-buffering exacerbates, rather than ameliorates, the direct excitotoxic effect of glutamate in vitro and similarly enhances ischemia induced cell death in vivo. It is possible that increased Ca^{2+}-buffering may promote the influx of Ca^{2+} by reducing the Ca^{2+}-dependent inactivation of voltage operated Ca^{2+}-channels.

571.5

DIMINISHED GLUTAMATE-INDUCED INFLUX OF EXTRACELLULAR CALCIUM IN THE EPILEPTOGENIC HUMAN HIPPOCAMPUS *IN VIVO*

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We have developed and characterized a microdialysis probe suitable for chronic implantion in the human hippocampus. These probes were implanted bilaterally in patients with intractable complex partial epilepsy. To test the hypothesis that clinical, spontaneous-onset seizures elevate glutamate to potentially excitotoxic concentrations and that repeated exposure to these concentrations results in a loss of glutamate receptor-mediated responses, we measured extracellular fluid glutamate during spontaneous-onset seizures and the flux in Ca^{++}_o following the local perfusion of exogenous glutamate.

During seizures, extracellular glutamate reached concentrations which are potentially neurotoxic (>100μM). Moreover, the increase was greater and sustained in the epileptogenic hippocampus. Furthermore, glutamate-induced calcium influx was diminished in the epileptogenic hippocampus (39±6% decrease in the non-epileptogenic vs. a 17±7% decrease in the epileptogenic side) consistent with prior excitotoxic injury and suggesting loss of receptors and/or cells which express glutamate-gated calcium channels.

Supported in part by the NIH.

571.6

KINETICS OF ^{45}Ca FLUX IN BRAIN SLICES: EFFECTS OF GLUTAMATE AND THICKNESS.

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Glutamate and excitatory neurotransmitters stimulate calcium influx into neurons and glial cells. Excess calcium accumulation may underlie ischemic brain injury as well. To study kinetics of calcium influx and efflux in response to glutamate or ischemia, we measured ^{45}Ca uptake and washout from hippocampal brain slices. ^{45}Ca kinetics was studied in 500μ thick control slices, 500μ slices in the presence of 0.1, 1, 10, 100, 1000 μM glutamate, or in 1000μ thick hippocampal slices which serve as our model of the ischemic penumbra.

We have derived three and four compartment kinetic models but report results only for a serial three compartment, four parameter model fit by weighted least squares analysis. Results with this kinetic model show that glutamate increases influx of calcium into the first tissue compartment without affecting efflux from the first compartment or either parameter for the second tissue compartment. Results with ischemic 1000μ slices show that first compartment constants are the same as control 500μ slices but that entry into the second compartment is significantly increased. Concentration-response curves for the stimulation of calcium influx into 500μ slices revealed relatively little variation with concentration, with a trend toward highest calcium influx at or below 1 μM.

Our results suggest that calcium accumulation in brain during ischemia occurs as a result of factors not solely related to glutamate excitotoxicity, but also to factors that increase calcium in intracellular compartments which are not affected by glutamate under physiological conditions. Further work will be done to clarify the kinetic models, study lower glutamate concentrations and receptor analogues and extend the method to autoradiography.

571.7

POST-ISCHEMIC GLUCOCORTICOID EXPOSURE DOES NOT AUGMENT EXCITATORY AMINO ACID OVERFLOW IN THE RAT HIPPOCAMPUS.

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Exposure to glucocorticoids (GCs) can enhance hippocampal neuron loss following ischemia in rodents. Recent findings from our lab suggest that GCs, when present both before and after kainic-acid infusion, increase the extracellular level excitatory amino acids (EAAs) in the rat hippocampus (J. Neurochem, 1992). This implies that GCs may aggravate injury by exacerbating excitotoxic cascades. In light of this, we asked whether post-ischemic exposure to GCs could influence the extracellular levels of EAAs in the rat hippocampus after transient forebrain ischemia. Rats (n=10) were surgically prepared for 4-vessel occlusion, adrenalectomized, and food-deprived overnight while given access to 0.9% saline. The next day, animals were subjected to 20' of global forebrain ischemia under halothane anesthesia. Rectal temperature was maintained at 37°C with heating lamps and cortical EEG amplitude was monitored throughout the experiment. Immediately after occlusion, half of the rats received injections of corticosterone (CORT) resulting in plasma CORT levels that matched those seen in intact rats following a 20' ischemic insult; control rats received equivalent vehicle injections. Hippocampal microdialysis samples were collected during and for 6 hours after ischemia and then analyzed for selected amino acids by HPLC. A large transient rise in EAAs was seen in both treatment groups during the ischemic period, but no differences between groups were detected at any time point for any amino acid. These results suggest that post-ischemic GC exposure in ADX rats does not endanger hippocampal neurons by elevating interstitial levels of EAAs.

571.8

A MASS-FRAGMENTOGRAPHIC SEARCH FOR 6-HYDROXYDOPAMINE (6HO-DA) IN THE RAT BRAIN FOLLOWING THE ADMINISTRATION OF PSYCHOSTIMULANTS: A NEGATIVE FINDING.

F. Karoum* and R.J. Wyatt. NPB, NIMH, St. Elizabeth Hospital, Washington, D.C. U.S.A.

Non-enzymatic formation of 6HO-DA from dopamine following the administration of psychostimulants has been hypothesized to mediate their neurodegenerative effects. Direct support for this hypothesis came from the report of a 6-OH-DA-like substance in the rat striatum (ST) following the administration of high doses of methamphetamine (Pharmacol. Biochem. Behav. (1984), 21, 29-31). Although the identity of this 6-OH-DA-like substance was established by indirect pharmacological approaches, its structure has not been confirmed by direct methods. We used a mass-fragmentographic method to search for 6-OH-DA in the rat frontal cortex (FC) and ST following the administration of 6-hydroxydopa and a number of drugs that stimulate dopamine release. 6-OH-DA identity was based on the presence of specific fragments in it spectra produced by positive and negative chemical ionization. While both I.P. and I.C.V. administrations of 6-hydroxydopa (2 mg/kg and 200 μg respectively) produced measurable concentrations of 6-OH-DA in the FC and ST, no 6-OH-DA was detected after 25 or 50 mg/kg of methamphetamine, amphetamine or after 20 mg/kg of cocaine. It is concluded that if these agents can cause the formation of 6-OH-DA, the concentrations produced are below the detection level of our assay (less than 50 pg/mg protein).

571.9

TRANSNEURAL MECHANISM OF SELECTIVE DEATH OF CA1 NEURONS BY AN NMDA RECEPTOR AGONIST, L-CCG-IV.

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We have previously reported that intracerebral injection with a potent NMDA receptor agonist, the (2S,3R,4S) isomer of α-(carboxycyclopropyl)glycine (L-CCG-IV) induced selective death of the CA1 neurons in the rat hippocampus. Although the injection site was unilateral CA1, neuronal death frequently occurred in the bilateral CA1 sector. Therefore, we aimed to investigate whether or not the severance of corpus callosum would influence the events in the contralateral side. We employed three groups of adult rats under halothane anesthesia; (1) injection of L-CCG-IV (50nmole in 1μl vehicle) into the unilateral CA1, (2) severance of corpus callosum followed by injection of L-CCG-IV and (3) injection of a metabotropic receptor agonist, the (2S,3S,4S) isomer (L-CCG-I) (50nmole in 1μl vehicle) into the unilateral CA1. After 1 week, the brain was examined for histopathology. The intact neurons in the CA1 sector were counted. As shown below, L-CCG-IV significantly killed neurons ipsilateral to injection. There was also massive reduction in cell number contralateral to injection, though statistical significance could not be obtained. By contrast, when combined with severance of corpus callosum, there was no neuronal death in the bilateral CA1 sector. L-CCG-I did not cause neuronal death either, as we previously reported. We conclude that transneural mechanism is a prerequisite for selective death of CA1 neurons by glutamate excitotoxicity. The intrinsic neuronal circuitry in and around the hippocampus would underlie this phenomenon.

Neuronal Density in the CA1 Sector (cells/mm)	(n)	ipsilateral	contralateral
L-CCG-IV	(5)	19±14 sig.p<0.01	76±93
Severance of Corpus Callosum & L-CCG-IV	(11)	160±28	164±30
L-CCG-I	(6)	171±30	162±29

571.10

HOW LONG A PERIOD OF DEPOLARIZATION INDUCED BY HIGH EXTRACELLULAR POTASSIUM IS NEEDED TO KILL RAT AMYGDALA NEURONS *IN VIVO*?

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In vivo perfusion of rat amygdala with a microdialysis probe containing 100 mM KCl for 3 h results in edema and neuronal necrosis up to 370 μm from the probe, as well as large increases in extracellular aspartate, glutamate and taurine (Fujikawa *et al., Soc. Neurosci. Abstr., 17:* 789, 1991). To determine the minimal time required to damage neurons, we shortened the exposure times to 20 and 60 min. Bilateral intracranial guide cannulae were implanted in adult Wistar rats, and the next day probes were inserted into the basolateral amygdaloid nuclei and perfused with Krebs-Ringer-bicarbonate (KRB) solution for 2 h. One side was switched to 100 mM KCl in modified KRB for 20 (n=4) or 60 min (n=5). At a probe efficiency of 6%, this delivered 6 mM K^+ to the tissue, increasing the extracellular K^+ ($[K^+]_o$) immediately adjacent to the probes to about 10 mM. Rats then underwent brain perfusion-fixation for histological examination. After 60 min of high-$[K^+]_o$, severe edema extended up to 121 ± 20 μm (mean ± S.E.), compared to mild edema up to 54 ± 0 μm on the control (KRB) side (p=0.03). In 108 x 540 μm areas adjacent to each probe, there were only 8 ± 3 normal neurons on the high-$[K^+]_o$ side, compared to 18 ± 3 on the control side (p=0.05). No differences between the high-$[K^+]_o$ and control sides were found after 20 min. Thus, the minimal high-$[K^+]_o$ time needed to kill amygdala neurons is between 20-60 min. A 40-min exposure time is currently under investigation. (Supported by the VA Research Service.)

571.11

NEURODEGENERATION MEDIATED BY PLATELET-ACTIVATING FACTOR (PAF) RECEPTORS P.V. Desai, M.V. Hogan*, E. Kornecki[1], and Y.H. Ehrlich CSI/IBR Ctr. Dev. Neurosci. and Dev. Disab., CUNY at Staten Island, NY 10301 and [1]Dept. Cell Biol. Anat. SUNY Brooklyn.

Platelet-Activating Factor (PAF) is a naturally occurring alkyl-ether phospholipid which serves as an intercellular messenger in a variety of physiological and pathological processes. The use of antagonists in experimental models has implicated PAF in a wide range of diseases, including brain damage induced by anoxia and ischemia. The ability of PAF to cause an increase in intraneuronal levels of calcium-ions (Kornecki and Ehrlich, Science, 240: 1792, 1988; Lipids, 26: 1247, 1991) has suggested that, in analogy to the action of glutamate, PAF may have excitotoxic activity. In the present study we have examined this possibility directly by testing the effects of PAF on primary cultures of central nervous system neurons. CNS neurons were cultured from the telencephalon of 7-day chick embryos and maintained in-vitro for a 5-day period during which they undergo differentiation and maturation. Neurodegeneration was observed microscopically and quantitated by measuring release of lactate dehydrogenase (LDH). PAF-induced degeneration of these cultured CNS neurons was found to be both concentration-dependent (1nM to 1μM) and time-dependent (30 min to 96 hrs). At saturation, PAF caused 35% neurodegeneration. Presumably, only those neurons in the heterogenous population that have PAF receptors are sensitive to its excitotoxic action. This possibility was supported by the finding that two structurally different antagonists of PAF receptors, the thienotriazolodiazepine WEB2086 and the ginkgolide BN52021, completely blocked the neurodegenerating effects of PAF. Antagonists of PAF may prove useful in the treatment and/or prevention of neuronal degeneration induced by stroke and spinal cord injury. Supported in part by a grant from the American Paralysis Association .

571.12

INTRATHECALLY ADMINISTERED ACROMELIC ACID INDUCES LONG-LASTING SPASTIC PARAPARESIS WITH DAMAGE OF SPINAL NEURONS IN THE RAT. Kwak S.*, Aizawa H., Nakamura R.. National Institute of Neuroscience, Kodaira, Tokyo 187, Japan.

Since systemic administration of acromelic acid (ACRO) induces selective damage of spinal interneurons in the rat, direct neurotoxic activity of ACRO was investigated in the spinal neurons in vivo. ACRO, kainic acid and AMPA were injected intrathecally at a constant rate for 2 h through a small tube placed in the L5/6 subarachnoid space of adult rats. ACRO induced sequential behavioral changes in a dose dependent manner at the concentrations above 2 μM; forced extension of hindlimbs, defecation, fasciculation and tremor of hindlimbs and flaccid paraplegia. At the concentrations exceeding 8 μM of ACRO, some rats developed spastic paraparesis on the next day with degenerating small or medium sized neurons in the caudal spinal segments, as seen in the spastic rats induced by systemic administration of ACRO. The paraparesis remained unchanged for months. Co-injection of 1 mM CNQX but not APV ameliorated the behavioral and neuropathological changes induced by ACRO. Kainic acid also induced similar behavioral and neuropathological changes but is more than 10 times weaker than ACRO. AMPA induced quite different behavioral changes; rats extended their hindlimbs periodically at concentrations above 1 mM with pathological changes in the spinal neurons. This study suggests that ACRO has potent kainate-like, rather than AMPA-like, excitotoxic activity on the spinal neurons in vivo. ACRO-induced neuron damage provides a useful tool for investigating neuronal death of spinal neurons and also for the investigation of spastic paraparesis of spinal origin.

571.13

THE EFFECTS OF IBOTENIC ACID LESIONS OF THE VENTRAL TEGMENTUM ON BRAIN STIMULATION REWARD. R. Anderson*, M. Trzcinska and E. Miliaressis. Behavioural Neurophysiology Lab., Univ. of Ottawa, Sch. of Psy., 275 Nicholas St., Ottawa, Ontario, K1N 6N5.

Until recently, it was not possible to differentiate between the role of cell bodies and fibers of passage in brain stimulation reward (BSR). If BSR within the ventral tegmentum (VT) is due to the activation of cell bodies, an intra-VT injection of ibotenic acid (IBO, a neurotoxin that destroys cell bodies while presumably sparing fibers of passage) should then decrease the rewarding efficacy of the stimulation. We obtained the rate-frequency functions (RF) of VT self-stimulation (relating barpressing rate to the frequency of cathodal pulses) for a series of pulse intensities, before and after an intra-VT injection of 4 ug of IBO in rats implanted with a chemitrode (a combination of electrode and injection cannula). The injection of IBO shifted the RF functions toward higher frequencies, indicating a decrease in rewarding stimulation efficacy. If subsequent histology reveals no primary demyelination, the data will point to an important role of VT cell bodies in BSR.

571.14

SYNAPTIC SPECIFICITY OF EXCITOTOXICITY IN ISCHEMIC HIPPOCAMPAL SLICES. G. Capocchi*, M. Cecconi, G. Della Torre§, M. Zampolini. Inst. Neurol. and §Inst. of Human Physiol., Univ. Sch. of Med., Perugia, 06100, Italy

The activation of the excitatory amino acids plays an important role in the physiopathology of ischemic damage in the central nervous system (CNS) mediating the increase of intracellular levels of Ca^{++}. This increase could be due to both synaptic (NMDA receptors) and extrasynaptic (Ca++ voltage-gated channels) mechanisms. To understand the synaptic specificity of excitotoxicity we stimulated a synaptic input with a patterned high frequency stimulation (HFS) in ischemic hippocampal slices.

The experiments were carried out in rat hippocampal slices maintained in vitro with Krebs-bicarbonate solution at 32°C gassed with 95% O_2 and 5% CO_2. Stimulation electrodes (St1 and St2) were applied in the Ca1 area in order to activate different groups of Schaffer collateral and to evoke field potentials in Ca1 pyramidal cells. In stable conditions of the recorded potentials, evoked by low frequency stimulation, 1 st every 20 sec (LFS) the O_2 was substituted with N_2 (95%). After 5 minutes patterned HFS was applied in St1 and LFS in St2. In normal conditions, patterned HFS (10 bursts of 35 msec at 100 Hz) induces LTP. In ischemic conditions the HFS in St1 induced a greater synaptic potentiation than in normal conditions while no changes were observed in St2. After 1-2 minutes there was a rapid and very similar decrease of the potential in both St1 and St2. When the decrease reached 90% of the pre-ischemic conditions, the N_2 was substituted with O_2 (95%). In these conditions the potentials increased and after 20-30 minutes stabilized at pre-ischemic levels without significant differences between St1 and St2.

In ischemic conditions, the decrease of both potentials after HFS of one of them indicates that the damage is not only synaptic but the synaptic activation also induces a more general cellular damage

571.15

AMINOOXYACETIC ACID (AOAA) POTENTIATES EXCITOTOXIC BRAIN INJURY IN PERINATAL RATS. J.W. McDonald* and D.D. Schoepp. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285

AOAA, an inhibitor of mitochondrial malate-aspartate shunt, was assessed for its ability to potentiate excititoxic brain injury in perinatal rats. Unilateral intrastriatal stereotaxic injection (AP=0, L=2.5, D=4 relative to Bregma) of AOAA in PND 7 rats produced prolonged, dose-dependent tonic-clonic seizures and unilateral brain injury over a 1-4 μmol dose range. Intrastriatal injection of 1 μmol/0.5 μl AOAA on PND 7 resulted in significant brain injury when assessed on PND 12 by comparison of cerebral hemisphere weight disparities ($p<0.05$ ANOVA,1.5±0.3% damage,mean±SEM, n=5, AOAA treated vs vehicle treated, 0.1±0.5% damage, n=5). Co-intrastriatal injection of 1μmol AOAA with subtoxic doses of either NMDA, AMPA or 1S,3R-ACPD markedly potentiated the severity of behavioral seizures and resulting brain injury (see table). Co-administration (i.p.) of 1 mg/kg MK-801, but not the AMPA antagonist GYKI 52466 (20 mg/kg), prevented the brain injury induced by intrastriatal injection of 4 μmol AOAA when assessed by hemisphere weight disparities.

	%Damage		
	NMDA (1nmol)	AMPA (1nmol)	1S,3R-ACPD (250 nmol)
Vehicle	-0.5 ± 0.5	1.0 ± 0.7	0.9 ± 0.5
AOAA 1 μmol	3.6 ± 0.2*	3.0 ± 0.3*	3.1 ± 0.2*

*1-way Anova $p<0.001$, Duncan Post-hoc Test $p<0.05$ vs vehicle co-injection.

The results indicate that AOAA markedly potentiates excitotoxic brain injury mediated by the selective excitatory amino acid agonists NMDA, AMPA and 1S,3R-ACPD. AOAA has been shown to disrupt mitochondrial electron transport and intracellular energy metabolism. The data suggest that impairment of intracellular energy metabolism augments excitotoxic injury in perinatal rats.

571.16

EFFECT OF SOMAN AND ACh ON GLUTAMATE-INDUCED NEUROTOXICITY IN CULTURED CEREBRAL CORTICAL NEURONS. S.S. Deshpande, R. Ray* and M.G. Filbert, Neurotoxicology Branch, Pathophysiology Division, USAMRICD, Aberdeen Proving Ground, MD 21010-5425.

Soman (pinacolylmethylphosphonofluoridate), an irreversible inhibitor of acetylcholinesterase, causes seizure-related brain damage in animals. Extensive neuropathology has been observed in the pyriform cortex, hippocampus, amygdala and thalamus of rats and guinea pigs (Churchill et al, NeuroToxicology 6, 81-90, 1985; Sparenborg et al, NeuroToxicology, 11, 509-520, 1990). To investigate the link between soman-induced neuropathology and glutamate (GLU) excitotoxicity, we used dissociated cell cultures derived from rat embryonic (E17 days) cerebral cortex. After 14 days in culture, the cells were exposed to GLU (50 uM) alone or in combination with ACh (100 uM) and soman (100 nM). Cytotoxicity was assessed after 24 hr by trypan blue dye uptake and LDH release to the extracellular medium. Complete inhibition of AChE by 100 nM soman had no effect on the cell viability. Exposure to a nontoxic concentration of GLU (50 uM) in the presence of 100 uM ACh produced a 3 fold increase in LDH release confirming earlier observations of Mattson (Brain Res., 497, 402-406, 1989) that ACh lowers the threshold for GLU-induced neurotoxicity. Exposure to 50 uM GLU plus 100 uM ACh in combination with 100 nM soman contrary to the expectation did not significantly potentiate cytotoxicity further. In conclusion, soman does not exert a direct toxic effect on cerebral cortical neurons in culture. The degeneration-potentiating effect of ACh emphasizes the importance of multiple transmitter inputs involved in neuronal degeneration.

572.1

ISOLATION OF NOVEL METABOTROPIC GLUTAMATE RECEPTOR CDNAS FROM RAT OLFACTORY BULB. J. A. Saugstad, T. P. Segerson, E. R. Mulvihill+ and G. L. Westbrook*. Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences Univ., Portland, OR 97201 and +Departments of Molecular and Cellular Biology and DNA Chemistry, Zymogenetics, Seattle, WA 98105.

Glutamate receptors are grouped into two broad classes; the ionotropic receptors which are cation-selective ion channels, and the metabotropic receptors (mGluRs) which are G protein-coupled receptors. Several distinct metabotropic receptor cDNAs have been isolated to date (Masu *et al.*, 1991; Houamed *et al.*, 1991; Tanabe *et al.*, 1992). Characterization of these receptors reveal that they couple to different G proteins to effect various intracellular transduction mechanisms. In addition, pharmacological data has shown that in brain, L-AP4 binds to a presynaptic glutamate receptor that inhibits transmitter release at several pathways. This inhibition is blocked by PTX, suggesting a direct interaction via a Gi/Go protein with voltage dependent calcium channels. L-AP4 does not activate mGluR1. Therefore, this receptor appears to be pharmacologically and functionally distinct. In physiological experiments (Trombley and Westbrook, 1992) the AP4-specific receptor(s) appear to be abundant on mitral cells of the olfactory bulb. In order to identify and characterize the AP4-specific receptor, we have amplified rat olfactory bulb cDNA using the polymerase chain reaction and degenerate oligonucleotide primers to the conserved transmembrane domains three and six of mGluR1 and mGluR4. Sequence analysis of the amplification products revealed the presence of at least three cDNAs that are distinct, but related to the previously isolated metabotropic receptor cDNAs. We are currently screening a rat olfactory bulb cDNA library with the amplification products to isolate full-length cDNA clones. As several of the previously cloned mGluRs show distinct expression patterns in the olfactory bulb, isolation and expression of these related receptors should provide a basis for exploring the function of these receptors in the olfactory system. Supported by the Klingenstein Foundation and NIH T32 DK07680.

572.2

INHIBITION OF cAMP FORMATION BY METABOTROPIC RECEPTOR AGONISTS IN BRAIN SLICES: DEVELOPMENTAL PROFILE AND PHARMACOLOGICAL CHARACTERIZATION. A.A. Genazzani, G. Casabona, G. Aleppo, M. Di Stefano, E. De Bernardis, M.A. Bortino* and F. Nicoletti. Institute of Pharmacology, University of Catania, Italy.

$1_S,3_R$ACPD inhibited forskolin-stimulated cAMP formation in slices from adult rat hippocampus and hypothalamus. Quisqualate and ibotenate were much less efficacious than $1_S,3_R$ACPD in inhibiting forskolin-stimulated cAMP formation, whereas BMAA, AMPA or NMDA were inactive. Stimulation of cAMP formation by forskolin was attenuated by the enzyme adenosine deaminase (ADA), which depletes the endogenous adenosine. The inhibitory action of ACPD was no longer visible when forskolin was added to the slices in the presence of ADA. In addition, ACPD failed to inhibit forskolin-stimulated cAMP formation in hippocampal or hypothalamic slices prepared from rats at 1, 8 or 15 days of postnatal life. These results suggest that hippocampal or hypothalamic slices express a metabotropic receptor subtype that is negatively coupled to adenylate cyclase, is preferentially activated by ACPD, is expressed in the adult life and interacts with endogenous adenosine through a mechanism that remains to be elucidated.

572.3

METABOTROPIC GLUTAMATE RECEPTOR-MEDIATED INCREASES IN CYCLIC AMP ACCUMULATION IN THE HIPPOCAMPUS. P.J. Conn* and D.G. Winder. Dept. of Pharmacol. and Program in Neuroscience, Emory Univ., Atlanta, GA 30322.

The most well characterized metabotropic glutamate receptor (mGluR) subtype is coupled to activation of phosphoinositide hydrolysis. However, little is known about other second messenger systems that are activated by mGluRs. Thus, we performed a series of experiments to test the hypothesis that mGluR activation leads to increases in cAMP accumulation in rat hippocampus. We found that the selective mGluR agonist (1S,3R)-1-aminocyclopentane-2,3-dicarboxylic acid (1S,3R-ACPD) induces a concentration-dependent increase in cAMP accumulation in cross-chopped hippocampal slices. Furthermore, 1S,3R-ACPD enhanced the cAMP response to activation of other receptors that activate adenylate cyclase. As with other responses to mGluR activation, the effect of 1S,3R-ACPD was not mimicked by 1R,3S-ACPD. However, 1S,3S-ACPD stimulated the response with similar potency and efficacy to 1S,3R-ACPD. The response to 1S,3R-ACPD was blocked by the mGluR antagonists L-serine-O-phosphate (L-SOP), L-2-amino-3-phosphono- propionic acid (L-AP3), L-2-amino-4-phosphonobutyric acid (L-AP4), but not by selective antagonists at ionotropic glutamate receptors. Taken together, these data suggest that 1S,3R-ACPD-stimulated increases in cAMP accumulation are mediated by activation of mGluRs.

572.4

ENDOGENOUS ADENOSINE MEDIATES INCREASES IN cAMP ACCUMULATION INDUCED BY ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS D.G. Winder* and P.J. Conn. Department of Pharmacology and Program in Neuroscience, Emory University, Atlanta, GA 30322.

We have found that activation of metabotropic glutamate receptors (mGluRs) results in an increase in cAMP accumulation in rat hippocampal slices. Previous studies suggest that cAMP increases induced by other excitatory amino acids may be mediated by adenosine. Thus, we tested the hypothesis that adenosine mediates the 1S,3R-ACPD-stimulated increase in cAMP levels in rat hippocampal slices. We report that the selective mGluR agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD) potentiates adenosine-stimulated increases in cAMP accumulation. Adenosine receptor antagonists inhibited 1S,3R-ACPD-stimulated cAMP accumulation with an order of potency that corresponds to their affinity at A_2 adenosine receptors. Additionally, an adenosine uptake blocker potentiated the cAMP increases induced by 1S,3R-ACPD. Finally, preincubation of slices with adenosine deaminase abolished the cAMP response to 1S,3R-ACPD and this response was restored by addition of concentrations of 2-chloroadenosine that did not increase cAMP levels in the absence of 1S,3R-ACPD. These data support the hypothesis that 1S,3R-ACPD increases cAMP accumulation in the hippocampus by potentiating responses to endogenous adenosine.

572.5

THE METABOTROPIC GLUTAMATE RECEPTOR IS COUPLED TO ADENYLATE CYCLASE IN PRIMARY CULTURES FROM MOUSE CEREBRAL CORTEX. E. Ratti[1], P. Michieli[1], F. Ferraguti[1], F.Th.M. van Amsterdam[1], G. Gaviraghi[1], E. Cavicchini[1]* and F. Nicoletti[2]. (1) *Glaxo* Research Laboratories, Verona, Italy; (2) Institute of Pharmacology, University of Catania, Italy.

Recent evidence has suggested the existence of multiple subtypes of metabotropic glutamate receptors (Tanabe et al, Neuron, 8, 1992). Whereas the mGluR1 subtype is coupled to polyphosphoinositide (PPI) hydrolysis, the mGluR2 subtype is negatively linked to adenylate cyclase. *In situ* hybridization demonstrated that mGluR1 and mGluR2 receptors differ in their anatomical distribution, with the latter being expressed at high density in the cerebral cortex. We now report the presence of a metabotropic receptor negatively coupled to adenylate cyclase in primary cultures from mouse cerebral cortex. Cultures at 14 days of maturation *in vitro* were stimulated with forskolin or isoproterenol, which both increase cAMP formation. This stimulation was largely attenuated by the metabotropic receptor agonists, quisqualate and 1S,3R-ACPD, which also reduced basal cAMP levels. These results support the existence of a metabotropic receptor subtype coupled to adenylate cyclase in cortical cells.

572.6

TRANS-1-AMINOCYCLOPENTANE-1,3-DICARBOXYLIC ACID-INDUCED PHOSPHOINOSITIDE HYDROLYSIS AND MODULATION OF NEURONAL EXCITABILITY DO NOT UNDERGO PARALLEL DEVELOPMENTAL REGULATION. V. Boss*, M.A. Desai, T.S. Smith and P.J. Conn. Pharmacology Dept., Emory University, Atlanta, GA.

The selective metabotropic glutamate receptor agonist, *trans*-1-aminocyclopentane-1,3-dicarboxylic acid (*trans*-ACPD), stimulates phosphoinositide hydrolysis and elicits a number of electrophysiological responses in hippocampus. If these effects are mediated by the same receptor, then they should undergo parallel developmental regulation. Therefore, we compared the phosphoinositide hydrolysis response and the electrophysiological responses to *trans*-ACPD at two different developmental stages. *Trans*-ACPD-stimulated phosphoinositide hydrolysis was much greater in hippocampal slices from immature (6-11d old) rats than from adults. In contrast, *trans*-ACPD elicited decreases in spike frequency adaptation and in the amplitude of the slow afterhyperpolarization in roughly equal percentages of immature and adult CA1 pyramidal cells. Similar results were obtained using the putative endogenous agonist, glutamate. These data support the hypothesis that certain electrophysiological effects of *trans*-ACPD are mediated by a metabotropic glutamate receptor that is distinct from the phosphoinositide hydrolysis-linked glutamate receptor.

572.7

MODULATION OF NMDA RECEPTOR-MEDIATED TRANSMISSION IN CEREBELLAR GRANULE CELLS BY METABOTROPIC GLUTAMATE RECEPTOR AGONISTS AND NITRIC OXIDE.

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Excitatory synaptic transmission at the mossy fiber (MF)-granule cell (GC) synapse in cerebellum is mediated by both AMPA and NMDA subtypes of glutamate receptor. However, little is known of the mechanisms which regulate synaptic efficacy at this synapse in situ. We have examined the actions of metabotropic glutamate receptor (mGluR) agonists and the NO donor nitroprusside (SNP) in both intact turtle cerebellum and thin slices of rat cerebellum *in vitro*. Turtle Purkinje cells were impaled with sharp microelectrodes, and the disynaptic EPSP evoked by MF stimulation was recorded. Bath application of quisqualate (1 μM) and the 1S,3R- and 1S,3S-isomers of the mGluR agonist ACPD (1-50 μM) reversibly potentiated the MF-evoked EPSP, but were without effect on the monosynaptic parallel fiber-evoked EPSP. These effects were blocked by the NMDA antagonist D-AP5 (50 μM), but were not blocked by forskolin (25 μM), calphostin C (1 μM), the phorbol ester TMA (10 μM), L-AP3 (1 mM), 1R,3S-ACPD (25-500 μM), nor SNP (1 mM), although calphostin C, TMA and SNP reduced the initial EPSP amplitude. By contrast, in rat GCs recorded using the perforated patch-clamp technique, 25 μM 1S,3R-ACPD did not affect the MF-evoked EPSC, nor responses to NMDA, but both responses were reversibly blocked by SNP (100-250 μM). However, in the majority of cells the response to NMDA was potentiated by higher doses (100 μM) of 1S,3R-ACPD. These results demonstrate opposing roles for the regulation of NMDA sensitivity of GCs by mGluR agonists and the NO donor SNP. (Supported by USPHS Grant NS17489 to N.T.S.)

572.8

ACTIVATION OF METABOTROPIC EXCITATORY AMINO ACID RECEPTOR POTENTIATES AMPA AND NMDA RESPONSES OF SPINAL DORSAL HORN NEURONS.

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In freshly isolated spinal dorsal horn neurons (laminae I-IV) of the young rat, the effects of activation of metabotropic glutamate receptor with trans-ACPD [(±)-trans-1-aminocyclopentane-1,3-dicarboxylic acid], 1S,3R-ACPD and 1R,3S-ACPD on inward currents induced by glutamate (GLU), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and N-methyl-D-aspartate (NMDA) were studied under whole-cell voltage-clamp conditions. When the cells were clamped to a holding potential of -60mV, application of 1S,3R-ACPD (100μM) reversibly enhanced the peak amplitude of the initial transient component of the glutamate-induced current. The enhancing effect lasted up to 40 min after removal of 1S,3R-ACPD. In addition, 1S,3R-ACPD (140.8 ± 13.9%, 10/12 cells), 1R,3S-ACPD (124.4 ± 6.5%) and (±)-trans-ACPD potentiated both the initial transient and the steady-state components of AMPA-induced current in most of tested cells. These effects were Ca^{2+}-sensitive and lasted 10-45 min depending upon both dose and length of application. (±)-Trans-ACPD also reversibly enhanced (146.1 ± 7.8%, 11/12 cells) the peak amplitude of the initial fast component of NMDA-induced current. In a smaller proportion of dorsal horn neurons, the enhancing effect was preceded by a transient depression of the responses to GLU, AMPA and NMDA. These results are consistent with the possibility that the activation of metabotropic glutamate receptor may contribute to the regulation of the strength of excitatory amino acid-mediated primary afferent neurotransmission, including nociception. (Supported by BNS 8418042).

572.9

ACTIVATION OF METABOTROPIC RECEPTORS DIFFERENTIALLY MODULATES EXCITATORY AND INHIBITORY SYNAPTIC TRANSMISSION BETWEEN RAT HIPPOCAMPAL NEURONS IN CULTURE.

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Glutamate receptors can be classified into ionotropic and metabotropic receptors; the latter possibly mediating their effects through an increase in membrane phosphoinositides hydrolysis. We have studied the effects of metabotropic receptor activation on synaptic transmission in hippocampal cultures using the whole-cell voltage-clamp technique.

Application of 1S,3R-ACPD (100 μM) (in the presence of appropriate ionotropic receptor antagonists) reversibly decreased the amplitude of spontaneous NMDA and non-NMDA receptor-mediated EPSCs with no consistent change in frequency. By contrast, 1S,3R-ACPD as well as glutamate (2-5 μM) and quisqualate (2-5 μM), produced a reversible increase in the frequency and a transient increase in the amplitude of IPSCs. The origin of these metabotropic effects seems to be at a presynaptic site since postsynaptic responses to pressure applied NMDA, AMPA and GABA did not change. Preliminary paired cell recordings also reveal a similar potentiation effect of quisqualate on evoked IPSCs. The effects of 1S,3R-ACPD on IPSCs were only partially blocked by L-AP3 (1 mM) and L-aspartate-ß-hydroxamate (100 μM), two potent inhibitors of IP_3 turnover. Activation of presynaptic glutamate receptors by L-AP4 (50-100 μM) decreased the amplitude and frequency of IPSCs while decreasing amplitude of EPSCs with little change in frequency.

The differential modulation of excitatory and inhibitory synaptic transmission by metabotropic receptor activation, with a net result of enhanced inhibition may have important implications for development, synaptic plasticity and some neuropathological states. (Supported by GM 34781)

572.10

METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION EVOKES THE RELEASE OF ENDOGENOUS GABA FROM RAT CORTICAL SYNAPTOSOMES.

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Metabotropic glutamate receptor (mGluR) mRNA is expressed by many cells of the mammalian cerebral cortex, including interneurones (Masu *et al*, Nature 349,760-765, 1991), and it is likely that the major inhibitory GABA-ergic basket cells, may also do so. Therefore, *in vivo* activation of mGluRs by endogenous glutamate may directly influence the release of GABA from these interneurones.

The effects of mGluR activation on GABA release were examined using an *in vitro* preparation of superfused cortical synaptosomes. Endogenous GABA efflux was measured by HPLC with pre-column derivatization and fluorescence detection. Following a 5 min exposure to the selective metabotropic agonist t-ACPD (300 μM, racemic mixture), cortical synaptosomes showed a 52.1 ± 10.8 % (n=12) increase over basal levels. A control stimulus of 40 mM KCl, which is known to open voltage sensitive calcium channels, evoked a 294.9 ± 33.5 % (n=8) increase under the same conditions. Omission of Ca^{++} from the superfusion medium significantly attenuated but did not abolish the t-ACPD-evoked GABA release, (35.9 % reduction, $p<0.05$), possibly indicating the mobilization of Ca^{++} from internal stores.

These data suggest that activation of the mGluR on GABA-ergic nerve terminals may provide a mechanism for local inhibitory GABA-ergic feedback onto excitatory cortical neurones.

LDA gratefully acknowledges the support of the British Council.

572.11

POTENTIATION OF IONOTROPIC GLUTAMATE RECEPTOR RESPONSES BY METABOTROPIC RECEPTORS IN THE RAT DORSAL HORN.

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Using both acutely isolated dorsal horn neurons or slices of the adult rat spinal cord (9-16 days) we have examined the effects of the metabotropic glutamate receptor agonist (1S,3R) 1-amino cyclopentane-1,3-dicarboxylic acid ((1S,3R)-ACPD) (1-50μM) on responses mediated by the ionotropic glutamate receptor agonists, N-methyl-D-aspartate (NMDA), kainate (KA) and α-amino 3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA). We measured $[Ca^{2+}]_i$ in isolated neurons using fura-2 based microfluorimetry and found that in approximately 50% of neurons examined, (1S,3R)-ACPD (threshold concentration 20μM) produced increases in the $[Ca^{2+}]_i$ which could be prevented by blockers of voltage sensitive calcium channels. In addition, increases in $[Ca^{2+}]_i$ produced by NMDA (100μM), KA (1-10μM) and AMPA (1-10μM) were markedly potentiated by (1S,3R)-ACPD (1-50μM). Potentiation required simultaneous application of both (1S,3R)-ACPD and the ionotropic agonist. However, potentiation was observed at concentrations of (1S,3R)-ACPD which did not increase responses produced by elevated concentrations of KCl (10-50mM). (1S,3R)-ACPD potentiated ionotropic responses in all cells examined and reversed rapidly upon washout of (1S,3R)-ACPD.

Dorsal horn neurons of the spinal cord slice preparation were whole cell voltage clamped. In these cells (1S,3R)-ACPD also potentiated inward currents evoked by the pressure application of AMPA, NMDA and KA, an effect which was also rapidly reversible. These short term effects of (1S,3R)-ACPD may play an important role in the regulation of ionotropic responses mediated by glutamate in the spinal cord.

572.12

INTERACTIONS BETWEEN PHOSPHOLIPASE C-COUPLED AND NMDA RECEPTORS IN CULTURED CEREBELLAR GRANULE CELLS: PROTEIN KINASE C-MEDIATED INHIBITION OF NMDA RESPONSES.

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The NMDA receptor of rat cerebellar granule cells in primary culture is inhibited by phospholipase C-coupled receptor activation. In the absence of NMDA, stimulation of muscarinic, metabotrophic glutamate or endothelin receptors induces an elevation of the cytoplasmic free calcium ($[Ca^{2+}]_c$) monitored with the fluorescent probe fura-2. The response is consistent with the ability of phospholipase C-coupled receptors to release an intracellular pool of Ca^{2+} and induce subsequent Ca^{2+} entry into the cell; both responses can be abolished by discharge of intracellular Ca^{2+} stores with low concentrations of ionomycin or thapsigargin. In cells stimulated with NMDA the $[Ca^{2+}]_c$ response to the phospholipase C-coupled agonists is complex and agonist-dependent; however in the presence of ionomycin each agonist produces a partial inhibition of the NMDA component of the $[Ca^{2+}]_c$ activator 4β-phorbol dibutyrate. It is concluded that NMDA receptors on cerebellar granule cells are inhibited by phospholipase C-coupled receptors via activation of protein kinase C.

572.13

EFFECTS OF THE METABOTROPIC AGONIST *t*-ACPD ON PYRAMIDAL NEURONS IN RAT PIRIFORM CORTEX SLICES. M. Fejtl*, N. Hori, and D.O. Carpenter. NYS Dept. of Health, Wadsworth Center, and School of Public Health, Albany, NY 12201.

The metabotropic subtype of glutamate receptor in mammalian CNS is thought to be exclusively coupled to a receptor protein mediating second messenger processes such as an increase of $[Ca^{2+}]_i$ and activation of PKC. Here we report that activation of the metabotropic receptor leads to an increase of the iontophoretically evoked AMPA and QUIS responses, whereas NMDA responses are unaffected.

Piriform cortex slices (450 μm) were taken from male rats (150-200g) and incubated in Ringer solution for 2h. After transfering the slices into the recording chamber, intracellular recordings were obtained from pyramidal neurons using 60-80 MΩ electrodes filled with 3M KAc. 50 μM *t*-ACPD, a selective agonist for the metabotropic receptor, 1-10 mM AP-3, and 1 mM Ni^{2+} were bath applied, whereas AMPA, QUIS and NMDA were applied iontophoretically (5-20 nA for 1s, 30 s autocycle).

t-ACPD caused a depolarization accompanied by an increase in input resistance and membrane noise. Although the depolarization was blocked by 10 mM AP-3 the increase in noise was not. Neither hyperpolarization nor depolarization appeared to alter the noise increase. Surprisingly it is blocked by 1 mM Ni^{2+} and this may reflect the involvement of presynaptic Ca^{2+} channels which trigger other postsynaptic mechanisms. *t*-ACPD also enhanced the iontophoretically applied AMPA and QUIS but not NMDA responses, possibly due to increased sensitivity of the AMPA receptor caused by phosphorylation.

Our studies in adult rats and in piriform cortex differ from those reported from neonatal hippocampus. Thus these results may reflect either developmental or regional differences of the metabotropic receptor(s). Supported by NIH NS 23807 (DOC) and the Austrian Science Foundation, "Erwin Schrödinger" fellowship # J-0607-MED (MF)

572.14

METABOTROPIC GLUTAMATE RECEPTOR MEDIATED AFTERDEPOLARIZATION IN RAT NEOCORTICAL NEURONS. C.C. Greene, P.C. Schwindt, and W.E. Crill*. Dept. of Physiology & Biophysics, University of Washington, Seattle, WA. 98195.

Metabotropic receptors (mGluR) are a family of G-protein linked glutamate receptors. Like muscarinic agonists, metabotropic agonists block the afterhyperpolarization (AHP) in hippocampal pyramidal neurons (Stratton et al, 1989). We used intracellular recording to determine if mGluR mediate a similar effect on the spike-evoked slow AHP (sAHP) of rat neocortical neurons in a submerged brain slice preparation. Slices were bathed in artificial CSF containing kynurenic acid (2 - 4 mM) or CNQX (50 μM) and APV (50 μM) to block ionotropic receptors, atropine (5 μM) to block muscarinic receptors, and s-propranolol (15 μM) and phentolamine (30 μM) to block adrenergic receptors. Medium and slow duration AHPs followed 20 action potentials individually evoked by current pulses at 100 Hz. When 1-aminocyclopentane-1,3-dicarboxylic acid, 1S,3R-ACPD (14 - 50 μM), a relatively selective metabotropic agonist, was added, a large afterdepolarization (ADP) replaced the sAHP. Glutamate (0.1-1 mM) and quisqualate (0.1 - 5 μM), but not the ionotropic agonist AMPA (0.5 μM), elicited a similar ADP. The ADP represents a new type of mGluR mediated excitatory response which caused persistent self-sustained firing following the cessation of the repetitive firing stimulus in several neurons and could contribute to pathophysiologic conditions like epilepsy. We have previously shown that muscarinic agonists induced a comparable ADP in cat Betz cells. Hence, our finding supports the hypothesis that metabotropic receptor activation mimics many of the effects of muscarinic agonists (Miller, R.J., 1991). Supported by PHS grants NS 16792 and GM 07266 and the W.M. Keck Foundation.

572.15

TRANS-ACPD AND QUISQUALATE CAN INHIBIT EPILEPTIFORM ACTIVITY IN THE RAT NEO-CORTEX. M.J. Sheardown*. Novo Nordisk A/S, CNS Division, DK-2760 Maaloev, Denmark.

The compounds (±)-trans-ACPD and quisqualate are agonists at the so-called metabotropic quisqualate receptor (mGluR). Recently, the sequences of four mGluR's have been published, one ($mGluR_1$) which stimulates inositol polyphosphate formation. This study shows the effects of trans-ACPD and quisqualate on the spontaneous epileptiform spike activity observed in rat neo-cortex slices bathed in magnesium-free medium containing 10 μM NBQX. (±)-trans-ACPD and quisqualate reduce the frequency of the spikes in a concentration-dependent fashion, the IC_{50} values being 16 and 26 μM, respectively. These responses were not inhibited by AP3 at concentrations upto 1 mM. The order of potency of the agonists was (±)-trans-ACPD > quisqualate suggesting that the $mGluR_1$ receptor is not involved and that the $mGluR_{2,3\ or\ 4}$ receptor subtypes may be responsible. As this spiking activity is Ca^{2+}-dependent and sensitive to TTX and NMDA receptor blockade, it is possible ACPD and quisqualate are acting by reducing glutamate release.

572.16

ACTIVATION OF POSTSYNAPTIC METABOTROPIC GLUTAMATE RECEPTORS BY TRANS-ACPD EVOKES A MEMBRANE HYPERPOLARISATION IN NEURONES OF THE BASOLATERAL AMYGDALA (BLA). **D. G. Rainnie* and P. Shinnick-Gallagher. Dept. of Pharmacology, Univ. of Texas Medical Branch, Galveston, TX 77550.**

Intracellular recordings were made from 51 neurones of the BLA *in vitro*. Application of the metabotropic glutamate receptor agonist (±)-1-amino-1,3-cyclopentane-trans-dicarboxylic acid (trans-ACPD, 100-200 μM) and its active isomer, (1S, 3R)-1-aminocyclopentane-1,3-dicarboxylic acid (1S, 3R ACPD, 100 μM), evoked either membrane hyperpolarisation (29/51 neurones) or hyperpolarisation and subsequent depolarisation (22/51 neurones). The hyperpolarisation was associated with a decrease in neuronal input resistance (R_N) and the depolarisation with an increase in R_N. The hyperpolarisation persisted in the presence of tetrodotoxin (TTX, 0.5 μM) and was unaffected by the ionotropic glutamate receptor antagonists, 2-amino-5-phosphonovaleric acid (APV, 50 μM) or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM), suggesting a direct action at postsynaptic metabotropic glutamate receptors. The reversal potential of the agonist evoked hyperpolarisation was -83 ± 5 mV (n = 6), close to that of potassium (-87 mV, estimated from the Nernst Equation). Hyperpolarising responses to both agonists were abolished in neurones impaled with microelectrodes containing either the non-hydrolysable GTP analogue, guanosine 5'-O-(3-thiotriphosphate) (GTP-γ-S, 10mM) or the calcium chelator, 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA, 200 mM). These data suggest that the initial hyperpolarising response of BLA neurones to postsynaptic metabotropic receptor activation might result from a G-protein mediated increase in a calcium-dependent potassium conductance. Supported by NS-24643.

572.17

SPECIFIC IONOTROPIC AND METABOTROPIC GLUTAMATE RECEPTOR AGONISTS MODULATE FREE INTRACELLULAR CALCIUM IN ACUTELY DISSOCIATED EMBRYONIC RAPHE CELLS. N. König * [1], M.J. Drian [1], M. Pariat [1], O.J.J. Manzoni [2] and F. Sladeczek [2], [1] EPHE-INSERM U336, USTL, Place E. Bataillon, and [2] CCIPE, Rue de la Cardonille, 34095 Montpellier Cedex, France.

There is increasing evidence that glutamate receptors not only play a prominent role in postnatal development, but that they are present already in the embryonic brain. We dissected the basal plate parts of the rostral and the caudalmost rhombencephalon (including the raphe) from rat embryos at 13 and 14 days of gestation. The cells were dissociated, plated, and loaded with the calcium-marker FLUO-3 after 1 to 5 h *in vitro*. Relative changes of fluorescence were evaluated using video or confocal microscopy. When the tissue was taken from day 13 embryos, all the agonists used (kainic acid, AMPA, NMDA, and 1S,3R-ACPD) were able to elicit rises in free intracellular calcium. However, the number of responding cells was quite low. When the tissue was taken at day 14, the proportion of responding cells was higher, and the responses were more stable and reproducible. Some cells responded to more than one agonist. In conclusion, ***functional* ionotropic as well as metabotropic receptors** seem to be expressed in early developing regions of the embryonic rat brain **by day 13,** which rises the question about their role at these early stages.

572.18

METABOTROPIC RECEPTOR-SPECIFIC AGONISTS STIMULATE POLYRIBOSOME AGGREGATION IN SYNAPTONEUROSOMES. I.J. Weiler*, W.S. Childers, W.T. Greenough, Depts. Psychol. and Cell & Struct. Biol, Neurosci. Prog, and Beckman Inst.,Univ. Illinois, Urbana-Champaign, IL 61801.

We showed (Mol.Cell. Neurosci.2:305, 1991) that K+ stimulation of synaptoneurosome preparations triggers rapid aggregation of polyribosomes and transient acceleration of incorporation of methionine into TCA-precipitable polypeptides. We now show that this response is elicited by the neurotransmitter glutamate and have investigated its analogs.

At a dose of 10 μM, quisqualic acid, ibotenic acid, and the selective metabotropic receptor agonist trans-ACPD are all effective in stimulating polyribosomal aggregation. NMDA is ineffective at this concentration; the NMDA antagonist APV does not block the response to K+. CNQX, a selective inhibitor for the ionotropic quisqualate response, does not hinder aggregation, indicating that the metabotropic quisqualate activity is still present..

The cell-permeant diacylglycerol analog OAG (1-oleoyl-2-acetyl-glycerol) was able to mimic the effect of glutamate in inducing a typical polyribosomal aggregation response. This suggests that the phosphoinositol second messenger system may influence protein synthesis near nerve terminals. Supported by ONR N0014-89J-1556 and MH35321.

572.19

FURTHER CHARACTERIZATION OF THE INDUCTION OF GLUTAMINE SYNTHETASE ACTIVITY BY *trans*-ACPD IN CULTURED ASTROCYTES. S. Miller[1]*, C.W. Cotman[1], and R.J. Bridges[2]. Departments of Psychobiology[1] and Neurology[2], Univ. of California, Irvine, CA 92717.

Glutamine synthetase is a glial-specific enzyme which catalyzes the conversion of glutamate and ammonia to glutamine, playing a crucial role in nitrogen and excitatory amino acid metabolism. We have recently reported that 1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD), an agonist of phosphoinositide-coupled metabotropic glutamate receptors, induces glutamine synthetase activity in cultures of rat cortical type I astrocytes (J. Neurochem. *in press*). The action of *trans*-ACPD, which is dose-dependent, stereoselective, and sensitive to cycloheximide suggests a role of metabotropic glutamate receptors in the induction mechanism. However, other excitatory amino acid agonists, such as β-L-ODAP, can also induce glutamine synthetase activity yet have little activity at metabotropic glutamate receptors. These pharmacological inconsistencies prompted us to investigate the hypothesis that the induction of glutamine synthetase by *trans*-ACPD may be mediated at an alternative site. We found that dihydrokainate, an inhibitor of excitatory amino acid transport blocks the induction of GS activity by 100 μM *trans*-ACPD. The induction was inhibited in a concentration-dependent manner and completely blocked with 10 mM dihydrokainate. It is possible that this effect of dihydrokainate is mediated by inhibition of phosphoinositide hydrolysis, an action which has been reported for other transport inhibitors (Littman et al. Soc. Neurosci. Abs. 17: 1338, 1991). Alternatively, a more parsimonious explanation is that *trans*-ACPD has activity at an intracellular site, indicating that the effects of this agonist may not be limited to activation of cell-surface metabotropic glutamate receptors.

572.20

PROPERTIES OF A GLUTAMATE AUTORECEPTOR IN RAT NEOSTRIATUM D.M. Lovinger*, E. Tyler and A. Merritt, Dept. Mol. Physiol./Biophys., Vanderbilt Univ. Med. School, Nashville, TN 37232

The neostriatum receives a large glutamatergic afferent input from the neocortex. Transmission at striatal glutamatergic synapses is modulated presynaptically by a putative glutamate autoreceptor activated by the metabotropic receptor agonist t-ACPD (Lovinger, Neurosci. Lett., 129, 17-21, 1991). We have examined this receptor using field potential recording from adult rat neostriatal slices and whole-cell recording from neurons in slices from 2-4 wk. old rats. In adult slices, a synaptically-driven population spike (PS) evoked by local electrical stimulation was decreased by bath application of 5-100μM t-ACPD (IC_{50} of 50μM). This is similar to the potency with which t-ACPD depressed the amplitude of EPSPs in striatal neurons. Depression of the PS was stereospecific with 100μM 1S,3R-ACPD producing an 88±6% decrease in amplitude while 100μM 1R,3S-ACPD had no effect. The effect of t-ACPD on the PS or EPSP was not altered by the metabotropic receptor antagonist AP3 (100μM). Phorbol diacetate (PDAc), an activator of protein kinase C (PKC) reduced the synaptic depressant effect of 50μM t-ACPD (%inhib of PS=48±10 in the absence and 12±5 in the presence of 5.5μM PDAc, $p<0.005$; %Inhib of EPSP=74±4 in the absence and 39±5 in the presence of 3 or 5.5μM PDAc, $p<0.005$). Alpha-PMA, a phorbol ester which does not activate PKC, did not reduce the effect of t-ACPD (43±5% inhib of PS in 5μM alpha-PMA). t-ACPD inhibits voltage-activated Ca^{2+} current and activation of PKC inhibits this effect (Swartz & Bean, this meeting). The synaptic depressant effect of t-ACPD may involve an AP3-insensitive metabotropic glutamate receptor. We hypothesize that this receptor is negatively coupled to voltage-activated calcium channels via PKC-sensitive intracellular processes. Supported in part by grant #AA08986 from NIAAA.

PEPTIDES: RECEPTORS V

573.1

COMPARATIVE MODULATORY ACTION OF A GUANINE NUCLEOTIDE DERIVATIVE ON NEUROPEPTIDE Y_1 AND Y_2 RECEPTOR SUBTYPES IN RAT BRAIN. Y. Dumont[1]*, A. Fournier[2], S. St-Pierre[2] and R. Quirion[1] (1) Douglas Hospital Res. Ctr., McGill University, Montréal, Québec, Canada, H4H 1R3. (2) INRS-Santé, Pointe-Claire, Québec, Canada, H3R 1G5.

It has been suggested that the Y_1 and Y_2 neuropeptide Y (NPY) receptor subtypes are G protein-coupled. Consequently, we investigated the effect of the stable GTP analogue, GTPγS (100μM), on the competition profile of the specific Y_1 agonist [Leu^{31},Pro^{34}]-NPY and that of PYY for [^{125}I] PYY binding sites in homogenates prepared from the frontoparietal cortex or the hippocampus, two preparations respectively enriched with the Y_1 and Y_2 receptor subtype. As expected for G-protein coupled receptors, specific [^{125}I] PYY binding decreased in the presence of 100μM GTPγS in both the frontoparietal cortex (68 ± 15 to 39 ± 9 fmol/mg prot.) and the hippocampus (148 ± 9 to 98 ± 11 fmol/mg prot.). However, the slopes of the competition curves of [Leu^{31},Pro^{34}]-NPY and PYY were not altered by the nucleotide. A detailed computer analysis of the competition profile best fitted to a two binding site model in both membrane preparations and in the presence or absence of the GTP analogue. It thus appears that a certain proportion of Y_1 and Y_2 receptors in the two regions investigated exist in an affinity state (high) which can be shifted to a lower one by uncoupling from the relevant G-protein. Moreover, the still biphasic nature of the competition profiles observed in presence of GTPγS suggests the possible existence of additional sites which are not coupled to a G-protein in those regions of the rat brain. Supported by MRCC.

573.2

***IN VIVO* INTERACTION BETWEEN NEUROPEPTIDE Y, PEPTIDE YY AND SIGMA RECEPTORS IN THE MOUSE HIPPOCAMPUS.** Bouchard, P.*[1,3], Dumont, Y.[1], St-Pierre, S.[4], Fournier, A.[4] and Quirion, R.[1,2,3] (1) Douglas Hospital Research Center, Verdun, Que., Can. (2) Department of Psychiatry and (3) Neurology and Neurosurgery, Mc Gill Univ. and (4) INRS-Sante, Pointe-Claire, Que., Can.

Recently, it was proposed that neuropeptide Y (NPY) and peptide YY (PYY) could act as endogenous ligands for sigma binding sites since both NPY and PYY compete with high affinity (nM) for [^{3}H](+)SKF 10047 binding in rat brain membrane homogenates (Roman et al., 1990). However, several laboratories have failed to provide direct evidence for an interaction between NPY, PYY and sigma receptors. In order to clarify this apparent discrepancy, we investigated the effects of various peptides on *in vivo* [^{3}H](+)SKF 10047 binding parameters in the mouse brain. Mice were injected with haloperidol (i.p. 2 mg/kg) or saline to define specific binding. At t=15 min., animal received either a peptide injection (3 μl i.c.v.), or saline. At t=30 min., each animal were injected with 5 μCi [^{3}H](+)SKF 10047 (200 μl i.v.). Animal were sacrificed at t=60 min. and the binding of [^{3}H](+)SKF 10047 measured. In the hippocampus, haloperidol competed for up to 90% of [^{3}H](+)SKF 10047 labelling. At a dose of 1500 pmol, NPY, NPY_{2-36}, [$Leu^{31}Pro^{34}$] NPY and PYY competed for 20 to 40% of specific [^{3}H](+)SKF 10047 binding. Other peptides, like neurotensin and VIP, did not compete with [^{3}H](+)SKF 10047 binding but surprisingly, CGRP competed for up to 30% of the total binding. It thus appears that NPY, PYY and related peptides, as well as CGRP may interact with sigma binding sites *in vivo*. It now remains to be established if the effects of these differents peptides families at sigma sites, are mediated via similar or different mechanisms.

573.3

IDENTIFICATION OF RECEPTOR BINDING PHARMACOPHORES OF GROWTH HORMONE-RELEASING FACTOR IN RAT ADENOPITUITARY. L.Lefrançois and P. Gaudreau*. Neuroendocrinology Laboratory, Notre-Dame Hospital Research Ctr., Dept. Medicine, University of Montreal, Montreal, Canada, H2L 4M1.

Previous structure-activity studies on growth hormone-releasing factor (GRF) have mainly been carried out in pituitary cell culture assays. In such systems, the molecular features necessary to increase GRF receptor affinity cannot be fully differentiated from those that improve proteolytic resistance. To assess the affinity of GRF analogues, we have recently characterized [^{125}I-Tyr^{10}]hGRF(1-44)NH_2 binding to rat adenopituitary, developing a reliable binding assay in which GRF carboxamide-related peptides are stable (Abribat et al. Brain Res., 528:291, 199Q). In the present study, we have synthesized two series of analogues in which the entire sequence of hGRF(1-29)NH_2 was scanned with alanine and D-amino acid substitutions. An Alanine substitution at Tyr^1, Asp^3, Ile^5, Phe^6, Thr^7, Tyr^{10}, Arg^{11}, Lys^{12}, Arg^{20}, Lys^{21}, Leu^{23} or Gln^{24} decreased GRF affinity while it did not induce major changes at Asn^8, Val^{13}, Leu^{14}, Gln^{16}, Leu^{17}, Ser^{18}, Leu^{22}, Asp^{25}, Ile^{26}, Met^{27} or Arg^{29}. Interestingly, it increased GRF affinity at Ser^9, Gly^{15} and Ser^{28}. A D-amino acid substitution in position 3, 4, 5, 6, 7, 11, 13, 14, 17, 18, 19, 20, 23, 25, 26, 27 or 28 decreased GRF affinity but had little influence in position 1, 2, 9, 10, 12, 15, 16, 21, 24 or 29. It increased GRF affinity in position 8 and 22. Altogether these results allow to pinpoint residues that are involved in a structural role and those responsible for receptor contacts.

573.4

CEREBELLAR NUCLEI EXPRESS A NEW TYPE OF SOMATOSTATIN RECEPTORS WHICH EXHIBIT LOW AFFINITY FOR OCTREOTIDE AND MK 678. P. Leroux*, C. Bucharles and H. Vaudry. European Inst. Peptide Res., Lab. Molecular Endocrinology, CNRS URA 650, UA INSERM, University of Rouen, 76134 Mont-Saint-Aignan, France.

The presence of selective somatostatin (SS) receptor subtypes (SSR1 and SSR2) has been demonstrated in different brain areas on the basis of differential binding potency of octreotide. Both SSR1 and SSR2 bind with high affinity the SS analogs [Tyr^0,$DTrp^8$]S14 and [Leu^8,$DTrp^{22}$,Tyr^{25}]S28. In a previous study, we have observed preferential binding of iodinated [Leu^8,$DTrp^{22}$,Tyr^{25}] S28 in a few brain areas. In the present study we have characterized these binding sites in the lateral cerebellar nuclei (LCN) by quantitative autoradiography. The binding of [Leu^8,$DTrp^{22}$,^{125}I-Tyr^{25}]S28 in the LCN was saturable (Bmax= 219 fMol/ mg Prot.) and of high affinity (KD= 0.50 nM). In contrast, no labeling was detected in LCN using [^{125}I-Tyr^0,$DTrp^8$]S14. However both S14 and S28 analogs could displace [Leu^8,$DTrp^{22}$, ^{125}I-Tyr^{25}]S28 binding, indicating that these sites do not represent S28 preferring receptors. The efficiency of both octreotide and MK 678 in competition tests was very low. The order of potency for 10 SS analogs to compete with the binding of [Leu^8,$DTrp^{22}$,^{125}I-Tyr^{25}]S28 in the LCN was: S14 (2.02 ± 0.51 nM) > [Tyr^0]S28 (2.25 nM) > [Leu^8, $DTrp^{22}$,Tyr^{25}] S28 (2.66 ± 0.13 nM) > S28 (5.15 ± 1.76 nM) > [Tyr^{11}]S14 (7.34 nM) > [Tyr^0, $DTrp^8$]S14 (8.52 ± 2.64 nM) > [Tyr^0]S14 (15.9 ± 3.4 nM) > N-Ahep(7-13)S14Bzl (564 ± 229 nM) > Octreotide (> 1 μM) > MK 678 (55.2 ± 15.7 uM). In contrast to [^{125}I-Tyr^0,$DTrp^8$]S14 binding, divalent cations were not required to obtain maximal binding of [Leu^8,$DTrp^{22}$,^{125}I-Tyr^{25}]S28, and the potency of guanine nucleotides to inhibit the binding was much lower (IC50 > 50 μMfor GTP and GDP). The present results show that the SS receptors observed in the LCN represent a pure population of sites which are distinct from SSR1 and SSR2 receptor types present in the cortex and hippocampus.

573.5

UP-REGULATION OF CORTICAL SOMATOSTATIN RECEPTOR BINDING SITES FOLLOWING LONG TERM UNILATERAL LESION OF THE NUCLEUS BASALIS IN THE RAT. D. Cécyre*, J.C. Martel and R. Quirion, Douglas Hospital Research Center and Dept. of Psychiatry, McGill University, Montréal, Québec, Canada, H4H 1R3.

It has recently been reported that cortical somatostatin-like immunoreactivity (SRIF-IR) is altered following long term uni- or bilateral ibotenic acid lesions of the nucleus basalis magnocellularis (nbm) in the rat (Arendash *et al*, Science 238, 1987; *ibid*, Neurochem. Res., 14, 1989). Earlier data had shown that, contrary to expected results, both SRIF-IR and SRIF receptor binding sites were decreased in Alzheimer's brains (Beal *et al*, Science 229, 1985). Using quantitative *in vitro* receptor autoradiography, we examined the potential effects of long term changes in cortical SRIF levels observed following nbm lesions on its binding sites. Uni- and bilateral ibotenic acid lesions of the nbm were performed in three-months old rats. Eighteen months later, animals were sacrificed and brain sections prepared for receptor autoradiography. As reported elsewhere (Krantic *et al*, Neuroscience 39, 1990), [^{125}I]-Tyr^0,D-Trp^8-SRIF$_{14}$ ([^{125}I]-SRIF$_{14}$) binding sites are concentrated in various cortical laminae, the hippocampal formation and the amygdala body. A long term unilateral nbm lesion induces a significant (compared to sham control) up-regulation of [^{125}I]-SRIF$_{14}$ binding in the ipsilateral parietal and temporal cortices while no modification in labelling profile and intensity was detected in the amygdala. Surprisingly, the bilateral lesion failed to induce any significant change in [^{125}I]-SRIF$_{14}$ labelling. Thus, as observed for SRIF-IR, cortical alterations of [^{125}I]-SRIF$_{14}$ binding can be observed following long term lesions of the nbm but these are dependent on the nature of the lesion performed. Supported by MRCC.

573.6

MODULATION OF NEUROPEPTIDE FF RECEPTORS IN RAT BRAIN AND SPINAL CORD MEMBRANES BY GUANINE NUCLEOTIDES AND CATIONS.

K. PAYZA and H.Y.T. YANG. LBG, NIMH Neuroscience Center, WAW-113, St. Elizabeth's Hospital, Washington, D.C. 20032.

Neuropeptide FF (NPFF) is a mammalian FMRFamide-related peptide of the sequence FLFQPQRFamide. NPFF antagonizes morphine analgesia and appears to be involved in morphine tolerance and dependence. In this study, guanine nucleotides, NaCl and $MgCl_2$ were tested for modulatory effects on NPFF receptor binding in membrane preparations of rat brain and spinal cord. Specific binding of ^{125}I-YLFQPQRFamide to NPFF receptors in both brain and spinal cord preparations was stimulated by NaCl and $MgCl_2$ and inhibited by GTP and its nonhydrolyzable analogs. The GTP effect was observed in the presence and absence of Na^+ and Mg^{2+}. The specificity of the GTP effect (GTP[γ]S>GppNHp>GTP>GDP; no effect of GMP or ATP) suggests that NPFF receptors are coupled to G-proteins in these tissues. The ionic effects suggest that Na^+ and Mg^{2+} ions are required for maximal receptor binding but not for receptor-G-protein coupling.

573.7

DISTRIBUTION OF NEUROPEPTIDE FF RECEPTORS IN RAT BRAIN AND SPINAL CORD.

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Neuropeptide FF (NPFF, FLFQPQRF-NH_2) is an endogenous neuropeptide with anti-opiate activity. NPFF is unevenly distributed in the rat CNS with the highest concentrations in the spinal cord and hypothalamus. In this study, the distribution of NPFF receptors in rat CNS was examined using radioligand binding assays.. The specific ^{125}I-YLFQPQRFa bindings (dpm/mg protein/dpm total) in various regions of rat CNS were: dorsal spinal cord: 0.123±0.020, medulla oblongata: 0.1044±0.008, hypothalamus: 0.0998±0.0023, midbrain: 0.0789±0.0086, striatum: 0.0396±0.0035, hippocampus: 0.0163±0.0019, cerebellum: 0.0161±0.0072, cortex: 0.0037±0.0024, ventral spinal cord: 0±0.023. The radioligand binding in midbrain, medulla oblongata and dorsal spinal cord was inhibited by GTP[γ]S showing that the binding was NPFF receptor specific. The receptor distribution in this study correlated with the NPFF distribution in various regions of brain and spinal cord.

573.8

NEUROMEDIN B RECEPTORS AND FIBROBLAST GROWTH FACTOR ARE PRESENT IN C6 GLIAL GRAFTS. T.W. Moody*, F. Cuttitta , J. Battey, K. Engleka , T. Maciag and J. Rosenstein. Depts. Anatomy, Biochemistry & Mol. Biol., George Washington Univ. Med. Ctr., Washington, D.C. 20037, DPCP, NCI, 5516 Nicholson Lane, Kensington, MD 20895, Lab. Neurochemistry, NINDS, NIH, Bethesda, MD 20892 and Dept. Mol. Biol., Holland Lab., American Red Cross, 15601 Crabbs Branch Way, Rockville, MD 20855.

Rat glioma C6 cells have functional neuromedin B (NMB) receptors. Previously, we reported that NMB bound with high affinity, elevated cytosolic Ca^{2+} and stimulated the growth of C6 cells in tissue culture. Here NMB receptors were characterized in C6 transplants into the rat forebrain. Cultured C6 glioma cells (10^6) were transplanted into the rat forebrain. After a postoperative time of 1-3 weeks, the tumors and surrounding host brain were analyzed for NMB receptors and fibroblast growth factor (FGF) by immunocytochemistry using the PAP method. Antisera to the NMB receptor was elicited against a C-terminal fragment (S-16-L) conjugated to hemocyanin. The NMB receptor antisera strongly reacted with numerous cellular elements but not blood vessels within the C6 glioma. Also, using autoradiographic techniques high grain densities were present for NMB receptors in the graft. In contrast, antisera to FGF strongly reacted with blood vessels within the tumor and adjacent brain. The FGF may stimulate angiogenesis and the NMB receptors facilitate growth within the C6 tumor. Supported in part by NSF grant BNS88-15133 (T.W.M.) and NIH grants NS-17468 (J.R) and HL-32348 (T.M.).

573.9

RICIN CYTOTOXIN CELL TARGETING REVEALS UNIQUE ROLES FOR ANP AND CNP IN WATER DRINKING AND PROLACTIN SECRETION. W.K.Samson* and R.J.Fulton. Anatomy/Neurobiology,U MO Schl Med, Columbia MO 65212 and Inland Labs, Dallas TX 75207.

Centrally administered A-type and C-type natriuretic peptides (ANP and CNP) exert opposite actions on water intake and prolactin (PRL) secretion. While ANP is inhibitory, CNP is stimulatory suggesting actions of the peptides via unique receptors. ANP and CNP were conjugated to the A chain of the plant cytotoxin ricin and conjugates injected icv in adult rats. Two weeks later rats received icv injections of maximally effective doses of either ANP or CNP. In rats pretreated with ANP-ricin A chain, the inhibitory action of ANP on PRL secretion was absent yet the stimulatory effect of CNP was still present. The opposite was true in rats pretreated with CNP-ricin A chain conjugate. Following 18h water deprivation, rats pretreated with ANP-ricin A chain conjugate drank significantly more water than controls, while those pretreated with CNP-ricin A chain consumed significantly less. These results indicate that the opposing actions of these two members of the natriuretic peptide family are expressed via unique receptors, in all likelihood the ANPR-A and ANPR-B subtypes.

574.1

PRIMARY STRUCTURE OF RF-AMIDE NEUROPEPTIDE PRECURSORS FROM COELENTERATES C.Schmutzler, D.Darmer, R.K. Reinscheid, and C.J.P.Grimmelikhuijzen*; Centre for Molecular Neurobiology, University of Hamburg, Martinistr. 52, D-2000 Hamburg 20, F.R.G.

In the simple and evolutionary old nervous systems of coelenterates neuropeptides are highly abundant and play an important role in neurotransmission. From several coelenterate species we have isolated a family of neuropeptides with the common C-terminus Arg-Phe-NH_2 (RFamide). Thus, these peptides belong to the class of the RFamide peptides which have been found throughout the animal kingdom, the prototype being the molluscan FMRFamide.

We have cloned cDNAs encoding precursor proteins for the RFamide neuropeptide Antho-RFamide (<Glu-Gly-Arg-Phe-NH_2) from the anthozoans *Anthopleura elegantissima, Calliactis parasitica,* and *Renilla köllikeri* which show a highly repetitive organization (containing 13, 19, and >36 copies of immature Antho-RFamide, respectively). We also cloned the precursor proteins for the hydrozoan RFamide heptapeptides Pol-RFamide I and II from *Polyorchis penicillatus* and Hydra-RFamide I, II, and IV from *Hydra magnipapillata.*

At the C-terminal side of these peptides precursor cleavage occurs at conventional processing sites (mono- or dibasic residues). However, we have to postulate "non-classical" mechanisms to explain correct peptide processing at the N-terminus.

574.2

CHARACTERIZATION OF OPIOID MATERIAL IN THE PERIOESOPHAGAL GANGLIA OF *HELIX ASPERSA*. M. Sanchez-Alvarez+, M. Leon-Olea+, E. Piros, E. Talavera+, K. F. Faull*, C. J. Evans Division de Inv. en Neurociencias+, Instituto Mexicano de Psiquiatria. AP. 14370, Mexico, D.F. Department of Pshychiatry. UCLA School of Medicine. Los Angeles, CA 90024.

In molluscs, the first evidence for the existence of opioid peptides was presented by Martin *et al.* (1). In *H. aspersa* the anatomical distribution of enkephalin-like immunoreactivity has been mapped using immunohistochemical techniques (2), and the amount of enkephalin-like material has been determined by RIA experiments (3). The majority of the opioid-like material in invertebrates has been detected with antibodies raised against vertebrate enkephalins. Therefore, we decided to characterize the opioid-like material present in the perioesophageal ganglia of *H. aspersa* using an immunoassay that identifies all the vertebrate opioid peptides. Guanidine isothiocyanate extracts of 176 ganglia were used and co-chromatographed on Sephadex G-50. The gel filtration chromatography indicates the presence of opioid-like immunoreactive material with a molecular weight between the 1 to 1.5 KD range, which is under further characterization.

1. Martin, R. *et al.*, Neuropeptides. 2: 141, 1981.
2. Leon-Olea, M. *et al.*, Soc. Neurosci. Abs. 17: 385-18. 1991.
3. Gutierrez, R. *et al.*, Comp. Biochem. Physiol. 100:609, 1991.

574.3

N^{α}-ACETYLATED VASOPRESSIN IN THE RAT PITUITARY GLAND AND HYPOTHALAMUS.

F.M. de Bree[1], F.W. van Leeuwen* and J.P.H. Burbach[1]. [1]Rudolf Magnus Institute, University of Utrecht, 3521 GD Utrecht, The Netherlands; *Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.

Recently N^{α}-acetyl-vasopressin (Ac-VP) and N^{α}-acetyl-oxytocin (Ac-OT) were purified and identified from bovine pineal gland. Indirect determination of these forms by HPLC combined with a radioimmunoassay using a C-terminal specific VP antiserum revealed nanogram amounts of the acetylated forms in the rat neurointermediate lobe (NIL), while the brain itself contained only picogram amounts. The extent of acetylation differed markedly: in the NIL only a few percent of the peptide was acetylated, whereas in the pineal gland about 70% was acetylated. This indicates a possible regulatory role for this new kind of modification of VP and OT. In order to analyze and localize Ac-VP directly, antisera were raised against this peptide. Cross-reactivity studies in a radioimmunoassay and dot-tests revealed Ac-VP recognizing antibodies with no or low cross-reactivity for VP, Ac-OT, OT, N^{α}-acetyl-ß-endorphin and αMSH. One of the antisera (#F5) was used in a radioimunoassay to determine Ac-VP in NIL and hypothalamus. In the NIL F5 detected only Ac-VP and no VP indicating a high specificity of F5. The hypothalamus contained no measurable amounts of Ac-VP. In immunocytochemistry F5 showed after preadsorption with VP and Ac-OT a fibre-like staining in the posterior lobe. F5 showed no staining in the hypothalamus after preadsorption. In conclusion VP might be acetylated during axonal transport.

574.4

AN ANTISENSE OLIGODEOXYNUCLEOTIDE INHIBITS PROOPIOMELANOCORTIN TRANSLATION IN AtT-20 CELL LINE. S. Spampinato*, M.Canossa, L.Carboni, G.Campana, and S. Ferri. Dept. Pharmacology, Univ. Bologna, Irnerio 48, 40126 Bologna, Italy.

Gene expression in mammalian cells can be suppressed by nucleic acid sequences complementary to endogenous transcripts. In actual fact, these "antisense" sequences can hybridize to primary RNA transcripts and prevent translation of the target gene at ribosomal level. We employed this strategy to obtain a significant reduction of the synthesis of peptides derived from the precursor proopiomelanocortin (POMC) in a murine neuroendocrine cell line (AtT-20) that highly expresses this gene. Cells grown in suspension in DMEM supplemented with 10% fetal calf serum were seeded at $4x10^6$ cells per dish and exposed to a 30-base pair oligodeoxynucleotide TACGGTGGCTTCATGACCTCCGAGAAGAGC complementary to mRNA nucleotides which are translated into the first ten aminoacids of mouse ß-endorphin, an opioid peptide derived from POMC. In alternative, AtT-20 cells were treated with a sense 30-base pair oligonucleotide. After 24 h cells were harvested and ACTH (another peptide spliced from POMC and positioned upstream to ß-endorphin) was evaluated, by RIA, in acetic acid extracts of the cells. A significant reduction of ACTH content was observed in cells treated with the antisense oligonucleotide [26.62 ± 2.2 vs 12.03 ± 0.18 ng/10 cells (n=7); $p < 0.01$]. These results indicate that antisense oligonucleotides block the synthesis of neuropeptides in cell lines and may be useful in delineating their functions.

574.5

SELECTIVE RELEASE OF THE NEUROPEPTIDE VIP IN SHEEP EXPOSED TO THREE LUNG DAMAGING ORGANOHALIDES: CORRELATION WITH CYCLOOXYGENASE METABOLITES. [1]A.W. Assaad, [2]A. Absood, [2]M. Trotz, [1]C. Woodard, [1]W. Baze, [1]D. Martin, [1]J.P. Apland*, and [2]S.I. Said. [1]U.S. Army Med. Res. Inst. of Chemical Defense, Edgewood, MD 21010; and [2]S.U.N.Y. at Stony Brook, N.Y 11794.

Certain neuropeptides, e.g., tachykinins, can promote inflammation while others, e.g., vasoactive intestinal peptide (VIP), can modulate inflammation. We have examined the release of VIP and 3 cyclooxygenase metabolites (CM), after the inhalation of 3 different organohalides that cause lung toxicity. Three groups of sheep (n=10 each) were exposed for 10 min to 323 ppm/min of perfluoroisobutylene (PFIB), a pyrolysis product of Teflon, or 364 ppm/min of bis(trifluoromethyl)disulfide (TFD), a pesticide, or 767 ppm/min of phosgene, an industrial precursor. Plasma was collected immediately before and at 15, 30, 60, 120, 180 & 240 min after exposure, and assayed by RIA for VIP and metabolites of PGI_2, TXA_2 & PGF_{2a}. All three gases produced acute lung injury and elevated CM levels ($p<0.05$) for up to 60 min. VIP levels increased only with TFD ($p<0.05$) and paralleled those of CM. These data suggest that: 1) the release of VIP was selective, whereas that of CM occurred with all three gases; 2) the release of CM and VIP was both immediate and sustained with TFD; and 3) the mechanism of TFD-induced VIP release is unexplained, but it may be an attempt to modulate the injury.

574.6

MICRODIALYSIS OF OPIOID PEPTIDE RELEASE FROM THE NUCLEUS ACCUMBENS AND VENTRAL PALLIDUM OF THE FREELY MOVING RAT M. Bertolucci*, C. J. Evans and N.T. Maidment. Department of Psychiatry, NPI and BRI, UCLA, Los Angeles, CA 90024.

We previously described a method for the measurement of opioid peptides in basal ganglia microdialysates of the anesthetized rat (Maidment et al., 1989, Neuroscience, 33: 549-557). We have now extended these studies to the freely moving animal and to limbic regions in view of the proposed role of these structures in reward processes. Basal release of opioid peptides was detectable in the nucleus accumbens and the ventral pallidum for at least 3 days after the dialysis probe was inserted in the brain, although the quantity of material recovered was progressively reduced with time. Similar to previous studies in anesthetized rats the extracellular levels of the opioid peptide were enhanced by reverse dialysis of veratridine (50 uM) by approx 10-fold - an effect blocked by tetrodotoxin (2uM). However, contrary to the situation in anesthetized animals, the basal efflux was significantly reduced by both tetrodotoxin and the calcium ion chelator EGTA (10 mM) indicating a synaptic origin of the peptides. Having thus validated the methodology, studies are currently in progress to evaluate the inflence of abused drugs on this system.

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574.7

SIMULTANEOUS MEASUREMENT OF CCK AND NEUROTENSIN FRAGMENTS IN MICRODIALYSATES OF THE RAT FOREBRAIN: EFFECTS OF MIDBRAIN 6-HYDROXYDOPAMINE LESIONS. N. Villafranca, J.D. Barchas* and N.T. Maidment, Department of Psychiatry and Biobehavioral Sciences, N.P.I. and B.R.I., UCLA School of Medicine, Los Angeles, CA 90024.

We previously described a procedure for simultaneous determination of CCK and neurotensin fragments in rat dialysates of the caudate nucleus and posterior nucleus accumbens (Maidment et al., 1991, Neuroscience, 45: 81-93). In view of the well documented localisation of these peptides within subpopulations of midbrain dopamine neurons we sought to determine what proportion of the CCK and neurotensin measured extracellularly originated from dopamine terminals. As a first step in this process we injected 5 rats with 6-hydroxydopamine (10ug in 2ul) unilaterally in the substantia nigra and ventral tegmental area. Four rats received vehicle alone. Three weeks later the animals were tested for contralateral rotational behavior following injection of apomorphine (0.5mg/kg, I.P.). Four weeks following lesioning the animals were re-anesthetized and microdialysis conducted in the posterior nucleus accumbens and medial caudate nucleus ipsilateral to the lesion. Release was evoked by reverse dialysis of veratridine (50uM for10min) for two 30 min samples separarated by 2h. Dialysates were analyzed sequentially for the peptides by solid-phase radioimmunoassay. Veratridine induced an approximate 10-fold increase in extracellular CCK release in both regions. The neurotensin response in the nucleus accumbens was smaller (approx 3-fold) and less consistent and no stimulation was observed in the caudate. The preliminary data failed to demonstrate a clear effect of the lesion on the release of either peptide in the regions studied. This may reflect a non-dopaminergic origin of the peptides measured extracellularly or a compensatory increase in the releasable pool of the peptides within spared dopamine neurons similar to that described for dopamine itself (Robinson and Wishaw, 1988, Brain Res., 450: 209-224).

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574.8

STEROID DEPENDENCY OF GALANIN NEURONS IN THE BED NUCLEUS OF THE STRIA TERMINALIS BY IN SITU HYBRIDIZATION. M.A. Miller*, P.E. Kolb, and M.A. Raskind, Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98195.

We have recently observed that the majority of vasopressin (VP) synthesizing cells in the bed nucleus of the stria terminalis (BNST) also express galanin (GAL) mRNA. VP neurons in the BNST have been implicated in a variety of functions including learning and memory and antipyresis. We have previously reported that VP gene expression in the BNST is steroid dependent (Endocrinology 125(5):2335-2340, 1989). Castration of adult male rats results in a decline in VP gene expression in these cells which is reversed by testosterone replacement. To determine whether testosterone also regulates GAL gene expression in the BNST, we have used in situ hybridization and quantitative autoradiography to measure GAL mRNA levels in neurons in the BNST of intact (plasma T level: 5.2±1.2 ng/ml; n=7), castrated (plasma T level: 0.05±0.01 ng/ml; n=7), and castrated adult male rats treated with testosterone (plasma T level: 2.5±0.2 ng/ml; n=7).

Frozen brain sections (20μm) were hybridized with a cRNA probe complementary to GAL mRNA, coated with NTB2 emulsion, and developed. Sections through the BNST were anatomically matched (4/animal) and read blindly. The number of labeled cells (unilateral) and the average number of grains/cell (based on readings of up to 10 cells/slide) were compared across groups.

Castration significantly decreased ($p<0.05$) the number of GAL expressing cells in the BNST (X±SEM: 156±8, intacts; 115±13, castrates) and reduced ($p<0.001$) the average number of grains/cell (X±SEM: 98±5, intacts; 58±3, castrates). Testosterone treatment of castrated rats reversed the effects of castration on cell number (175±9) and grains/cell (103±6). These results indicate that GAL gene expression in the BNST, like VP gene expression, is regulated by testosterone and/or its metabolites. Although the function of GAL in the BNST is not known, these results suggest that it may be influenced by steroid state.

574.9

EFFECTS OF LENGTH OF STORAGE AND STORAGE CONDITIONS ON THIOL LEVELS IN RAT SCIATIC NERVE. K. K. Nickander, N. Lendvai and P. A. Low*. Neurophysiology Laboratory, Department of Neurology, Mayo Clinic, Rochester, MN 55905.

Comparison of the effect of length of storage and storage conditions on rat sciatic nerve levels of reduced and oxidized glutathione (GSH and GSSG, respectively) and the corresponding ratio was studied. Two groups of rat sciatic nerves were homogenized (in either 0.25 M perchloric acid or 5% 5-sulfosalicylic (SSA) acid) and centrifuged. A third group was excised and frozen immediately in liquid nitrogen. Samples were stored at -70° C for 0, 7, or 26-28 days. The thiols were measured using reverse phase high pressure liquid chromatography and electrochemical detection (Stein et al., 1986). GSH was decreased 56% by 7 days and 72% by 26-28 days when stored as a 0.25 M $HClO_4$ supernate. Consequently, the increase in level of GSSG was 806% by 7 days and remained high (765%) at 26-28 days. The decrease in the GSH/GSSG ratio was also highly significant at these time points (95% and 94% respectively). The changes were much less significant when stored as a 5% SSA supernate. The thiol levels and ratio remained unaltered when frozen immediately in liquid N_2 and stored at -70° C. Therefore, we conclude when storage of rat sciatic nerve tissue is necessary, to freeze immediately in liquid N_2 and store at -70° C until analysis. Homogenization should then be done in 5% SSA for best preservation of the thiol levels. (Reference: Stein AF, Dills RL, Klaasen CD. J. Chromatogr. 381:259-270, 1986.)

574.10

RAT BRAIN TRANSGLUTAMINASE AND RELATED PROTEINS. Y. Takeuchi*, P.J. Birckbichler, M.K. Patterson, Jr., K.N. Lee, M. Maxwell and B. Howell. Biomedical Division, The Samuel Roberts Noble Foundation, Ardmore, OK 73402.

To obtain basic information of transglutaminase (TGase) in mammalian brain, a 100,000 x g supernatant fraction was prepared from whole rat brain homogenate. SDS-PAGE analysis followed by immunoblotting of this soluble fraction gave 75 kDa/79 kDa proteins and 48 kDa protein that were detected by polyclonal antibodies against human erythrocyte TGase and guinea pig liver TGase, respectively. However, these immunopositive proteins were catalytically inactive and were separated from protein with TGase activity by DEAE Sephadex ion exchange chromatography. TGase activity which did bind to the ion exchange column was eluted with a gradient of NaCl and then applied to a GTP-agarose affinity column. TGase activity was recovered in both the KCl eluate and the GTP-unbound fraction. When the latter fraction was reapplied to the affinity column, the TGase activity was again recovered in the unbound fraction. These results suggest the presence of two different types of TGase in rat brain using the criterion of affinity for guanine nucleotide. It is interesting to note that an immuno-positive 48 kDa protein was also shown to bind GTP.

574.11

PLASMA CYCLO (HIS-PRO) IN AFFECTIVE DISORDERS. G.K. Tsuboyama, C. Prasad, R.C. Young, T. Kakuma and G.P. Smith*, Cornell U.Med. College, White Plains, New York 10605, and Louisiana State University, New Orleans, Louisiana 70112.

Cerebrospinal fluid thyrotropin-releasing hormone (TRH) is elevated in major depression. The metabolism of TRH ultimately yields acid TRH and cyclo (His-Pro), peptides with intrinsic biologic activity. Therefore, we have begun to measure cyclo (His-Pro) in affective disorders.

An unselected group of inpatients with major depression (n=5) or bipolar disorder, manic (n=4) was studied during treatment with antidepressants or mood stabilizers, respectively, and while on a low-monoamine diet. The mean concentration of plasma cyclo (His-Pro) determined by radioimmunoassay was 2557 +/- 212 pg/ml. This concentration is significantly higher than that reported by Hilton et al (Neuropeptides 13:65, 1989) in euthyroid control subjects (829 +/- 64 pg/ml, n=14, $p< 0.0005$). No difference between the two patient groups was found.

Limitations and confounds such as varying illness state and treatment make interpretation premature.

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574.12

COMPUTATIONAL SIMULATION OF NEUROPEPTIDE-LIPID INTERACTIONS: A NEW CONCEPT OF DIRECT EFFECT BY ENDOTHELIN-1 AT NEURONAL MEMBRANES. *D.F. Weaver*, S.T. Kim and P.M. Gross*, Departments of Chemistry, Medicine, Surgery & Physiology, Queen's University & Kingston General Hospital, Kingston, Canada K7L 3N6

Neuroactive agents may exert their effects on transmembrane ion channels specifically via channel protein receptors or nonspecifically by direct interactions with the neuronal membrane (e.g., local anesthetics). To explore potential membrane interactions for the neuropeptide endothelin-1 (ET), we applied computer-assisted conformational analyses to: 1) ascertain a family of low-energy ET conformers, 2) study the effects of different environments on the shape of ET, and 3) simulate ET's interaction with a model neuronal membrane under physiological conditions. Commencing with the NMR conformation of ET, we sought to identify the shape of the molecule having the lowest energy by applying molecular mechanics calculations. MM2, AMBER and DREIDING-2 semi-empirical force-field equations were combined with procedures for both first- and second-derivative energy minimization. Since ET was demonstrated to be a highly flexible molecule, its conformational space was further scanned using molecular dynamics calculations that required several hundred hrs of CPU time on an IBM RS/6000 550 RISC computer. Both vacuum and aqueous environments were simulated at 37°C. Finally, a fully hydrated ET conformer with the lowest energy was positioned adjacent to a phospholipid membrane surface and its molecular motions were simulated randomly over a period of 100 psec. These calculations demonstrated that the acyclic hexapeptide tail of ET inserted among the alkyl chains of the lipid membrane in an energetically favorable orientation that affected the shape and dynamic properties of the lipid molecules. The simulation thus revealed a novel mechanism of instantaneous insertion by a neuropeptide into a neuronal membrane. In addition to receptor binding, therefore, ET may invade lipid bilayers directly.

574.13

CCK PARTIAL AGONISTS : DIFFERENTIAL FUNCTIONAL ACTIVITY IN THE RAT. R. Simmons*, J. Zongrone, R. Julien, F. Kaiser and J. Rosamond, Depts. of Biology and Chemistry, Fisons Pharmaceuticals, Rochester, New York, 14603.

CCK peptide analogs were evaluated for selective CCK-A receptor agonist activity both in vitro, using pancreatic amylase release and phosphatidyl inositol (PI) turnover assays, and in vivo, using a 21 hour fasted rat feeding inhibition assay. The compounds tested showed a full range of in vitro activity from potent full agonists to weak partial agonists as compared to CCK-8, (CCK= HpaSE[MePhe^6 > Boc[Lys^2,MePhe^4] CCK-4 > Boc CCK-4 > Boc[MePhe^4] CCK-4. The feeding inhibition potency and efficacy were compared with the in vitro functional activity which was further correlated with the Ki binding values for the CCK-A, (pancreatic membranes) and CCK-B receptor (cortical membranes). A good correlation exists between CCK-A but not CCK-B binding affinity, in vitro functional potency and in vivo anorectic potency. However, for partial agonists, in vitro and in vivo efficacy did not correlate. This is probably not related to a difference in receptor subtype interaction (CCK-A vs. CCK-B) since the CCK-A selective antagonist (MK-329) but not the CCK-B antagonist (L365,260) completely inhibits CCK induced P.I. turnover and feeding inhibition. Differences in activity could therefore reflect differences in tissue receptor reserve between the CCK-A receptors in pancreas and those involved in the mechanism of CCK-8 induced feeding inhibition.

574.14

SUPER-REACTIVITY OF THE SERINE^4 RESIDUE IN GONADOTROPIN-RELEASING HORMONE AGONISTS IS NOT EVIDENT IN ANTAGONISTS. B.T. Miller*, T.J. Collins and A. Kurosky. Depts. of Anatomy & Neurosciences and of Human Biological Chemistry & Genetics, University of Texas Medical Branch, Galveston, TX 77555.

The critical roles of gonadotropin-releasing hormone (GnRH) in reproductive physiology have led to intensive study of the chemistry, structure and function of this peptide. We have previously demonstrated that the Ser^4 hydroxyl in native GnRH and several GnRH agonist peptides displays unusually high intrinsic reactivity toward activated esters of biotin (Miller et al, 1992, J Biol Chem 267, 5060). In this study, we report that the Ser^4 residues of two peptide analogs which are potent GnRH antagonists are virtually unreactive. Neither [D-Phe^2,Pro^3,D-Phe^6]GnRH nor [D-pGlu^1,D-Phe^2,D-Trp^{3,6}]GnRH reacted significantly with N-hydroxysuccinimide-biotin esters, whereas native GnRH and its agonists were readily O-acylated at Ser^4 under identical conditions. These results further emphasize the importance of the seryl residue in structure/function considerations of GnRH and its related peptides. (Supported by NIH grant NS 29261 and the Robert A Welch Foundation H-1190)

OPIATE RECEPTORS: INTERACTIONS WITH OTHER SYSTEMS

575.1

μ and K OPIOID AGONISTS ALTER IN VIVO 3H RACLOPRIDE (D2) BINDING: OPPOSING EFFECTS IN THE E21 RAT FETUS AND P10 PUP. P. Kehoe[1], K. M. Ward[1], S. L. Andersen[2], S. M. Umphress[2], S. R. Robinson[2] and W. P. Smotherman*[2]. [1]Department of Psychology, Trinity College, Hartford, CT 06106; [2]Laboratory of Perinatal Neuroethology, Center for Developmental Psychobiology, SUNY-Binghamton, Binghamton, NY 13902-6000.

The endogenous opioid system is thought to interact with the dopamine system in the adult; different classes of opioid ligands produce different effects on dopamine activity. Evidence from in vivo dialysis of adult rats has shown that μ opioid agonists promote dopamine release while K agonists suppress release. Recent developmental studies of neonatal and fetal rats confirm the early existence of this opioid-dopamine interaction. The present study employed an in vivo binding technique with a tritiated ligand for the D2 receptor (3H-raclopride) to further examine the development of interactions between the opioid and dopamine systems in the term rat fetus (E21) and rat pup (P10). Subjects were injected with μ or K agonists prior to administration of 3H-raclopride. Specific D2 activity was measured in tissue samples from the striatum, septum and hypothalamus. In P10 pups, μ stimulation resulted in decreased raclopride binding while K stimulation increased specific binding relative to saline-injected controls. Changes in specific binding imply that μ receptor stimulation promoted D2 receptor occupation (i.e., increased dopamine release) and K stimulation suppressed D2 receptor occupation. In sharp contrast, μ receptor stimulation resulted in increased specific binding at the D2 receptor in E21 fetuses, while K stimulation produced no change in binding relative to controls. These data suggest a developmental discontinuity in the pattern of interaction between opioid and dopamine receptors during the first 1-2 weeks after birth.

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575.2

EFFECTS OF INTRANIGRAL APPLICATION OF THE KAPPA OPIATE AGONIST U50,488 ON LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) AND ROTATIONAL BEHAVIOR IN THE RAT. A.G. Hohmann*, R.W. Clement and J.M. Walker. Schrier Research Laboratory, Department of Psychology, Brown University, Providence, RI 02912.

Unilateral microinjection of kappa-selective opiates in the substantia nigra induces contralateral circling behavior via actions on nondopaminergic neurons in the pars reticulata. However, the functional pathways activated downstream from the substantia nigra are poorly understood. The present study investigates changes in regional metabolic activity and rotational behavior following intranigral application of U50,488 using the 2-deoxy-D-[1-^{14}C]glucose ([^{14}C]DG) method as adapted for use in freely moving rats. Animals were unilaterally microinjected with 10 or 20 nmol U50,488 or normal saline just prior to the infusion of [^{14}C]DG. Behavioral data were collected concurrently via microcomputer. U50,488 induced dose-dependent contralateral circling, confirming the potent locomotor stimulation produced by this ligand. ANOVA revealed significant depressions in local cerebral glucose utilization (LCGU) in basal ganglia and related circuitry following intranigral U50,488. Bilateral decreases in LCGU were observed in the pedunculopontine n., substantia nigra pars compacta, substantia nigra pars reticulata and cingulate cortex. The effects suggest extensive collateralization in these regions. By contrast, significant decreases in LCGU were only observed ipsilateral to the injection site in the pontine n., fastigial n., inferior colliculus, ventral tegmental area and red n. Structures exhibiting significant contralateral decreases in LCGU included the vestibular n., globus pallidus, anterior caudate n. and parietal cortex. The observed motor activation and alterations in glucose utilization are consistent with a kappa-mediated inhibition of GABAergic reticulata cells and consequent decrease in the afferent supply to output structures. (Supported by PHS Grant DA04988).

575.3

MU AND DELTA OPIOID-REGULATED ADENYLYL CYCLASE ACTIVITY IN RAT CAUDATE-PUTAMEN AND NUCLEUS ACCUMBENS. B. Büzás, S. Izenwasser, and B. M. Cox*. Uniformed Services University, Bethesda, MD.

Activation of opioid receptors leads to inhibition of adenylyl cyclase activity. Adenylyl cyclase activity was examined in crude membrane preparations of nucleus accumbens and the rostral portion of the caudate-putamen using a cAMP radioligand binding assay. Basal adenylyl cyclase activity (expressed relative to membrane protein) in the caudate-putamen was twice that in the nucleus accumbens. The μ-agonists DAMGO and TAPS maximally inhibited approximately 30% of basal adenylyl cyclase in both brain regions. Both of these μ-opioid ligands were less potent for inhibiting adenylyl cyclase activity in the nucleus accumbens than in the caudate-putamen. DPDPE was equally effective in both brain regions, inhibiting less than 30% of basal adenylyl cyclase activity. DSLET, however, inhibited almost 40% of basal cyclase activity in both brain regions. U69,593 had no effect on adenylyl cyclase in either brain region. The μ-opioid selective antagonist CTOP blocked the inhibition of adenylyl cyclase by DAMGO or TAPS, but had no effect on inhibition by DSLET, suggesting that μ-opioid receptor activation did not contribute significantly to the DSLET effect. When the animals were pretreated with naloxonazine (30 mg/kg, s.c. 24 hours prior to assay), the effects of DAMGO and TAPS were attenuated but there were no changes in inhibition of cyclase in the caudate putamen by either DPDPE or DSLET. Similarly, β-FNA pretreatment (20 μg, icv, 24 hours prior to assay) attenuated the effects of DAMGO and TAPS but had no effect on DSLET. These findings suggest that both DAMGO and TAPS act predominantly through a naloxonazine-sensitive μ-opioid receptor to inhibit adenylyl cyclase in these brain regions. Furthermore, DSLET had a greater inhibitory effect than either DAMGO or DPDPE, but was not antagonized by any of the μ-opioid receptor antagonists. (Supported by a grant from NIDA).

575.4

REGULATION OF OPIATE RESPONSES IN BRAIN NORADRENERGIC NEURONS BY THE cAMP CASCADE: CHANGES WITH CHRONIC MORPHINE. R. Shiekhattar*, G. Aston-Jones. Department of Mental Health Sciences, Division of Behavioral Neurobiology, Hahnemann University, Broad and Vine, Philadelphia, PA 19102, U.S.A.

Intracellular recording from locus coeruleus (LC) neurons in brain slices revealed that the μ-opiate receptor-mediated responses of these neurons are regulated through the adenylate cyclase cascade. The hyperpolarization induced by 30 μM morphine was enhanced by forskolin (FSK, 20μM), an activator of adenylate cyclase, (+36%, p=0.008). Furthermore, intracellular diffusion of adenosine 3',5'-monophosphate (cAMP) from the microelectrode potentiated morphine responses by 44% (p=0.004). Application of FSK also potentiated the response of every cell examined with 0.3-30 μM enkephalin (+25+5%, p<0.001; n=7). The inactive FSK analog 1,9 dideoxyforskolin had no effect on enkephalin responses (p=0.5, n=3). The potentiation of the opiate response was blocked by agents (H-8, KT5720, RpcAMPS) that inhibit cAMP-dependent protein kinase (PKA). Moreover, the inhibitors reduced the opiate response below baseline values indicating that μ-opiate responses are regulated by a basal kinase activity. The response to 50 nM clonidine was also potentiated (p=0.005, n=4). Only 2 of 8 neurons showed potentiation of opiate responses by FSK following chronic morphine treatment and overall this was not significant (p=0.4). Furthermore, appliaction of H8 alone decreased the baseline enkephalin response in chronically morphine treated animals, similar to results for naive animals. These results indicate that opiate responses in LC are regulated by the cAMP-PKA system, and that such regulation is subject to change by chronic opiate treatment.

575.5

INHIBITION OF SYNAPTIC TRANSMISSION BY EXOGENOUS AND ENDOGENOUS OPIOID IN THE SUPERIOR CERVICAL GANGLION OF THE CAT. C. Zhang*, M. Bachoo and C. Polosa. Dept. of Physiology, McGill University, Montreal, Quebec, Canada H3G 1Y6.

Effects of opioid agonists and of heterosynaptic preganglionic conditioning stimulation on nicotinic transmission were studied in the superior cervical ganglion (SCG) of anesthetized, paralyzed and artificially ventilated cats. The cervical sympathetic trunk was dissected and split into two bundles, one for conditioning stimulation and the other for testing stimulation. The compound action potential was recorded from the external carotid nerve. A heterosynaptic stimulus train (5 Hz, 40 s) inhibited nicotinic transmission. The inhibition was antagonized by naloxone, suggesting mediation by endogenous opioids. Agonists selective for μ, δ and κ opioid receptors, injected through the lingual artery, also produced naloxone-sensitive inhibition. The synaptic inhibition was occluded by those produced by μ-, δ-, but not κ-selective agonists. The synaptic inhibition was antagonized by the δ-selective antagonist ICI 174,864, but not by the κ-selective antagonist Nor-BNI. The μ-selective antagonist CTAP produced a partial antagonism of the inhibition. It is concluded that all three main types of opioid receptors are present in the SCG but only μ and δ receptors are involved in the endogenous opioid-mediated inhibition of ganglion transmission. Supported by the Medical Research Council of Canada.

575.6

INHIBITORY EFFECTS OF OPIOID PEPTIDES ON NEONATE RAT SYMPATHETIC PREGANGLIONIC NEURONS *IN VITRO*. H. Tan* and N. J. Dun. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Whole-cell-patch recordings were made from sympathetic preganglionic neurons (SPNs) in transverse spinal cord slices and the effects of opioid peptides on these neurons and on synaptic transmission were studied. Two types of responses were detected. First, bath or pressure application of met-enkephalin (Enk 5,10 μM) induced an outward current or hyperpolarization accompanied by increased membrane conductance; the reversal potential was close to E_K. The δ and μ receptor agonists D-PEN and DAMGO and the antagonists ICI 174,864 and CTOP mimicked and blocked the response. Second, superfusion of Enk (1-10μM) depressed the excitatory postsynaptic currents (EPSCs) evoked by stimulation of either dorsal roots or lateral funiculus, without affecting the inward current induced by pressure ejection of glutamate. This inhibition of EPSCs could also be mimicked by either the δ or μ agonist. The results indicate that opioids inhibit the activity of SPNs by either a postsynaptic mechanism in opening K channels or by a presynaptic mechanism in reducing the transmitter release. (Supported by NS18710)

575.7

THE SUCKLING INDUCED PROLACTIN INCREASE IS MEDIATED BY β-ENDORPHIN THROUGH THE KAPPA OPIATE RECEPTOR SUBTYPE. Rebecca Parman, James Janik and Phyllis Callahan*, Miami University, Zoology Dept, Oxford, Ohio 45056

Administration of β-endorphin to virgin female rats, at doses as low as 25 ng, produced a prolactin secretory response which is the same order of magnitude as the suckling induced prolactin increase. This response was completely abolished by nor-Binaltorphimine (nor-BNI), a specific κ receptor antagonist. The purpose of this study was to determine whether or not β-endorphin played a physiologically signficant role in the suckling induced prolactin increase.

Post-partum lactating female Sprague-Dawley rats were used for all experiments. On day 2 post-partum, animals were implanted with chronic intraventricular (ivt) cannula into the lateral ventricle. Following recovery, each animal was implanted with a chronic jugular cannula. On the day of the experiment, pups were separated from the dams for 6 hours. One group of females received vehicle or Nor-BNI (10 nmol, ivt) 45 minutes prior to the pups return. A second group of females received vehicle or antiserum to β-endorphin (3, 7.5 or 15 μg total protein, ivt) immediately prior to pup return. Blood samples were withdrawn immediately prior to and 15, 30, 45, and 60 minutes after suckling was initiated.

Both β-endorphin antiserum and Nor-BNI pretreatment totally abolished the suckling induced prolactin increase in post-partum female rats. These results indicate that β-endorphin is involved in the suckling induced prolactin secretory response via its activity at the kappa opiate receptor subtype.

575.8

NALOXONAZINE BLOCKS β-ENDORPHIN INDUCED PROLACTIN SECRETION IN FEMALE RATS James Janik* and Phyllis Callahan, Miami University, Zoology Dept, Oxford, OH 45056

The prolactin secretory response to β-endorphin administration was determined in post-partum female rats and during the diestrous stage of the estrous cycle. The effects of Naloxonazine (NAZ) pretreatment were also determined since this μ_1 antagonist effectively blocks the morphine induced prolactin increase.

Female Sprague-Dawley rats were used for all experiments. Virgin females in diestrus and post-partum lactating females were implanted with chronic intraventricular (ivt) cannula into the lateral ventricle. Lactating females were implanted on day 2 post-partum. One day prior to the experiment, animals were surgically implanted with a chronic jugular cannula. On the day of the experiment, pups were separated from the dams for 2 hours.

The lowest dose of β-endorphin to produce a prolactin secretory response was 25 ng and this dose elicited a prolactin increase which is the same order of magnitude as the suckling induced prolactin increase. This was true in both post-partum female and virgin female rats. Lower doses of β-endorphin, i.e. 2.5, 5 and 10 ng, did not produce a change in circulating levels of prolactin. This prolactin increase was completely abolished by NAZ.

These results indicate that β-endorphin is a potent stimulus for secretion and may act in an "all or none" manner. In addition, it seems that β-endorphin, like morphine, can elicit a prolactin secretory response via its activity at the μ_1 opiate receptor subtype.

575.9

POSSIBLE ROLE OF PHOSPHATIDYLINOSITOL METABOLISM IN THE MECHANISM OF KAPPA OPIOID- AND MK-801-INDUCED NEUROPROTECTION IN PRIMARY RAT CORTICAL NEURONAL CULTURES. D.L. Yourick*, M.A. DeCoster and F.C. Tortella. Dept. Med. Neurosci., Walter Reed Army Inst. Research, Washington, D.C. 20307-5100.

The kappa agonists, CI-977 and PD117302 and the noncompetitive NMDA antagonist MK-801, have been shown to be neuroprotective (reduced LDH release) in primary rat cortical neuronal cultures treated with exogenous glutamate. These kappa agonists have also been shown to reduce glutamate-stimulated inositol phosphate accumulation while slightly stimulating phosphatidylinositol metabolism when used alone. Glutamate (40 μM), in the presence of phenol red, elevated inositol phosphate accumulation to approximately 400% of basal metabolism, and CI-977 (50 nM) and PD117302 (50 nM) reduced this response by as much as 47%. It was the purpose of the present study to evaluate whether MK-801 could also reduce glutamate-stimulated inositol phosphate accumulation. MK-801 (50 nM) did not reduce glutamate-stimulated phosphatidylinositol metabolism and did not alter basal inositol phosphate accumulation. However, when given alone at higher concentrations (100-500 nM), MK-801 reduced basal inositol phosphate accumulation by approximately 20-50%. In conclusion, the effects of the kappa opioid agonists and MK-801 are different with respect to basal and glutamate-stimulated phosphatidylinositol metabolism. While CI-977 may prevent neuronal death, in part, by reducing glutamate-stimulated inositol phosphate accumulation, the only apparent effect of MK-801 was on basal metabolism, implying an interaction between the metabotropic glutamate receptor subtype and the NMDA receptor channel complex.

575.10

MU AND MU/DELTA INTERACTIONS MEDIATE OPIOID-INDUCED RELEASE OF ADENOSINE VIA N-TYPE Ca^{2+} CHANNELS FROM DORSAL SPINAL CORD SYNAPTOSOMES. C.M. Cahill*, T.D. White and J. Sawynok. Dept. of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7.

The release of adenosine (ADN) from the spinal cord contributes to spinal antinociception by morphine (TIPS 10:186,1989). Morphine (1-100 μM) has been shown to release ADN from dorsal spinal cord synaptosomes (JPET 243:557,1989), but more recently, a nanomolar component of release was revealed when synaptosomes were partially depolarized with K^+ (unpublished). Release of ADN by morphine is Ca^{2+}-dependent. The present study examined the opioid receptor subtypes involved in the release of ADN from dorsal spinal cord synaptosomes and the Ca^{2+} channels involved in such release. DAMGO (a μ agonist) evoked two components (nM and μM concentrations) of ADN release similar to the biphasic response with morphine. DPDPE (a δ agonist) had little effect on the release of ADN by itself but when combined with DAMGO, a synergistic effect was evident as 1 μM DPDPE eliminated the trough component in the DAMGO dose-response curve. Combinations of a range of DAMGO and DPDPE doses significantly enhanced the release of ADN, and this was inhibited by ω-conotoxin (100 nM), which previously was shown to inhibit morphine evoked release of ADN. This study demonstrates that both μ and δ receptors are involved in the release of ADN from spinal cord synaptosomes, and that such release appears to involve Ca^{2+} entry via activation of N-type Ca^{2+} channels. *(Supported by MRC Canada).*

576.1

A DELTA OPIOID AGONIST REDUCES GABAERGIC IPSPS EVOKED BY LOW-, BUT NOT HIGH-INTENSITY, ELECTRICAL STIMULATION IN RAT HIPPOCAMPUS IN VITRO. C.R. Lupica*, and T.V. Dunwiddie. Dept. Pharmacology, Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262.

We have reported that μ opioid receptor activation reduces IPSP amplitudes and increases EPSPs in CA1 pyramidal neurons. However, when the δ-selective agonist DPDPE was used EPSPs were increased, spontaneous IPSPs decreased, and evoked IPSPs were unaffected. We concluded that while δ opioid receptor activation increased pyramidal neuron excitability by reducing GABAergic inhibition, this inhibition was difficult to elicit and characterize. The present study was conducted to determine the conditions under which this δ-sensitive inhibition could be characterized. Whole-cell recordings of synaptic potentials, elicited by low-intensity stimulation, were obtained from CA1 pyramidal neurons in rat hippocampal slices. In agreement with Turner (J. Physiol., 422:333,1990), at low stimulus intensities, we found that IPSPs could be evoked in the virtual absence of significant EPSP components (IPSP= -1.88$\pm$.08mV, EPSP= .26$\pm$.04mV, n=6). Bath application of DPDPE (.1μM) reduced these IPSPs (to -.93$\pm$.06mV) and increased the EPSPs (to 1.01$\pm$.1mV), but had no effect upon IPSPs evoked with more intense stimulation. To evaluate the possibility that these IPSPs were mediated *via* low-threshold excitatory afferents to interneurons, glutamate receptors were blocked using the antagonists APV (10μM) and DNQX (40μM). Under these conditions monosynaptic IPSPs generated by low-, but not high-intensity stimuli were again reduced by DPDPE. These data suggest that either 1. δ opioid agonists reduce a novel form of low-threshold feed-forward inhibition, or that 2. δ agonists hyperpolarize interneurons and reduce IPSPs at low-stimulus intensity, but at a higher stimulus intensities interneurons contributing to the IPSP are reliably activated, in spite of the δ-induced hyperpolarization. This work supported by NIDA grants DA 02702, DA 07725 and the V.A. Medical Research Service.

576.2

ELECTROPHYSIOLOGIC EFFECTS OF SELECTIVE OPIOID AGONISTS AND NALOXONE IN THE DENTATE GYRUS. JH Mayer*, SC Steffensen, SJ Henriksen. Dept. Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037

We have studied in vivo the electrophysiologic responses of two selective opioid agonists and systemic naloxone in the dentate gyrus of the hippocampus. [D-Ala^2, NMe-Phe^4, Gly-ol]-Enkephalin (DAGO), a selective mu receptor agonist, and U50488, a selective kappa receptor agonist, were applied iontophoretically in anesthetized rats. This study represents a continuation of a previous study in which effects of systemic morphine and preliminary data on the effects of locally applied DAGO were reported. The effects of these opioids on spontaneous single cell activity and on activity evoked by stimulation of the perforant path were recorded. Cells were classified electrophysiologically as either dentate granule cells (DGCs) or dentate interneurons (INTs) using stringent criteria. The responsiveness of the dentate to stimulation of the perforant path was assessed by measuring population spike amplitudes and constructing input/output curves (I/O) and via paired-pulse (PP) paradigms. The effect of naloxone on long term potentiation (LTP) was also studied. There was a tendency for DAGO to produce a decrease in the spontaneous activity of DGCs. This opioid had little consistent effect on the spontaneous activity of INTs; however, DAGO did tend to decrease the number of post-stimulus interneuronal discharges, induced by perforant path stimulation. As previously reported, DAGO increased the responsiveness of the dentate gyrus to evoked field potential activity. As opposed to DAGO, U50488 was found to have little significant effect on either the spontaneous activity of DGCs and INTs, or on evoked activity. Naloxone, administered systemically, readily reversed the effects of DAGO on both single unit firing and on population spikes, and, in preliminary studies, reduced perforant path-induced long term potentiation in the dentate gyrus. (Supported by DA 00143 to JHM)

576.3

ENDOGENOUS KAPPA OPIOIDS INHIBIT EXCITATORY TRANSMISSION IN THE DENTATE GYRUS OF THE GUINEA PIG HIPPOCAMPUS. J.J. Wagner* and C. Chavkin. Dept of Pharmacology, Univ of Washington, Seattle, WA. 98195.

Our previous pharmacological studies demonstrated that kappa$_1$ receptor activation inhibited excitatory synaptic transmission in the dentate gyrus. Those observation have been extended using whole-cell voltage clamp recordings of dentate granule cells performed in guinea pig hippocampal slices (500μm). EPSCs were isolated with CsCl (120 mM) in the recording pipet (to block K^+ currents postsynaptically) and biccuculline (10 μM) in the perfusion buffer (to block spontaneous and stimulus evoked IPSCs). A stimulating electrode placed in the outer molecular layer was used to elicit monosynaptic, (CNQX-sensitive) EPSCs from perforant path (PP) fibers. U69,593 (500 nM), a kappa$_1$ selective opioid agonist, decreased the amplitude of the PP EPSCs by 45%. This effect was reversed by the kappa opioid antagonist norbinaltorphimine (100 nM) (NBNI). Application of NBNI (100 nM) alone resulted in a 30% increase in the amplitude of the PP EPSC compared to predrug control values. This result suggests that tonic release of endogenous kappa opioids (i.e. dynorphins) also regulate glutamate release from the perforant path afferents. In order to evoke the release of additional endogenous kappa opioids, a second stimulating electrode was placed in the hilus to antidromically activate granule cells via their mossy fibers. After establishing a baseline PP EPSC response, a single high frequency train (10-50 Hz, 1 sec) (HFS) given in the hilus resulted in a 15% decrease in the amplitude of the PP EPSC. This effect was blocked by naloxone (1 μM) or NBNI (100 nM). We conclude that the stimulated release of dynorphins from dentate granule cells can act to inhibit EPSCs at the PP synapse. Supported by DA04123.

576.4

KAPPA OPIOIDS INHIBIT LONG-TERM POTENTIATION IN THE DENTATE GYRUS OF THE GUINEA PIG.HIPPOCAMPUS G.W. Terman*, J.J. Wagner, A. Schatzki and C. Chavkin. Departments of Anesthesiology and Pharmacology, University of Washington, Seattle, WA. 98195.

We have previously demonstrated a kappa opioid-mediated inhibition of excitatory synaptic transmission in the dentate gyrus of the guinea pig hippocampal slice. In this series of studies we evaluated the effect of kappa opioids on the induction of long-term potentiation (LTP) in this preparation.

Our initial studies defined the stimulus properties of tetanic stimulation in the lateral perforant pathway sufficient to produce LTP in the granule cell layer of the dentate gyrus bathed in artificial CSF and 10 μM bicuculline. A tetanus paradigm producing significant but sub-maximal LTP was chosen for the remainder of our studies. Three 20msec 100Hz trains of 0.3msec 300μA pulses were given at 10 sec intervals and the effect on the population spike amplitude was measured 30 min later. Whereas, this stimulus produced a significant ($p<0.01$) LTP when compared to untetanized controls, this effect was blocked by pre-treatment with 1 μM of the kappa selective opiate agonist U69593. Moreover, pretreatment with the opiate antagonist naloxone (1μM) significantly ($p<0.01$) enhanced LTP from this stimulus.

Thus, kappa opioids appear to inhibit long-term potentiation either when exogenously applied or when released from endogenous stores. Supported by DA04123 and GM07604.

576.5

HERPES SIMPLEX VIRUS-1 RECOMBINANTS FOR EXPRESSION OF THE PROENKEPHALIN GENE IN NEURONS. **C Gravel, C Meaney, M Comb and X O Breakefield*, Dept of Neurology, & Neurosci Ctr, Mass Gen Hosp, & Neurosci Prog, Harvard Med Sch, Boston, MA.**

The enkephalins are a class of endogenous opioid peptides thought to be involved in many physiological functions in the nervous system. In particular, many lines of evidence point to their role in the modulation of nociception. In order to assess the actions of enkephalins both *in vivo* and in culture, and to develop potential means to modulate nociception *in vivo*, we have devised a series of herpes simplex virus-type 1 (HSV) recombinants aimed at expressing the human proenkephalin gene in neural cells following infection. We and others have previously shown that HSV recombinant viruses can be used to confer long term expression of a foreign gene in post-mitotic neurons either in culture, or directly in the nervous system (Dobson et al, 1991; Andersen et al, submitted.)

To construct the recombinant viruses, the human proenkephalin cDNA, under the control of various promoter elements, was inserted into the coding region of the viral thymidine kinase gene (*tk*) in a plasmid vector. These transcriptional units were then inserted into the viral genome by homologous recombination between plasmid and viral DNA at the *tk* site. Some of the proenkephalin constructs were fitted with a minimal human proenkephalin promoter coupled to one or multiple copies of a cAMP/pKC-inducible enhancer element. Alternatively, a proenkephlain construct bearing the Moloney murine leukemia virus LTR promoter/enhancer element was also constructed. Different HSV viral genomes have been selected for insertion of the proenkephalin transcription units within their *tk* gene: wild-type KOS virus, and also mutant viurses with compromised replication or reduced cytopathogenicity. Preliminary results will be presented on the expression characteristics of these viral vectors in cell culture and in sensory neurons *in vivo*.

576.6

PREPROENKEPHALIN mRNA EXPRESSION IN THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS AND LUMBAR DORSAL HORN OF FEMALE RATS FOLLOWING GONADAL STEROIDS AND FORMALIN INJECTION . **D.A. Holtzman*, P.J. Brooks, D.W. Pfaff and S. Schwartz-Giblin. Rockefeller University, N.Y., N.Y. 10021.**

This study examines the interactions between estrogen (E), progesterone (P) and a noxious stimulus, 5% formalin s.c. (FORM), on preproenkephalin (PPE) mRNA expression in the ventromedial nucleus of the hypothalamus (VMH) and dorsal horn of the lumbar spinal cord (DH), as measured by in situ hybridization. 36 ovariectomized (OVX) rats were divided equally into 3 hormone groups: E, E+P, and OVX. Rats receiving steroids had 100% E silastics implanted 2 weeks prior to stimulus injection. P was injected 4 h prior to sacrifice. From each hormone group, 6 rats received a saline (SAL) injection (s.c.) and 6 received a FORM injection (s.c.) into a hindfoot pad, and rats from all 6 groups were sacrificed 24 h afterward. Frozen sections were hybridized with a single-stranded, ^{3}H-DNA probe complementary to rat PPE mRNA encoding amino acids 1-68. To date, mean grains (pixels)/cell have been analyzed from a minimum of 113 cells/group for the VMH with 2-4 rats/group and from at least 157 cells/group for the DH with 2-3 rats/group. At 24 h, no difference in PPE mRNA levels were seen between sides or between SAL and FORM injections in either the DH or VMH. 6 OVX rats that received no injection have been added as a stimulus control. So far, analysis of mean pixels/cell in laminae I-V of the DH from 2-3 rats/group shows no apparent effect of steroid treatment or injection solution alone but suggests a trend for an interaction between these factors ($p=0.07$). Similar analysis of the VMH data from 2-4 rats/group shows an effect of hormone treatment ($F=7.87$, $p=0.002$) but no apparent effect of injection solution or interaction between hormone and injection. In the VMH, post-hoc Tukeys show that the mean pixels/cell are greater in both E and E+P than in OVX rats ($p<0.05$). Mean pixels/cell are greater in OVX-FORM than OVX-SAL rats, suggesting that when PPE mRNA levels are low in the VMH, as in OVX rats, FORM can increase PPE transcription.

576.7

KAPPA OPIOID REGULATION OF THE SECRETION OF PROLACTIN AND α-MELANOCYTE-STIMULATING HORMONE IN MALE AND FEMALE RATS. J. Manzanares,* E.J. Wagner, K.E. Moore and K.J. Lookingland. Department of Pharmacology & Toxicology, Michigan State University, MI 48824.

Previous studies from our laboratory have demonstrated a sexual difference in the responsiveness of tuberoinfundibular dopamine neurons to kappa opioid receptor agonists and antagonists (Neuroendocrinol. 55: 301, 1992), but it is not known if this difference is reflected by similar differences in the secretion of prolactin. The purpose of the present study was to examine the effects of the kappa opioid agonist U-50,488 and antagonist nor-binaltorphimine (NOR-BNI) on the secretion of prolactin in male and diestrous female rats. For comparison the effects of U-50,488 and NOR-BNI on the secretion of α-melanocyte stimulating hormone (αMSH) were also examined. On the day of the experiment, rats were implanted with a right atrial cannula under diethylether anesthesia, and serial blood samples were taken -30, 0, 30, 60, 120, 240, 480 min relative to drug administration. Activation of kappa opioid receptors with U-50,488 (5 mg/kg; sc) caused a marked and time-dependent (30-120 min) increase in plasma prolactin concentrations in female rats, but produced only a transient increase in prolactin secretion in males. Blockade of kappa opioid receptors with NOR-BNI (25 μg/rat; icv) had no effect on plasma prolactin concentrations in female rats, but produced a pronounced and time-dependent (30-480 min) decrease in prolactin secretion in males. These results reveal a sexual difference in kappa opioid receptor-mediated regulation of prolactin secretion. In contrast, there was no sexual difference in kappa opioid regulation of αMSH secretion since administration of U-50,488 increased and NOR-BNI decreased plasma αMSH concentrations in both male and female rats. (Supported by ADAMHA Grant MH 42802).

576.8

INHIBITION OF THE TIME-DEPENDENT INWARD RECTIFICATION IN VASOPRESSIN CELLS OF THE GUINEA PIG SUPRAOPTIC NUCLEUS BY THE MU OPIOID AGONIST DAMGO. K.R. Erickson*, O.K. Ronnekleiv , A.R. Lagrange, S.A. MacMillan and M.J. Kelly . Dept. of Physiology, Oregon Health Sci. U., Portland, OR 97201-3098.

Guinea pig magnocellular neurosecretory cells (MNCs) exhibit a time-dependent inward rectification (TDR, or I_h) which depolarizes the cell from hyperpolarized membrane potentials (below -65 mV). This increases the excitability and, in vasopressinergic (AVP) neurons, leads to phasic firing (Erickson, et al., **Soc Neurosci.** 1991). In the present study, the μ-opioid agonist DAMGO (500 nM - 1 μM) was bath-applied to guinea pig supra-optic nucleus (SON) MNCs. Intracellular recordings were made in an in vitro slice preparation using biocytin-filled electrodes, thus permitting immunocytochemical identification. Six cells were identified as AVP-containing. Two others were not AVP positive. Five of the six AVP cells responded to DAMGO (0.5 - 1 μM), whereas neither of the two AVP-negative cells responded (χ^2, $p<0.05$). All five responding cells exhibited a TDR in current clamp or an I_h in voltage-clamp. DAMGO hyperpolarized and inhibited the TDR in current clamp (N=2); and in voltage clamp, DAMGO produced an outward current and inhibited the I_h on steady-state ramp I/V measures (N=2). Naloxone (1 mM) depolarized the membrane by 2 mV and increased the TDR in an AVP neuron from a morphine treated guinea pig, indicative of an antagonism of tonic opioid tone. The results suggest that the TDR (I_h) in guinea pig AVP MNCs is modulated by μ-opioid receptor activation. (Supported by PHS grant DA05158)

576.9

ESTROGEN AND PROGESTERONE MODULATION OF AN INTRINSIC OPIOID ANALGESIC SYSTEM(S). M.E. Dawson-Basoa* and A.R. Gintzler. Dept. of Biochemistry, SUNY Health Science Center at Brooklyn, N.Y. 11203, USA.

It has been demonstrated in rats as well as in humans, that pregnancy and parturition are associated with an opioid-mediated elevation in maternal pain threshold. This analgesia has been shown to involve a spinal opioid system(s). Simulation of the pregnancy blood profile of 17-β-Estradiol (E_2) and Progesterone (P) in non-pregnant, ovariectomized rats resulted in statistically significant elevations in pain threshold. The increase occurred at doses of E_2 and P that approximated that which occurs during late pregnancy (1-3 days before birth) and parturition, the time of actual pregnancy during which analgesia is also observed. Administration of pregnancy levels of P alone or E_2 with the delayed addition of P (starting with dose 3, the dose at which initial increases in pain threshold occur) is not sufficient to produce the increase in pain threshold. Therefore, the entire pregnancy profile of steroid hormones is responsible for the manifestation of analgesia. Administration of the narcotic antagonist naltrexone blocked the increase in pain threshold achieved during hormone-simulated pregnancy, indicating that it is mediated via an endogenous opioid system(s), as is the analgesia of actual pregnancy. The striking similarities between the analgesia of hormone-simulated pregnancy and actual gestation strongly suggest that the profile of change in plasma E_2 and P are parameters of the pregnant condition essential to the manifestation of elevated pain thresholds.

576.10

ROLE OF OVARIAN SEX STEROIDS IN THE REGULATION OF SPINAL CORD CONTENT OF DYNORPHIN, ENKEPHALIN AND PROENKEPHALIN. V. Medina*, M. Dawson-Basoa, L. Wang and A.R. Gintzler. Dept. of Biochemistry, SUNY Health Science Center at Brooklyn, N.Y. 11203, USA.

In laboratory animals and humans, pregnancy is associated with an opioid receptor-mediated increase in the threshold for maternal responsiveness to aversive stimuli. This analgesia results, at least in part, from the activation of a spinal cord dynorphin/kappa opioid receptor system. During late pregnancy, (day 22) there is a significant elevation in the content of dynorphin (1-17 or 1-8; 34 and 48%, respectively). This increase is specific to the lumbar cord. Elevated content of dynorphin (1-17) is also observed in the lumbar spinal cord obtained from non-pregnant, ovariectomized rats following simulation of the profile of change in the concentration of plasma estrogen and progesterone that occurs during actual pregnancy. As was observed during actual gestation, elevated levels of dynorphin (1-17) were not observed in the cervical or thoracic cord during hormone-simulated pregnancy. Levels of met-enkephalin remain unchanged in the lumbar cord on gestational day 22, but are elevated in the cervical and thoracic cord (28 and 23%, respectively). The level of the enkephalin precursor, proenkephalin, is also elevated on gestational day 22 in cervical and thoracic cord (64 and 60% respectively) and, to a lesser extent, in the lumbar region (26%). These results suggest that during late pregnancy at least two spinal opioid analgesic systems are activated. Changes in circulating levels of estrogen and progesterone appear to be at least one aspect of the pregnant condition responsible for the increase in lumbar dynorphin (1-17).

576.11

ESTROGEN RAPIDLY ATTENUATES THE RESPONSE OF GUINEA PIG HYPOTHALAMIC NEURONS TO μ-OPIOIDS. A.H. Lagrange, O.K. Rönnekleiv and M.J. Kelly*. Department of Physiology, Oregon Health Sciences U., Portland, OR 97201-3098.

Neurons of the hypothalamic arcuate nucleus are hyperpolarized by μ-opioids via an inwardly-rectifying potassium conductance. Ovariectomized guinea pigs (GPs) treated with estrogen 24 hours prior to sacrifice show a rightward shift in the dose response curve to the μ-opioid agonist, DAMGO, versus oil-injected controls. There is no change in the maximal response to DAMGO or the K_e for antagonism by naloxone. Presently, we have made intracellular recordings in hypothalamic slices prepared from ovariectomized, oil-treated GPs to show a more rapid effect of 17β-estradiol (E_2). Dose-response curves to DAMGO, followed by washout of the opioid and perfusion with 200nM E_2 for 20 minutes showed the same three-fold rightward shift when a second dose response curve to DAMGO was performed ($p<0.001$, n=8). The EC_{50} shifted from 80 ± 16nM to 236 ± 67nM . This effect did not appear to be homologous desensitization by DAMGO as multiple dose-response curves, both before and after estrogen, did not show this shift. Moreover, the change in sensitivity to DAMGO was seen in slices treated with E_2, without prior exposure to exogenous μ-opioids. The effect of E_2 could be seen for up to 3 hours after washout of this steroid. Histochemical double labelling identified a subpopulation of these cells as β-endorphin neurons. These actions of E_2 imply a direct effect on the receptor-G protein-K^+ channel effector system. (Supported by PHS grants DA05158 & HD00718)

576.12

SLEEP-WAKEFULNESS AND VEGETATIVE EFFECTS MEDIATED BY OPIATES IN THE NUCLEUS OF THE SOLITARY TRACT. F. Reinoso-Barbero and I. de Andrés*. Dpto. Morfología. Fac. Medicina, Univ. Autónoma. 28029 Madrid, SPAIN.

The Nucleus of the Solitary Tract (NST) has dense concentrations of opioid receptors and is involved in the control of both cardiovascular reflexes and electrocortical activity. We evaluated the involvement of the NST opioid system in hypnogenic and vegetative functions by making single microinjections of opiate agonists in NST, and monitoring sleep-wakefulness cycle (SWC) states, as well as heart and breath rates (HR and BR). Under general anesthesia, nine cats were implanted with a cannula stereotaxically aimed at the NST, and with EEG, EOG, EMG and lateral geniculate nucleus electrodes. Microinjections of 50 nl. of saline, morphine sulphate, morphicetine (specific μ agonist), D-pen-2-D-pen-5-enkephaline (∂ agonist) and U-50488H (κ agonist) were made with a 0.5 μl Hamilton syringe introduced in the implanted cannula. The doses ranged between 0.8-2.4 nmols. Following the microinjections, simultaneous sleep-wakefulness, EKG and BR recordings were obtained. Mu agonists produced a statistically significant increase of slow wave sleep (SWS) accompanied by a decrease in HR and BR in each state of the SWC. All these effects were blocked by prior intraperitoneal administration of naloxone. The ∂-agonist also produced a hypnogenic effect, but only a general decrease of HR and BR was observed because the enhancement of SWS. The κ-agonist caused no changes in HR, BR or SWC. These results indicate that μ and ∂ opioid receptors are involved in the syncronization of the EEG generated in NST, while only μ receptors seem to participate in the control of NST vegetative responses. Kappa receptors, by contrast, seem not to be involved in the functions explored.

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576.13

INCREASED OPIATE SENSITIVITY AND DECREASED RECEPTOR -G-PROTEIN COUPLING IN THE NEONATAL RAT. R.T. Windh and C.M. Kuhn*. Dept. of Pharmacology, Duke Univ. Med. Center, Durham, NC 27710.

Despite considerable research on the outcome of perinatal opiate exposure, ontogenic changes in u receptor function and biochemistry have not been reported. The present studies were undertaken to evaluate u receptor function, number and G-protein coupling in neonatal and mature rats. Rats 10, 15, 20 and 27 days old were tested for morphine and sufentanil antinociception in the paw lift assay, and ED50s for morphine and sufentanil were determined. Contrary to previous reports, neonatal rats were more sensitive to opiate antinociception than were mature animals. Analysis of serum and brain morphine levels after 5 mg/kg morphine on days 10 and 27 indicated that morphine elimination is slightly faster in neonates than weanlings, yet both brain and serum levels are higher and remain elevated longer in neonates, largely due to a more rapid redistribution phase in weanlings (t1/2= 26 min.) than in pups (t1/2= 2.5 hrs.). Additionally, comparing brain morphine levels at equipotent analgesic doses in each age revealed that the effect was acheived in neonates at brain morphine levels that were half those in weanlings. Radioligand binding studies indicated that the number of u receptors increased 2 fold without change in affinity between days 10 and 27, and the GppNHp was nearly twice as effective in shifting the u receptors from high to low affinity on day 27 than on day 10. This weaker GppNHp regulation of agonist binding affinity apparently contradicts the enhanced antinociceptive sensitivity in neonates, and may result from a larger spare receptor population relative to G-proteins. Supported by DA 02739.

576.14

THE CAPSAICIN FLARE OF VASA NERVORUM D.W. Zochodne*, L.T. Ho, Queen's University, Kingston, Ontario Canada K7L 3N6

In previous work we identified prolonged and intense hyperemia of rat sciatic endoneurial vasa nervorum from epineurial capsaicin. In additional work, we studied further pharmacological aspects of the flare using serial hydrogen clearance in anesthetized rats. The capsaicin flare was interrupted by: removal of the epineurial vascular plexus; topical coadministration with capsaicin of spantide, spantide II (SP antagonists), or hCGRP (8-37) (CGRP antagonist); desensitization by prior capsaicin; pretreatment with combined systemic H1 and H2 histamine receptor antagonists, cromolyn sodium or morphine. Combined systemic morphine and naloxone pretreatment restored the flare. The above treatments did not alter baseline endoneurial perfusion. Interruption of central afferent connections by sectioning the nerve proximally did not influence baseline perfusion or the flare. The findings suggest that capsaicin-induced endoneurial hyperemia is local, mediated through an intact epineurial plexus and involves SP, CGRP and mast cell release of histamine. Inhibition by morphine suggests that there are opiate receptors on peptidergic fibers innervating vasa nervorum.

576.15

POSSIBLE INTERACTION OF μ-OPIOID AND α_2-ADRENOCEPTORS IN DERMORPHIN-INDUCED BRADYCARDIA IN CONSCIOUS RATS. O.M. Adeyemo and A-L. Sirén*. Dept. of Neurology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Picomole doses of the selective μ-opioid agonist dermorphin (DM) increased mean arterial pressure (MAP) and heart rate (HR) after intracerebroventricular (i.c.v.) administration by a μ_1-opioid receptor mechanism while nanomole doses of DM i.c.v. induced bradycardia which was related to activation of μ_2-opiod receptors (Paakkari P. et al., Neuropharmacol.1992). Bradycardia is also associated with stimulation of central α_2-adrenoceptors. In the present study the influence of α_2-adrenoceptors on the cardiovascular effects of DM i.c.v. was examined in conscious male Sprague-Dawley rats (250-300 g, n=30) using the α_2-agonist clonidine (CLDN) and SK&F 86466 (6-chloro-N-methyl-2,3,4,5,-tetrahydro-1-H-3-benzazepine), a selective non-imidazoline α_2-adrenoceptor antagonist (Hieble J.P. et al.,J.Pharm.Exp.Ther.236: 90-96,1986). In order to block any μ_1-opioid receptor mediated effects, naloxonazine (NLZ), a μ_1-antagonist, (50 μg/rat, i.c.v.), was administered 20 h before the interactions between DM and the α_2-adrenoceptor drugs were tested. DM (3-10 nmol/rat i.c.v.) induced a dose-dependent bradycardia in NLZ treated rats. SK&F 86466 (1 μmol/rat, i.c.v., 30 min before DM) had no effect on MAP and HR but blocked the bradycardic effect of DM (3 or 10 nmol/rat). CLDN (10 μg/rat, i.c.v., 30 before DM) had no effect on heart rate and did not modify the DM-induced bradycardia. These data suggest that α_2-adrenoceptors interact with the μ_2-opioid receptor associated bradycardia.

576.16

THE EFFECT OF MORPHINE ON QUANTAL TRANSMITTER SECRETION FROM VISUALIZED SYMPATHETIC VARICOSITIES. Nickolas A. Lavidis* Neurobiology Lab., Dep. of Physiology, University of Sydney, NSW, Australia 2006.

Morphine reduces the amount of transmitter secreted by nerve terminals during electrical stimulation. Two mechanisms have been proposed to explain this action of opiates: (a) μ-type opioid receptors increase the conductance of K^+ ions resulting in hyperpolarization of neuronal cells (Morita & North, 1982, Brain Res. 242, 145-150). (b) κ-type opioid receptors decrease the influx of extracellular Ca^{2+} ions following nerve stimulation, resulting in a decrease in the amount of transmitter secreted (Bennett & Lavidis, 1980, Br. J. Pharmac.69, 185-191; Bixby & Spitzer, 1983, Nature 301, 431-432; Gross & MacDonald, 1987, Proc. Natl. Acad. Sci. 84, 5469-5473). At the amphibian motor nerve terminal, morphine was shown to decrease the probability of quantal secretion (p) uniformly along the length of terminal branches without affecting the action potential (Lavidis 1987, Int. Cong. Pharmac. 10, P164).

The effect of morphine on quantal secretion ($\bar{m}$),p and the propogation of the action potential along visualized sympathetic nerve varicosities of the mouse vas deferens has been evaluated. $DiOC_2$-fluorescence was used to visualize the varicosities while placing an electrode over a group of varicosities to record the terminal action potential and the excitatory junctional currents (Lavidis & Bennett, 1992, J. Physiol. In Press).

Morphine (0.5 μM) produced a 51±4% decrease in $\bar{m}$ and a 8±6% decrease in the amplitude of the terminal action potential (n=8). Morphine (1μM) produced a 72±3% decrease in $\bar{m}$ and only 2 of the 9 experiments showed a significant decrease in the action potential. This action of morphine was partly reversed by naloxone (1μM) or by increasing the frequency of stimulation from 0.1 Hz to 0.5 Hz or 1.0 Hz or by increasing the extracellular calcium concentration from 4 mM to 7 mM.

The significant decrease in $\bar{m}$ produced in the presence of morphine occurred without a significant change in the size of the terminal action potential. This decrease in $\bar{m}$ was mainly due to a decrease in the probability of quantal secretion from known numbers of varicosities.

CATECHOLAMINES: NOREPINEPHRINE

577.1

MONOAMINERGIC INNERVATION OF RAT PIRIFORM CORTEX

M. Ennis*, T.A. Rizvi and M.T. Shipley Department of Anatomy & Cell Biology, University of Cincinnati, Cincinnati, OH 45267.

Piriform cortex (PC), the main target of the olfactory bulb, plays a key role in olfactory information processing. PC receives noradrenergic (NE), dopaminergic (DA) and serotonergic (5-HT) afferents. The organization of these modulatory inputs to PC has not been characterized, however. Here, we directly compare the distributions and relative densities of NE, DA and 5-HT fibers in PC using antibodies against dopamine-B-hydroxylase (NE), tyrosine hydroxylase (DA) and 5-HT.

The densities of these monoamines in PC is generally greater than their innervation of hippocampus and neocortex. As in other cortical regions, 5-HT provides the densest innervation of PC, followed by NE, then DA. These systems also exhibited laminar specificity. Layers I and III of PC contain a moderate plexus of NE fibers; layer II is sparsely innervated. The density and laminar distribution of NE fibers is relatively uniform along the rostrocaudal axis of PC. By contrast, DA innervation exhibited a marked rostrocaudal gradient. Rostrally, DA fibers are relatively sparse and confined primarily to layer III. The density of innervation systematically increases at more caudal levels and DA fibers progressively invade more superficial layers. The 5-HT innervation of PC is very heavy in layer I and the superficial half of layer III.

In light of the potent modulatory actions of NE, DA, and 5-HT in hippocampal and neocortical circuits, these transmitters likely play important roles in olfactory information processing. Like PC, the olfactory bulb receives dense and laminar specific extrinsic NE and 5-HT inputs; by contrast, DA innervation of the bulb is derived exclusively from intrinsic DA neurons. Thus, NE and 5-HT may *coordinately* regulate olfactory circuits whereas DA *differentially* modulates neural processing in PC and MOB.

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577.2

PROJECTIONS FROM THE MEDIAL PREOPTIC AREA (MPO) TO NUCLEUS LOCUS COERULEUS (LC) AND THE PERICOERULEAR REGION. T.A. Rizvi*, M. Ennis, P. Luppi, G. Aston-Jones and M.T. Shipley. University of Cincinnati, Cincinnati, OH 45267; Hahnemann University, Philadelphia., PA 19102.

LC is the sole source of noradrenergic (NE) innervation of the olfactory bulb. NE plays an important role in olfactory learning, sexual behavior and pheromonal regulation of pregnancy. However, circuits linking the olfactory system *to* LC are unknown. Here, we report a novel pathway from MPO to LC. MPO, a structure critical to sexual behavior and neuroendocrine function, receives olfactory bulb inputs via the medial amygdala.

Injections of retrograde tracers in LC and peri-LC labeled neurons in MPO. Injections of the anterograde tracer, PHA-L, into MPO densely labeled fibers and terminals in the dorsolateral pontine central gray, including the Barrington's nucleus and the dorsolateral tegmental nucleus. Fiber labeling in LC proper was more modest but there was very heavy labeling in a focal zone rostral and medial to LC (rostromedial peri-LC). Our recent studies show that this zone contains a dense plexus of LC dendrites. This suggested that MPO axons may preferentially terminate on extranuclear LC dendrites rather than LC proper. Double labeling studies showed that MPO fibers (PHA-L) are densely aggregated in the focal plexus of TOH-immunolabeled LC dendrites in rostromedial peri-LC.

Olfactory stimuli evoke significant NE release in the bulb. Projections from both the main and accessory olfactory bulbs terminate in the medial amygdala which, in turn, projects heavily to MPO. The present findings, therefore, suggest a possible di-synaptic pathway by which olfactory bulb outputs activate LC neurons, thus releasing NE in the bulb. *(Support: NIH DC00347, NS20643, NS24698, NS29218 & NS29635).*

577.3

NUCLEUS PARAGIGANTOCELLULARIS STIMULATION REDUCES LATERAL OLFACTORY TRACT EPSPS IN THE DENTATE GYRUS

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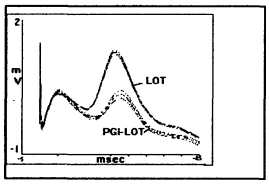
In vitro norepinephrine (NE) potentiates the dentate gyrus response to medial perforant path (PP) input, while depressing response to lateral PP input (Dahl & Sarvey, PNAS 86:4776, 1989). The present study asks whether a similar selectivity occurs with NE release via locus coeruleus (LC) activation in vivo.

Four .5 msec pulses at 333 Hz in the nucleus paragigantocellularis (PGi) were used to activate the LC input to dentate gyrus in urethane-anesthetized rats. Such PGi stimulation produces maximal potentiation of the medial PP population spike at latencies which are consistent with mediation by LC and this potentiation is blocked by propranolol as has been shown for direct LC and NE-induced potentiation.



To selectively activate the lateral PP input to dentate gyrus stimulating electrodes were placed in the lateral olfactory tract (LOT). Single LOT pulses were used to initiate field EPSPs in the dentate gyrus and were preceded by PGi pulses at intervals which would result in co-incident release of NE and lateral PP glutamate. PGi activation consistently reduced LOT EPSPs (see graph) and also reduced EPSPs evoked by stimulating lateral PP fibers directly. PGi stimulation in the same experiments potentiated the medial PP population spike. The data suggest LC-NE is a highly selective promoter of input-evoked responses in the dentate gyrus.

577.4

5-HYDROXYTRYPTAMINE NEURONS TONICALLY INHIBIT NOREPINEPHRINE NEURONS TERMINATING IN THE HYPOTHALAMUS

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The medial zona incerta (MZI) and dorsomedial nucleus of the hypothalamus (DMN), which contain cell bodies and terminals of the incertohypothalamic dopamine (DA) neurons, are innervated by norepinephrine (NE) neurons and 5-hydroxytryptamine (5-HT) neurons. The purpose of the present study was to examine the role of 5-HT neurons in the regulation of NE and DA neurons in the MZI and DMN in rats. Catecholamine neuronal activity in these brain regions was estimated by measuring the accumulation of 3,4-dihydroxyphenylalanine (DOPA) after administering a decarboxylase inhibitor and the concentrations 3,4-dihydroxyphenylacetic acid (DOPAC), indices of synthesis and metabolism, respectively. Inhibition of 5-HT neurons with the 5-HT_{1A} agonist 8-hydroxy-2-(DL-n-propylamino)tetralin (8-OH-DPAT) increased the accumulation of DOPA in the DMN and the concentration of DOPAC in the MZI and DMN, indicating an activation of either NE or DA neurons in these regions. The concentrations of 3-methoxy-4-hydroxyphenylethyleneglycol, the primary metabolite of NE, in the MZI and DMN were increased by 8-OH-DPAT or following destruction of 5-HT neurons, confirming that NE neurons in these regions were, indeed, activated following a decrease in 5-HT neuronal activity. After destruction of NE neurons projecting to the MZI and DMN, 8-OH-DPAT no longer increased DOPAC concentrations in these brain regions, indicating the lack of a direct effect of this drug on incertohypothalamic DA neurons. Taken together, these results reveal that 5-HT neurons tonically inhibit NE neurons terminating in the MZI and DMN, but not the incertohypothalamic DA neurons located in these regions. (Supported by NIH grant NS15911)

577.5

EFFECT OF LOCUS COERULEUS (LC) LESION ON C-FOS RESPONSE TO METRAZOL (PTZ) IN RAT BRAIN

Y. Zhang*, G. Bing, D. Filer and E.A. Stone. Dept. Psychiatry, New York Univ. Sch. Med., New York, NY 10016

The convulsant PTZ has been shown to greatly induce c-fos in the rat CNS. We and others have shown that norepineprine (NE) which has antiseizure activity and may be released during seizures also can induce brain c-fos. The present study was undertaken to determine if part of the c-fos response to PTZ is the result of the activation of the LC noradrenergic system. Rats were given unilateral lesions in the LC with 6-OHDA. After 7 days the animals were convulsed with an i.p. dose of PTZ (50 mg/kg) and after 2 hrs anesthetized and perfused for immunohistochemistry of c-fos and tyrosine hydroxylase (TH). Completeness of LC lesion was verified by TH immunohistochemistry. It was found, in agreement with previous research, that PTZ greatly increased c-fos immunoreactivity in various telencephalic structures on the side of the brain with the intact LC. The LC lesioned side showed a significant attenuation of the response to PTZ in the frontal, parietal and piriform cortices. These results support the notion that part of the c-fos response to PTZ is a result of noradrenergic activation as an apparent compensatory antiseizure response. Supported in part by grants AFOSR 89-0208, MH45265 and MH08618.

577.6

PROTECTIVE ACTION OF LOCUS COERULEUS NORADRENERGIC SYSTEM ON SUBSTANTIA NIGRA (SN) OF MICE TREATED WITH MPTP.

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In addition to its immediate neurophysiological functions, the LC noradrenergic system has been hypothesized to have trophic effects in the CNS (Behav. Brain Sci. 6:535,1983). Recently, in support of this, it has been found that LC lesions in the monkey greatly retard the recovery of SN lesions caused by the neurotoxin MPTP (Neurosci. 41:507,1991). Because of the potential clinical significance of these findings to Parkinsons's disease (PD) we have attempted to replicate them in the mouse MPTP model. Mice were given unilateral LC lesions with 6-OHDA and after a 10 day period challenged with MPTP at a dose known to be subtoxic to SN dopamine (DA) containing neurons. After an interval of 7 days the animals were sacrificed and immunohistochemical studies of tyrosine hydroxylase (TH) and Nissl staining of the SN and LC were undertaken. It was found that the LC lesion dramatically enhanced the toxic effect of MPTP as reflected in a marked reduction in the number of TH positive cells in the ipsilateral SN after subtoxic MPTP. Nissl staining studies confirmed the loss of neurons in the ipsilateral SN. DA levels in the striatum are currently under investigation. The results support the notion that the LC has marked trophic actions on the SN and may be involved in the etiology of PD. Supported in part by grants AFOSR 89-0208, MH45265 and MH08618.

577.7

SEXUALLY DIMORPHIC AND AGE-RELATED DIFFERENCES IN THE IMPACT OF A STRESSOR ON HYPOTHALAMIC NOREPINEPHRINE TURNOVER

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Previous studies have indicated an age-related influence on hypothalamic norepinephrine (NE) turnover in male rats. The decrease in NE levels following synthesis inhibition followed a markedly different pattern at 42 days of age (midpuberty) than at either 28 (juvenile) or 70 (young adult) days (Neurosci. Abstracts, 17, 415; 1991). Whereas at 28 and 70 days, NE levels decreased to reach 50% of basal values by 4 hours after synthesis inhibition, at 42 days, NE levels had decreased this amount by 60 min after inhibition. Moreover, in adult rats, exposure to a stressor (2 hours restraint) accelerated the loss of NE after synthesis inhibition. This present study investigated the impact of a stressor on hypothalamic NE neurons as a function of pubertal development and gender. Male and female rats at 28, 42, and 70 days of age were given alpha-methyl-p-tyrosine (MT; 250mg/kg) immediately before being placed in an acrylic restraint chamber for either 45 or 60 minutes. For males at all ages 45 min of restraint retarded the NE decrease in the presence of MT. NE levels increased over basal values, even in the presence of the inhibitor. At 28 and 70 days of age, hypothalamic NE levels in males began to decrease following 60 minutes of restraint plus MT. However, at 42 days NE levels continued to increase after 60 min of restraint plus MT. In contrast to males, hypothalamic NE levels in females of all ages decreased over time following restraint and synthesis inhibition with MT. This may suggest that in the hypothalamus there is a sexually dimorphic and age-related difference in kinetic aspects of the tyrosine-hydroxylase enzyme in response to stressors. Grant No DA07080.

577.8

PRAZOSIN ENHANCES *IN VIVO* NOREPINEPHRINE SYNTHESIS IN YOHIMBINE-TREATED RAT HIPPOCAMPUS AND LOCUS CERULEUS.

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The adrenergic pharmacology of norepinephrine (NE) synthesis was evaluated in rat hippocampus (HP) and locus ceruleus (LC, contained within dorsal pons-medulla) *ex vivo* by accumulation of DOPA in adult male rats given a decarboxylase inhibitor (NSD-1015, 100 mg/kg, ip, 5 min post-drug). Test agents were injected (ip) at 35-40 min prior to decapitation and brain dissection. DOPA content was assayed by HPLC with electrochemical detection. As expected, yohimbine (YOH) which blocks α_2-receptors and may directly stimulate NE release dose-dependently increased DOPA accumulation in HP and LC. The potent α_1- (and weak α_{2B}) receptor antagonist, prazosin (PRZ), increased NE synthesis only slightly, even at high doses. Low, ineffective doses of PRZ plus a maximally effective dose of YOH, however, enhanced DOPA accumulation significantly above levels obtained with YOH alone, indicating potentiation. In contrast, chlorpromazine, which has similar α-receptor affinities as PRZ, had no effect alone and failed to enhance YOH's effects, suggesting that α-receptor activity may not explain PRZ's effects, the basis of which remains unclear. [Supported by NIMH grants 14275, 31154, 36004, 47370 and Deutsche Forschungsgemeinschaft.]

577.9

COMPARISON OF AIMAX AND DIETHYLDITHIOCARBAMATE (DDC) ON RAT HYPOTHALAMIC MONOAMINE LEVELS. W-J. Chang[1], C.R. Barb[2], L.S. Leshin*[2], G.B. Rampacek[1], B. Johnson[2], R.R. Kraeling[2] and J.T. Wright[2]
[1]University of Georgia, Athens 30602 and [2]USDA-ARS, Athens, GA 30613.

DDC reduces brain norepinephrine (NE) content and subsequent LH secretion in the rat. AIMAX, also a carbamate compound, suppresses LH secretion. However, the mechanism of AIMAX action is unknown. Concentrations of monoamines in the hypothalamus were compared after DDC or AIMAX in ovariectomized rats primed with an injection of 50 μg estradiol benzoate followed 48 h later by 2.5 mg progesterone (d 0) sc. Treatments were: 1) injection sc of 650 mg DDC/kg BW (n=5) on d 0; 2) daily injection of 20 mg AIMAX/kg BW sc (n=6) from d-7 to d 0; 3) or saline (C; n=6). Injections were given between 1000 and 1030 h. Rats were decapitated on d 0 between 1530 and 1630 h. Monoamine content within the medial basal hypothalamus (MBH) and retrochiasmatic area (RCA) were determined. AIMAX reduced ($P<0.05$) NE and increased ($P<0.05$) dopamine (DA) content in MBH and RCA compared to C rats. Similar patterns in NE and DA levels were observed in DDC rats. However, DDC elevated epinephrine (EPI) content in MBH and RCA compared to AIMAX rats. Therefore, like DDC, AIMAX is an apparent NE synthesis inhibitor.

577.10

MONOAMINE LEVELS IN A RAT BRAIN SUBJECT TO A STATIC MAGNETIC FIELD. K.Hyodo* and K.Homma. Biomechanics Div., Mech. Eng. Lab., Agency of Industrial Science and Technology., Tsukuba, 305JAPAN

To study the rapid influence which a static magnetic field has upon brain metabolism by means of in vivo microdialysis , dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid(HVA), 5-hydroxyindoleacetic acid(5-HIAA) and norepinephrine(NE) in a rat caudate putamen were monitored during the application of a 250 gauss static magnetic field, applied to a whole body by a 2 tesla(T) MRI superconductive magnet. A 28 week old male Wister rat (n=7) was placed in a black cloth-covered free moving unit while monitoring. After 2 hours of monitoring, in 15 minute intervals, during the application of the magnetic field, the changes in monoamine levels were not significant when compared to normal changes in the absence of the field. By using a static magnetic field of 250 gauss, rapid influence was not determined by changes in monoamine levels in the rat caudate putamen. In response to the report that the B.B. barrier function is altered by a magnetic field, the change may not have been rapid or it may not have had a direct influence upon the monoamine levels in caudate putamen. If a magnetic field dose have an influence on the brain metabolism, not only for monoamine levels in the caudate putamen but also other metabolisms, it may appear after long time monitoring or in the presence of a stronger magnetic field.

577.11

EFFECTS OF S-2(3-METHYLAMINOPROPYLAMINO) ETHYLPHOSPHOROTHIOIC ACID (WR-3689) ON THE CONTENT OF CATECHOLAMINES IN MOUSE ADRENALS. D.L. Palazzolo*, W.A. McLean and K. Sree Kumar, Radiation Biochemistry Department, AFRRI, Bethesda, MD 20889.

Simultaneous administration of caffeine and WR-3689 mitigates impairment of locomotor activity in mice treated with WR-3689 alone. This combination of drugs also decreases the caffeine-induced increase of catecholamines in mouse hypothalamus. In the current studies, we determined the effect of *i.p.* injections of WR-3689 on the catecholamine content of the adrenals. Mice were treated with saline (control) or WR-3689 (100 or 200 mg/kg), adrenals removed at 0, 1, 2, 4, and 8 hrs after injections and dopamine (DA), norepinephrine (NE), and epinephrine (EPI) determined using HPLC. Treatment with 100 mg/kg WR-3689 had no effect on DA; 200 mg/kg WR-3689 increased ($p<0.05$) DA content from a control value of 29±5 to 37±5 ng/mg wet weight 1 hr after treatment. With both 100 and 200 mg/kg WR-3689, NE content decreased ($p<0.02$) from 664±50 to 396±59 and 437±80 ng/mg, respectively, 4 hrs after treatment. EPI content decreased ($p<0.04$) from 1178±179 to 852±122 ng/mg 4 hr after treatment with 100 mg/kg WR-3689, but increased ($p<0.03$) from 1315±149 to 1784±179 ng/mg 1 hr after treatment with 200 mg/kg WR-3689. These results indicate that WR-3689 alters catecholamine metabolism.

577.12

INTRACEREBROVENTRICULAR INJECTION OF ANGIOTENSIN II INCREASES PLASMA NOREPINEPHRINE LEVELS.

B Kimura, M I Phillips* and C Sumners. Dept. of Physiology, University of Florida, Gainesville, FL 32610.

Centrally mediated effects of Angiotensin II (AII) include increases in blood pressure, drinking and release of vasopressin (AVP). The increase in blood pressure has been postulated to occur both through the increase in AVP and an increase in peripheral sympathetic activation. We investigated the effect of intracerebroventricular (i.c.v.) injections of AII on the release of peripheral norepinephrine (NE) and epinephrine (E) in Sprague-Dawley male rats. NE and E were measured by radioenzymatic assay. To determine whether release of NE from the adrenal played a role in the response NE in plasma was measured after adrenalectomy. The result shows that i.c.v. injections of AII (50 ng - 500 ng in 2 μl) caused a significant increase in plasma NE levels. The highest dose of AII (500 ng) also caused an increase in E levels. Adrenalectomy lowered E concentrations to below the detection limit of the assay. The AII-stimulated increase in NE levels persisted in the adrenalectomized rats, and at a dose of 250 ng AII i.c.v. the effect was not significantly different from intact animals. However at 500 ng AII i.c.v. this response was significantly attenuated. The results confirm that central AII releases NE and E peripherally. The source of E appears to be by stimulation of the adrenal gland, but high doses of AII are required. The source of NE is the peripheral sympathetic nervous system at low doses and the adrenal medulla at the high dose.

577.13

THE EFFECTS OF CHRONIC OPIATE TREATMENT ON [^{3}H]NOREPINEPHRINE UPTAKE PROPERTIES OF NORADRENERGIC LOCUS COERULEUS NEURONS IN VITRO. H.K. Raymon* and F.M. Leslie, Dept. of Pharmacology, University of California, Irvine, CA. 92717.

Endogenous opioids have been implicated in the control of developmental events, such as cell proliferation and differentiation. The cell types that are under opioid control during development have not been clearly identified. The purpose of this study was to determine whether the maturation of noradrenergic locus coeruleus (LC) neurons in vitro could be influenced by chronic opiate treatment. LC cells were obtained from the rostral rhombencephalon of rats at embryonic day 14. Cells were dissociated and plated in either a serum-containing or serum-free, fully defined medium. [^{3}H]Norepinephrine (NE) uptake was used as a marker for the growth of noradrenergic cells in culture. LC cells treated chronically with fentanyl citrate for 4 days in serum-containing medium showed a significant decrease in [^{3}H]NE accumulation. The dose-response curve was U-shaped, with a maximal effect at 10 nM and a reduced effect at the highest dose (1 μM). Fentanyl inhibition of [^{3}H]NE uptake was reversed by the opiate antagonist, naloxone. Kinetic constants derived from uptake velocity curves for control and drug-treated cultures were, K_m = 206 nM and V_{max} = 65.3 fmol/min and K_m =302 nM and V_{max} = 67.4 fmol/min, respectively. This indicates a change in the affinity of the NE transporter with chronic opiate treatment. No significant differences in uptake were found in fentanyl-treated cultures grown in defined medium. The lack of effect in defined medium may be due to the development of tolerance. Experiments utilizing opioid inhibition of [^{3}H]NE release as an index for functional opioid receptor activity in serum-containing and defined medium are currently being undertaken to determine whether tolerance develops after chronic fentanyl treatment. Overall these data suggest that chronic opiate treatment influences monoamine transporter function in developing LC neurons. *Supported by NIH grants NS19319 and MH09737.*

578.1

INFLUENCE OF PROTEIN SYNTHESIS INHIBITORS ON THE NICOTINIC-MEDIATED INCREASE IN TYROSINE HYDROXYLASE (TH) GENE EXPRESSION IN BOVINE ADRENAL CHROMAFFIN CELLS. G.L. Craviso[1]*, R.W. Moore[1], V.B. Hemelt[2] and J.C. Waymire[2]. [1]Dept. Pharmacology, Univ. Nevada Sch. Med., Reno, NV 89557 and [2]Dept. Neurobiology and Anatomy, Univ. Texas Med. Sch., Houston, TX 77225.

In isolated bovine adrenal chromaffin cells, the selective nicotinic agonist dimethylphenylpiperazinium (DMPP; 1 μM) produces a rapid calcium-dependent pulse of increased TH gene transcription (2 to 3-fold within 30 min, with a return to basal levels by 2 hr), followed by a 2-fold rise in TH mRNA levels by 8-18 hr. The requirement for protein synthesis in this regulation of TH gene expression was examined by treating cells with either cycloheximide (5 μM), puromycin (100 μM) or anisomycin (100 μM) prior to addition of the agonist, and analyzing both TH gene transcription and TH mRNA levels. Basal TH transcription rates were either unaffected or slightly decreased by a 2 hr exposure to the protein synthesis inhibitors; longer exposure (8 hr) significantly reduced transcription. None of the inhibitors blocked the increase in transcription elicited by DMPP. Furthermore, the rapid downregulation of the transcriptional response was also not affected. This contrasts with the superinduction of *c-fos* transcription by DMPP in the presence of these inhibitors. Despite its inhibitory effect on basal transcription, cycloheximide elevated TH mRNA levels 2-fold, measured at 12 or 24 hr. This increase was not always additive with the rise in TH mRNA levels produced by DMPP. Our results suggest that protein factors may be involved in regulating basal TH transcription rates but not the increase produced by DMPP. The rise in TH mRNA levels by cycloheximide could be due to increased TH mRNA stability whereas the apparent blockade of the effects of DMPP on TH mRNA by this inhibitor may indicate a role for protein synthesis in pre-TH mRNA processing. USPH NS27550.

578.2

VIP, SECRETIN, AND PACAP ACT AT DISTINCT RECEPTORS TO INCREASE TYROSINE HYDROXYLASE GENE EXPRESSION IN PC12 CELLS. R. Basiboina, C. Hale, J.W. Haycock and R. Strong*. Departments of Pharmacological and Physiological Science and Internal Medicine, St. Louis University School of Medicine, and GRECC, St. Louis VA Medical Center, St. Louis, MO, 63125 and Dept. of Biochem. and Mol. Biol., Louisiana State Univ. Med. Ctr., New Orleans, LA.

As the rate limiting enzyme in catecholamine biosynthesis, tyrosine hydroxylase (TH) is important in determining sympathetic tone. Vasoactive intestinal polypeptide (VIP) has been established as a neurotransmitter within the splanchnic nerve which innervates the adrenal medulla (Wakade,89). We have reported that both VIP and secretin, homologous peptides within the glucagon family, increase TH gene expression in PC12 cells, through a cyclic AMP-dependent mechanism. Recently, PC12 cells have been shown to possess receptors for pituitary adenylate cyclase activating polypeptide (PACAP), a peptide that has 68% homology to VIP in the first 27 amino acids. Moreover, PACAP is several orders of magnitude more potent than VIP in eliciting increases in cAMP. We undertook a series of studies using VIP and secretin antagonists to determine whether VIP, secretin, and PACAP induce changes in TH gene expression through the same receptor.

Experiments were performed in PC12 cells, a rat chromaffin cell line. Preconfluent cells were incubated 6 hours with various concentrations of hormone. Following total RNA extraction, Northern and slot blots were performed using a full length TH.36 cDNA probe. We found that the order of potency for the compounds on TH gene expression was PACAP > secretin > VIP. The secretin antagonist (secretin [5-27]) reduced the effects of secretin but not VIP or PACAP. The VIP antagonist (VIP [10-28] inhibited the effects of VIP but not PACAP or secretin. Neither receptor antagonist had an effect by itself. These studies suggest that VIP, secretin and PACAP work through distinct receptors to increases TH gene expression.

578.3

ACUTE COLD STRESS LEADS TO AN INCREASE IN TYROSINE HYDROXYLASE GENE TRANSCRIPTION IN RAT ADRENAL MEDULLA MEASURED BY INTRONIC *IN SITU* HYBRIDIZATION. Maureen K. Hahn* and Thomas G. Sherman. Department of Cellular and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260-7700.

Tyrosine hydroxylase (TH), the rate-limiting enzyme that converts tyrosine to DOPA in the catecholamine biosynthetic pathway, is modulated in the rat adrenal gland by stress. Both the enzymatic activity of and mRNA levels for TH increase in response to various stressors. Because cold stress produces an increase in TH mRNA and another stimulus, nicotine, induces TH gene transcription, as measured by nuclear run-on assay, we sought to determine the extent to which acute cold stress itself will increase TH gene transcription using the novel method of intronic *in situ* hybridization. We have shown previously, in a different system, that measures of vasopressin gene transcription by intronic *in situ* hybridization yield changes that are quantitatively similar to those determined by nuclear run-on assay. Male Sprague Dawley rats were shaved, exposed to 5°C for 3 hr, decapitated and the adrenal glands removed. This stress paradigm is sufficient to cause an increase in mRNA for adrenal TH as measured by Northern and RNase protection assays. *In situ* hybridization was performed on post-fixed tissues at 54°C using either a 509 nucleotide TH cDNA riboprobe or a 365 nucleotide riboprobe coding for intron 2 of the TH gene. Using the riboprobe for TH intronic sequences, 3 hr of exposure to cold stress produces a 3-fold increase in medullary grain density. The signal for TH intron that appears in the adrenal glands from cold-stressed animals is virtually absent in control animals. This occurs in conjunction with a 61% ($p<.03$) increase in cytoplasmic TH mRNA as measured by *in situ* hybridization with the riboprobe coding for TH exonic sequences. These results indicate that the increase in TH mRNA seen in response to acute cold stress in the rat adrenal medulla is due to transcriptional activation of the TH gene. Supported by grant MH29670-14.

578.4

TRANSCRIPTIONAL AND POSTTRANSCRIPTIONAL CONTROL OF TYROSINE HYDROXYLASE (TH) GENE EXPRESSION BY OXYGEN IN PC12 CELLS. M.F. Czyzyk-Krzeska*, B.A. Furnari, E.E. Lawson and D.E. Millhorn. University of North Carolina, Chapel Hill, N.C.

We reported recently that TH mRNA is substantially enhanced in carotid body O_2 sensitive (type I) cells by acute (1-48hr) exposure to low oxygen (J.Neurochem 58: 1992). Carotid body type I cells closely resemble pheochromocytoma (PC12) cells with regards to their catecholamine phenotype, morphology and embryonic origin. The present study was performed to determine if TH gene expression is modulated in PC12 cells by low O_2 in a manner similar to that measured in type I cells We report here that TH mRNA is enhanced (3-4 fold) by acute exposure (1-48 hr) to low O_2 (5%). Experiments were performed to identify the mechanism by which TH gene is regulated by O_2 in PC12 cells. Findings from nuclear runoff assays revealed a 2-3 fold increase in nascent transcripts in response to exposures to low O_2 (1-48 hr). To identify the 5' flanking region of the TH gene that is responsible for transcriptional regulation during hypoxia, CAT assays were performed using different TH promoter constructs. We found that promoter region of TH gene that extends from -272 to +27 mediates the increased transcriptional activity associated with hypoxia. When constructs that contained sequence more upstream, the increase in transcription was repressed. This finding implies that transcription of TH gene during hypoxia is regulated by an interaction of enhancer and silencer factors. To determine if TH gene expression during hypoxia is also regulated posttranscriptionally, PC12 cells were pretreated with α-amanitin to block transcription, exposed to low O_2 (1-24 hr), and TH mRNA quantitated by northern blot analysis. We measured a substantial increase (3-5 fold) in TH mRNA over control (cells not exposed to low O_2) at all time points. This finding indicates that TH mRNA is stabalized during low O_2. Thus, the TH gene in PC12 is regulated by a dual mechanism involving both transcription and translation.

578.5

PARALLEL UPREGULATION OF CATECHOLAMINE SYNTHESIZING GENES IN ADRENOMEDULLARY TUMOR CELLS *IN VIVO*: OVEREXPRESSION OF TH, AADC, AND DBH BUT SUPPRESSION OF PNMT. T.C. Wessel*, K.S. Kim, H. Baker and T.H. Joh. Cornell Univ. Med. College at the Burke Medical Research Institute, White Plains, NY 10605.

Adrenomedullary cells are segregated into two distinct populations: the majority of medullary cells express all four catecholamine synthesizing enzymes and have the capacity to produce epinephrine, while a minority lack PNMT and produce norepinephrine. This second group of medullary PMNT-negative cells contains higher mRNA levels for TH, AADC, and DBH. Interestingly, catecholamine-secreting tumors of the adrenal medulla are frequently found in rats >12 months of age. *In situ* hybridization and immunohistochemical analysis of these tumors revealed that the mRNA and protein levels for TH, AADC and DBH are markedly increased, while PNMT expression is suppressed. In addition, TH enzyme activity in adrenal glands with visible tumors is increased >10-fold over age-matched controls. To delineate the mechanisms underlying the differential regulation of these enzyme genes, we examined the expression of several trans-acting factors known to be involved in catecholamine regulation. Message levels for CREB, c-fos, c-jun and glucocorticoid type 2 receptor examined on adjacent tissue sections as well as immunochemical staining for CREB and Fos protein revealed no differences between normal medullary and tumor tissue. These data suggest that increased enzyme gene expression is not correlated with altered steady-state levels of these trans-acting factors.

578.6

STUDIES ON THE COORDINATE REGULATION OF GENES ENCODING TYROSINE HYDROXYLASE AND TETRAHYDROBIOPTERIN (BH_4) BIOSYNTHETIC ENZYMES IN CULTURED PC12 CELLS. P.Z. Anastasiadis* and R.A. Levine. Lab of Molecular Neurobiology, Lafayette Clinic, and Cellular and Clinical Neurobiology Program, Wayne State University, Detroit, MI, 48207.

Tetrahydrobiopterin (BH_4) is the naturally occurring cofactor for tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH), which are the initial and rate-limiting enzymes in catecholamine (CA) and serotonin (5-HT) synthesis, respectively. Genetic deficiencies in BH_4 biosynthesis occur in newborns (atypical PKU), and altered BH_4- and CA-related gene expression may be involved in Parkinson's and Alzheimer's disease, familial dystonia, and depression. Elevation of cellular BH_4 levels increases TH and TPH activities, whereas inhibition of BH_4 synthesis causes CA and 5-HT deficits. Therefore, cultured pheochromocytoma (PC12) cells are being used to study BH_4 and catecholamine metabolic interactions with emphasis on the coordinate regulation of TH and BH_4-related gene expression. Incubation of PC12 cells with exogenous BH_4 caused a marked rise in intracellular BH_4 levels and a stimulation of dopamine (DA) synthesis. Inhibition of GTP cyclohydrolase and sepiapterin reductase, the initial and final BH_4 biosynthetic enzymes, by 2,4-diamino-6-hydroxypyridine (DAHP) and N-acetylserotonin, respectively, significantly reduced BH_4 levels and DA synthesis. Conversely, incubation of PC12 cells in the presence of nerve growth factor (NGF) for 24 hours significantly elevated BH_4 levels (180% of control). Using these and other pharmacological manipulations of BH_4 and CA metabolism, the hypothesis that the expression of TH, GTP cyclohydrolase, and sepiapterin reductase genes are coordinately regulated is being investigated by measuring mRNAs, enzyme content, enzyme activity, and end products of BH_4 and CA biosynthesis.

578.7

ELECTROCONVULSIVE SHOCK INCREASES TYROSINE HYDROXYLASE AND NEUROPEPTIDE Y MESSENGER RNA IN THE LOCUS COERULEUS.

S. Kapur*, M.C. Austin, M.D. Underwood, V. Arango and J.J. Mann . Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

Electroconvulsive shock therapy (ECT) is an effective antidepressant treatment for certain cases of depression that are not responsive to pharmacologic treatments. Elucidation of the mechanism of antidepressant action of ECT may permit development of more effective drugs. Multiple electroconvulsive shock (ECS) enhances noradrenergic activity by an increase in norepinephrine (NE) levels and tyrosine hydroxylase (TH) activity in the rat cortex and brainstem, respectively. A recent study found that multiple ECS increases TH gene expression in the rat LC suggesting an effect on gene expression may contribute to increased TH activity. ECS effects on neuropeptide Y (NPY), which coexists with NE in perikarya of the rat LC, are unknown. Using *in situ* hybridization, we sought to determine whether multiple ECS treatments regulate NPY gene expression and to determine the anatomical specificity of TH gene regulation by ECS. Male Sprague-Dawley rats were divided into three groups: a) ECS group received isoflurane anesthesia followed by 120 volts for 1s delivered through ear clips; b) sham group received anesthesia followed by attachment of ear clips with no electricity; c) control group received no anesthesia or ECS-related manipulation. Treatments were administered daily for 10 days. Rats were sacrificed 24h after the last treatment and coronal tissue sections of the LC and substantia nigra (SN) were processed for *in situ* hybridization. Multiple ECS produced an 80% increase in TH mRNA and a 32% increase in NPY mRNA in the LC ($p < 0.05$). No change in TH mRNA was observed in the pars reticulata or pars compacta of the SN of rats that received ECS. These results indicate that ECS selectively increases TH mRNA concentrations in the noradrenergic LC and, in addition, demonstrates that gene expression for the coexisting peptide, NPY, can be similarly regulated by ECS. (Supported by MH40695, MH46745)

578.8

LOCUS COERULEUS LESION REDUCES TYROSINE HYDROXYLASE IMMUNOREACTIVITY AND mRNA LEVELS IN RAT SUBSTANTIA NIGRA. Y. Watanabe*, G. Bing, Y. Zhang, B.S. McEwen and E.A. Stone Lab. of Neuroendocrinology, the Rockefeller University and Department of Psychiatry, NYU Medical Center, NY, NY.

Recent work has suggested that the noradrenergic (NA) system is involved in long term adaptive responses of the central nervous system to stress. It has also been found that the dopaminergic (DA) system may play an important role in the stress response of the CNS. A number of studies have shown the interrelations between these two systems. Since NA projections from the locus coeruleus (LC) to the substantia nigra (SN) are predominantly unilateral, the present study was designed to futher elucidate the NA-DA relationship by assessing the effects of unilateral lesions of the LC on DA containing neurons in the SN. Unilateral LC lesions were produced stereotaxically by injection of 2 μl (2μg/μl) 6-OHDA into the right LC. Seven days after the lesion, the animals were injected with yohimbine (5mg/kg) to release NA from non-lesioned terminals, so as to accentuate the difference in NA function between the two sides. Animals were sacrificed after 2 hours. Immunohistochemical assay using a monoclonal antibody against tyrosine hydroxylase (TH) revealed marked reduction in immunoreactivity in the SN on the side of LC lesion compared to the intact side. In situ hybridization using oligonucleotide probes from TH cDNA also demonstrated a significant reduction of TH mRNA in the ipsilateral SN. These reductions were especially dramatic in the anterior portion of the SN pars compacta. Thus, the NA system may exert a supportive and facilitative effect on DA substantia nigra neurons. Supported in part by: AFOSR 89-0208,MH45265,MH41256.

578.9

EFFECTS OF PREFRONTAL CORTICAL LESIONS ON TYROSINE HYDROXYLASE GENE EXPRESSION IN MIDBRAIN DOPAMINE NEURONS. H. E. Nye and A. Y. Deutch*. Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06508, and VA Medical Center, West Haven, CT 06516.

Retrograde tracer studies have demonstrated innervation of the ventral tegmental area (VTA) from a variety of sites, including the medial prefrontal cortex (PFC). In particular, the infralimbic (IL) and ventral prelimbic (PL) parts of the PFC project to the VTA. Both of these PFC areas increase Fos expression in response to stress, while other afferents to the VTA do not. Since stress increases dopamine (DA) metabolism and release in the PFC and VTA, and since ibotenic acid lesions of the PFC prevent the stress-induced increase in DA release in subcortical sites, similar lesions may decrease functional activity of certain midbrain DA neurons and may alter tyrosine hydroxylase (TH) gene expression. We therefore examined the effects of PFC lesions on TH mRNA in the VTA. The IL and PL were lesioned by microinjection of NMDA; NMDA lesions of the parietal cortex and sham-operated animals served as two types of controls. Animals survived for either 5-6 or 21-22 days before being sacrificed. TH mRNA was determined by Northern blots.

Lesions of the PFC involved the IL and ventral PL in all cases; in some cases lesions extended dorsally. Lesions of the PFC did not appear to alter TH mRNA in the total VTA (21 days survival). *In situ* hybridization studies are in progress to determine if PFC lesions alter TH mRNA in a restricted portion of the VTA, i.e., in those cells projecting to the PFC. Supported by MH-45124, TG GM-07324, and the VA National Centers for Schizophrenia and PTSD at the West Haven VA Medical Center.

578.10

EFFECT OF STRIATAL CELLS ON TYROSINE HYDROXYLASE GENE EXPRESSION IN CULTURED MESENCEPHALIC DOPAMINE NEURONS. Y. Solberg*, Y. Pollack and W.F. Silverman. Units of Morphology and Microbiology & Immunology, Corob Center for Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel.

Our previous work has shown a temporal correlation between establishment of dopaminergic mesostriatal connections and an increase in the expression of the tyrosine hydroxylase (TH) gene in selected portions of the substantia nigra (SN). We have prepared cultures of embyonic mesencephalon as well as co-cultures with embryonic neostriatal cells to test whether the presence of the striatal cells is required to produce this effect on TH gene expression. Mesencephalic and striatal cells were obtained by microdissection from E15 stage rat embryos and were cultured as pure mesencephalic cells or together with the striatal cells. Processes were first observed at about 48 hours extending from putative neurons within the cultures, and continued to increase with time, most dramatically in the co-cultures. *In situ* hybridization using a ^{35}S-labeled RNA probe complementary to TH mRNA was performed on 2, and 6 day old cultures. The amount of signal in selected, labeled neurons was quantified as autoradiographic grains per cell area. No difference was observed in TH gene expression in the pure SN cultures between 2 and 6 days. Cells from 2 day old co-cultures exhibited a higher amount of TH mRNA than their pure SN cultured counterparts, while at 6 days, this difference was yet more pronounced. These data support a role for the striatum on TH gene expression in mesencephalic dopamine neurons which project to it. Supported by Israel Academy of Science and Humanities Grant ## 285-90.

578.11

FOUR TYPES OF TYROSINE HYDROXYLASE mRNA TRANSCRIPTS ARE EXPRESSED IN ADULT AND IN FETAL MONKEY BRAINS OF DIFFERENT DEVELOPMENTAL STAGES. S.-Y. Tam*, J.D. Elsworth, D.E. Redmond, Jr., S.J. Galli, and R.H. Roth. Depts. of Pathology, Beth Israel Hospital and Harvard Med. Sch., Boston, MA 02215 and Depts. of Pharmacology and Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06510.

Tyrosine hydroxylase (TH) is encoded by a single gene in humans, but four distinct mRNA transcripts are generated by alternative splicing from a single transcript. In monkeys, two types of TH mRNA and protein isoforms have been reported to exist in the brain. As part of our effort to study the development of mesencephalic dopamine (DA) neurons in the primate, we have examined the expression of TH mRNA transcripts in brains of embryonic, fetal and adult African green monkeys. Total RNAs extracted from adult monkey tissues and fetal brains of 42, 59, 81, 91, and 150 days after gestation were reverse transcribed and the cDNAs were further amplified by PCR techniques using a pair of primers based on human TH sequences. In adult monkeys, four distinct PCR fragments corresponding to each of the four types of TH transcripts were detected in adrenal medulla and in midbrain regions such as the substantia nigra and the ventral tegmental area. The observed sizes of these PCR products were identical to those predicted for different human TH mRNA subtypes. DNA sequencing of these PCR fragments is in progress. In developing fetal monkey brains, TH transcripts were barely detectable at 42 and 59 days, but at 81, 91, and 150 days after gestation, four types of TH mRNA species were detected in the midbrain regions. In contrast to previous findings, these results indicate a lack of divergence in TH mRNA splicing pattern among primates, and that in the monkey, four types of TH mRNA transcripts are present in brains of both the adult and the developing fetus. Experiments are in progress to study the expression of some of the neurotrophic factors and their receptors whose actions may influence the development of the mesencephalic DA neurons. Supported by AI-22674, AI-23990, MH-14092 and the Axion Research Foundation.

578.12

MULTIPLE ISOFORMS OF TYROSINE HYDROXYLASE ARE SPECIFIC TO PRIMATES, BUT ONLY HUMANS HAVE FOUR ISOFORMS. John W. Haycock*. Dept. Biochem. & Molec. Biol., Louis. State Univ. Medical Center, New Orleans, LA 70119

Tyrosine hydroxylase (TH) is encoded by a single gene in all species studied to date. In subprimates, TH is synthesized from a single form of mRNATH. In humans, however, alternative splicing results in four mRNATH species--recently shown to be translated *in vivo* by the identification of all four TH protein isoforms (Types 1-4) in human adrenal medulla using isoform-specific anti-peptide antibodies. These antibodies have been used here in Western blots of mammalian adrenal samples to determine the evolutionary appearance and development of multiple TH isoforms.

Antibodies to Type 1 TH (-EAIMS^{31}PRF-) labeled a ~62 kDa band in all mammalian species tested except mouse, rat and hamster. Rodents have a slightly different amino acid sequence (-EAVTS^{31}PRF-) although, notably, guinea pig TH reacted with the Type 1 antibodies. The ~62-64 kDa Type 2 band was not detected in subprimates. Among primate species, Type 2 was present in members of Anthropoidea (humans, great apes, Old World and New World monkeys) but not Prosimii (5 species from 3 families). By contrast, Types 3 and 4 TH (66 kDa) were detected only in humans--both in adrenal medulla and brain. Neither a sensitive pan-specific antibody nor the isoform-specific antibodies detected the presence of these higher M, isoforms even after partial purification of monkey TH. Thus, one splicing variant (Type 2) arises with, and is expressed throughout, Anthropoidea whereas the additional splicing event that produces Types 3 and 4 occurs uniquely in humans.

578.13

GENERATION OF STATE-SPECIFIC ANTIBODIES TO A SEGMENT OF TYROSINE HYDROXYLASE (TH). J.Y. Lew, K.Y. Lee, M. Goldstein* and A. Y.Deutch. Neurochem. Res. Lab., N.Y. Univ. Med. Ctr., N.Y., N.Y. 10016 and Dept. Psychiatry, Yale Univ. Med. Sch., New Haven, CT. 06508

Previously we have reported that antibodies to a synthetic peptide, TH-16, corresponding to a serine (Ser)-40-containing segment of TH recognize catecholaminergic neurons and processes in the CNS (Lee, K.Y. et al., J. Neurochem. 53:1238-44, 1989). We have now generated and characterized polyclonal antibodies to TH-16 phosphorylated at Ser-40 by protein kinase A and designated them as anti-pTH-16. Anti-pTH-16 recognize the phosphorylated, but not the nonphosphorylated state of TH. Thus, a positive ELISA response against phosphorylated TH purified from PC12 cells and a negative response against the nonphosphorylated enzyme were obtained. Immunoprecipitation of TH with anti-pTH-16 results in a decrease of TH activity of the phosphorylated form, but not of the nonphosphorylated. Immunohistochemical studies reveal that anti-pTH-16 recognizes norepinephrine neurons in the locus coeruleus, as well as noradrenergic and adrenergic neurons of the ventrolateral medulla. It also recognizes a small subset of midbrain dopamine (DA) neurons, staining some neurons in the nucleus interfascicularis of the ventral tegmental area and in the posterior aspects of the A10 cell group. However, other DA neurons including the A9 (SN) and A8 (retrorubral field) cell groups were not, or very weakly, immunoreactive. In the forebrain there was weak or absent staining in the neostriatum and in the cerebral cortex. These results suggest that the phosphorylated form of TH recognized by anti-pTH-16 is present in relatively high concentrations in noradrenergic, but in low abundance in dopaminergic cells. These studies were supported in part by NIMH 43230 and NIMH 45124.

578.14

DELINEATION OF CNS AND PNS DNA RESPONSE ELEMENTS RESPONSIBLE FOR CELL-TYPE SPECIFIC EXPRESSION OF TYROSINE HYDROXYLASE. Y.J. Oh*, S.C. Wong, M. Moffat, D. Ullrey+, A.I. Geller+ and K.L. O'Malley. Dept. of Anat.& Neurobiol., Wash. U. Sch. of Med., St. Louis, MO 63110; +Div. of Endocrinology, Children's Hospital Boston, MA 02115.

In order to define cell-type specific elements that regulate expression of the tyrosine hydroxylase (TH) gene, we are using a defective Herpes Simplex Virus-1 vector system to deliver genes into both PNS and CNS neurons in primary culture. Deletion mutants of the rat TH promoter region were subcloned in front of the reporter gene, lacZ, and then packaged into viral particles. In preliminary studies, viral particles containing 685 bp of the TH promoter were used to infect primary neuronal cultures prepared from superior cervical and dorsal root ganglion in the PNS as well as mesencephalic and corpus striatal neurons in the CNS. One day after infection, expression of β-galactosidase was visualized by X-gal histochemistry. β-galactosidase activity was observed predominantly in the TH-expressing superior cervical ganglion neurons but infrequently in dorsal root ganglion neurons. In contrast, both mesencephalic and corpus striatal neurons showed a relatively similar level of β-galactosidase expression. These results seem to indicate that 685 bp of the rat TH promoter contain sufficient information for cell-type specific expression in the PNS, but not in CNS neurons. Experiments using other deletion mutants are being investigated to further delineate these elements. In the future, such sequences may aid in the targeting of relevant gene products in clinical models of catecholamine dysfunction.

578.15

PRESENCE OF TYROSINE HYDROXYLASE mRNA IN THE CEREBELLUM AND PITUITARY NEUROINTERMEDIATE LOBE OF ADULT RATS: POSSIBLE AXONAL LOCALIZATION. K.R. Melia*, A. Trembleau, P.P. Sanna, and F.E. Bloom. Neuropharmacology, Scripps Res. Inst., La Jolla, CA 92037.

Although both the cerebellum (CB) and the neurointermediate lobe (PIT) are densely innervated by catecholaminergic fibers, catecholamines or their biosynthetic enzymes have not been previously detected in the perikarya of adult CB or PIT tissue. However, we have recently detected mRNA for the biosynthetic enzyme, tyrosine hydroxylase (TH), in CB and PIT of adult male Sprague Dawley rats. Total RNA was extracted from these regions using a GIT/CsCl gradient. TH mRNA was first converted into single stranded cDNA using a specific downstream primer and then subjected to polymerase chain amplification (PCR) in the presence of oligo primers 5' and 3' to TH introns 11 and/or 12. PCR products were resolved by agarose gel electrophoresis, transferred to nylon membranes and probed with a ^{32}P-labeled oligo complementary to nucleotides 1233-1267 of the TH cDNA. Specific amplification products ranging in size from 127-429 bases were produced when various combinations of 4 different primers were used. Because the primers recognized sequences lying on different exons, products amplified from genomic DNA vs cDNA could be easily distinguished. The origin of TH mRNA in these regions is presently unknown. Based on preliminary non-radioactive in situ hybridization experiments it is possible that this message is contained within axons innervating these structures. Studies are in progress to evaluate the validity of this hypothesis and to examine whether levels of TH mRNA in the CB or PIT can be regulated by pharmacological stimuli.

578.16

IONIC FACTORS AFFECTING THE INTERACTION BETWEEN SOLUBLE TYROSINE HYDROXYLASE AND CHROMAFFIN GRANULE MEMBRANES. K.Morita* and H.Houchi. Dept. of Pharmacol., Tokushima Univ. Sch. of Med., Tokushima 770, Japan.

Tyrosine hydroxylase has previously been shown to be reversibly associated with chromaffin granule membranes under the experimental conditions which may approximate the intracellular environment of the resting cell. This enzyme has therefore been considered to be localized in association with the surface of the granules within the adrenal medulla cell. In the present study, the effects of ions and pH on the interaction of the soluble enzyme with the granule membranes were studied to elucidate an influence of the cytoplasmic environment alterations on the subcellular localization of the enzyme. The enzyme-membrane interaction was inhibited by elevating the Na+ concentration, but this interaction was not affected by increasing the Ca2+ concentration and the ATP level, or lowering pH in the mixture. These findings suggest that the association of tyrosine hydroxylase with chromaffin granules may be modulated by a rise in the Na+ level in the cell cytoplasm, thus proposing the possibility that stimulation of the cell may induce an alteration in the subcellular localization of the enzyme as a consequence of modulating the cytoplasmic ionic environment.

SEROTONIN RECEPTORS: BEHAVIORAL ACTIONS

579.1

FURTHER EVIDENCE FOR 5-HT-1C MEDIATED POTENTIATION OF APOMORPHINE-INDUCED LOCOMOTOR ACTIVITY. P.B. Hicks*, R.J. Zavodny and K.A. Young. Department of Psychiatry, Scott and White Clinic; Department of Medical Pharmacology and Toxicology and Department of Medical Anatomy & Neurobiology, Texas A&M College of Medicine, Temple, Texas 76508.

As reported previously (S. Neurosci. Abstr. 17, 407), the 5-HT-1C/2 antagonist mesulergine potentiated apomorphine-induced locomotor activity (AILA) at a low dose that had no effect on spontaneous locomotor activity. DOI, which has high affinity for 5HT-1C receptors as an agonist, reversed this potentiation. The current study further characterizes this putative 5HT-1C effect. Doses of mesulergine significantly lower than the AILA potentiating dose of 0.1 mg/kg SC had no effect on AILA, while mesulergine doses including and higher than 0.5 mg/kg supressed AILA. This information suggests that mesulergine maximally potentiates AILA between 0.05 and 0.3 mg/kg. The 5-HT-2 receptor antagonists ketanserine and MDL 100,907, tested over a wide range of doses, suppressed AILA at higher doses but did not potentiate AILA at low doses. Furthermore, DOI did not block suppression of AILA by the relatively specific 5HT-2 receptor antagonist MDL 100,907. These findings provide further evidence that mesulergine's potentiation of AILA is a 5HT-1C mediated effect and suggest an important modulatory role for 5HT-1C receptors in motor behaviors associated with DA neurotransmission.

579.2

EVIDENCE OF PRE OR POST-TRAINING ACUTE ADMINISTRATION OF 8-OH-DPAT IN ASSOCIATIVE LEARNING. A. Meneses* and E. Hong. Sección de Terapéutica Experimental,Departamento de Farmacología y Toxicología, CINVESTAV-IPN, México,D.F.

Diverse evidences suggest a role for 5-HT_{1A} receptor agonists in learning and memory. In the present work, it was determined the pre or post-training injection (ip) of 8-OH-DPAT (DPAT) on autoshaping lever press response, a model for associative learning. Animals were individually trained to find 15 US in the food magazine. Once that the animal ate the USs, one session began. Each session consisted of 20 trials and each trial consisted of illumination of a retractable lever for 8 sec (conditioned stimulus, CS) followed by the delivery of a food pellet (unconditioned stimulus, US) each 60 sec. If the animal pressed the lever (conditioned response, CR) the trial was shortened and the lever was retracted, the light was turned off and US was delivery. The results showed that DPAT improved the consolidation of CR when this was injected post-training, but impaired it when administered before training. Both effects were time-dependent. When DPAT was injected pre or post--training to free-feeding or pre-feeding rats, they did not learn the CR. When DPAT was administered to overtrained animals (90-100% of CR), the compound did not elicit any effect. These results strongly suggest an effect of DPAT on the consolidation of learning. The pre-training administration impaired the learning but also the food intake and exploration.

579.3

THE INTERACTION OF 5-HT_{1A} AND 5-HT_2 RECEPTORS IN THE RAT MEDIAL PREFRONTAL CORTEX: BEHAVIORAL STUDIES.
Martin I. Granoff,* Chau Lee, Adrian Jackson, Kavin Patel, Yanira Martinez, Charles R. Ashby, Jr. and Rex Y. Wang. Dept. of Psychiatry and Behavioral Sciences, SUNY at Stony Brook, Stony Brook, NY 11794.

A recent iontophoretic study indicated that there is an interaction between 5-HT_{1A} and 5-HT_2 receptors at the neuronal level in the medial prefrontal cortex (mPFc). In this study, we examined the head-twitching (HTW) response produced by injection of 5-HT_2 receptor agonists directly in the mPFc of freely moving, male Sprague-Dawley rats. One week prior to testing, bilateral cannula were implanted in the mPFc. Local injection of (-)DOB (1-4 µg in 1 µl each side), but not saline, dose-dependently increased the HTW. (-)DOB-induced HTW was prevented completely by the pretreatment with ritanserin (1mg/kg ip.). The effect of DOB is stereospecific, i.e. (-)DOB is much more potent than (+)DOB in eliciting HTW. The rank order of potency of various compounds tested is: (-)DOB ≥ (+)LSD > (+)DOB > lisuride = BL3912A. As a control, a group of animals were injected with (-)DOB in the ventral tegmental area. These animals exhibited no significant increase in HTW above the saline baseline, suggesting that the HTW induced by (-)DOB in the mPFc is not a result of drug-diffusion to the brainstem. To examine the effect of the 5-HT_{1A} agonist 8-OH-DPAT on (-)DOB-induced HTW, groups of rats were pretreated with increasing i.p. doses of 8-OH-DPAT 30 minutes prior to a 4 µg intracranial injection of (-)DOB. A dose-dependent supression of (-)DOB induced HTW was observed. These results, taken together, suggest that 5-HT_2 receptors in the mPFc may be important in the HTW response. This supports the hypothesis that there is a 5-HT_{1A} and 5-HT_2 receptor interaction in the mPFc. (Supported by USPHS grants MH-41440 and DA-07193)

579.4

THE INTERACTION OF 5-HT_{1A} AND 5-HT_2 RECEPTORS IN THE RAT MEDIAL PREFRONTAL CORTEX: IONTOPHORETIC STUDIES.
C.R. Ashby, Jr.* and Rex Y. Wang. Dept. of Psychiatry and Behavioral Sciences, SUNY at Stony Brook, Stony Brook, NY 11794.

Recent data obtained from behavioral and radioligand binding studies suggest that there is an interaction between 5-HT_{1A} and 5-HT_2 receptors in the rat brain. In this study, we examined the interaction between 5-HT_{1A} and 5-HT_2 receptors in the medial prefrontal cortex (mPFc) of anesthetized, male Sprague-Dawley rats. This was accomplished using the techniques of single cell recording and iontophoresis. The iontophoresis of the 5-HT_{1A} receptor agonist 8-hydroxy-di-n-propyl-aminotetralin (DPAT) produced a current-dependent suppression of mPFc cell firing. This effect was selectively antagonized by the 5-HT_{1A} antagonist NAN-190 but not by the antagonists eticlopride (D_2), atenolol (ß), prazosin (α_1), idazoxan (α_2), granisetron (5-HT_3) or SR 95103 ($GABA_A$). The suppressant action of DPAT was prolonged and potentiated by the iontophoresis of the 5-HT_2/5-HT_{1C} receptor antagonist ritanserin and the selective 5-HT_2 antagonist (+)-MDL 11,939. The systemic administration of ritanserin (0.1-0.5 mg/kg, i.v.) also potentiated and prolonged DPAT's action. In contrast, neither the iontophoretic nor systemic (0.1-0.5 mg/kg, i.v.) application of granisetron potentiated or prolonged DPAT's suppressant action. The iontophoresis of a low current of the 5-HT_2/5-HT_{1C} agonist (±)-DOI potentiated L-glutamate (GLU)-induced excitation of mPFc cells. This effect was significantly attenuated by the coiontophoresis of DPAT at currents that had little or no effect on L-GLU-induced excitation alone. Overall, these results indicate that there is an interaction between 5-HT_{1A} and 5-HT_2 receptors at a neuronal level.

579.5

CHARACTERIZATION OF PHOSPHOINOSITOL PHOSPHATE ACCUMULATION INDUCED BY THE 5-HT_3 RECEPTOR AGONIST 2-METHYL-5-HT.
R.Y. Wang,* C.R. Ashby, Jr. and E. Edwards. Department of Psychiatry and Behavioral Sciences, SUNY at Stony Brook, Stony Brook, NY 11794.

The aim of the present study was to examine the effect of manipulations of calcium (Ca^{++}) concentrations and activation of protein kinase C (PKC) on 2-methyl-5-HT-induced phosphoinositide (PI) hydrolysis in the rat medial prefrontal cortex. The omission of added Ca^{++} from the Krebs incubation medium significantly reduced the ^{3}H-inositol phosphate accumulation from pre-labelled phospholipids (from 41 ± 3% to 23 ± 1% above baseline level). Removal of Ca^{++} by pre-incubation with EGTA (0.5 mM) as well as the addition of the calcium channel blocker Lanthanum (10 µM) abolished the 2-methyl-5HT-stimulated PI response. By contrast, the calcium ionophores A 23187 and ionomycin stimulated PI hydrolysis and their effect was additive to that of 2-methyl-5-HT.

The increase in phosphoinositide hydrolysis induced by 2-methyl-5-HT was dose-dependently inhibited by phorbol dibutyrate (PDBu) and phorbol myristate acetate (PMA), but not by an inactive isomer 4α-phorbol, indicating that the activation of PKC may provide negative feedback to the PI response induced by 2-methyl-5-HT. The effect of PDBu on 2-methyl-5-HT stimulated PI metabolism was reversed by the PKC inhibitors staurosporine, calphostin and chelerythrine (10 µM) respectively. However, pertussis toxin (0.5 & 1 µg) had no effect on 2-methyl-5-HT stimulated PI hydrolysis suggesting that this response is not associated with the G_i or G_0 GTP binding protein. In conclusion, these results suggest that 2-methyl-5HT stimulated PI turnover is a Ca^{++}-dependent process. Furthermore, the process is subjected to a negative feedback inhibition via PKC. (Supported by USPHS grants MH-41440 and DA-07193).

579.6

FOOD INTAKE, LOCOMOTOR AND TEMPERATURE EFFECTS OF 5-HT_3 ANTAGONISTS AND AGONISTS.
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We studied the effects of various doses (0.1, 1.0 and 10.0 mg/kg) of MDL-72222 (a 5-HT_3 antagonist) and 1-(m-chlorophenyl)-biguanide (a 5-HT_3 agonist) on food intake using food-deprived paradigm, locomotor activity and rectal temperature in male Wistar rats.

Administration of MDL-72222 produced dose-dependent suppression of food intake. However, statistically significant decreases were observed with 1.0 and 10.0 mg/kg doses but not with 0.1 mg/kg dose. On the other hand, only the highest dose (10.0 mg/kg) of MDL-72222 produced significant decreases in locomotor activity. Administration of various doses of 1-(m-chlorophenyl)-biguanide did not have any significant effect on food intake except for a small (24%) nonsignificant decrease at the highest dose (10 mg/kg) during the first hour. However, there was a significant increase (56%) in food intake during 1-4 hours with the highest dose only. These findings suggest that the locomotor effects of the 5-HT_3 antagonists and agonists may partially contribute to their effects on food intake. We will also present data on changes in rectal temperature following administration of various doses of 5-HT_3 antagonists and agonists.

579.7

HINDLIMB SCRATCHING IN RATS DICRIMINATES AMONGST SUMATRIPTAN AND OTHER 5-HT1D AGONISTS.
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There is a lack of reliable animal models to screen in vivo 5-HT1D ligands. Recently, the hindlimb scratching (HS) induced by serotonergic compounds in rats has been suggested to involve peripheral 5-HT1D-like receptors although no selective 5-HT1D ligands were used. We have compared the HS inducing properties of the non-selective 5-HT1D agonists: 5-carboxamidotryptamine (ED50 mg/kg s.c.; 5-CT=5.1), 5-methoxytryptamine (5-MeOT=3.5) and ergotamine (ERG=0.4), with those of the selective 5-HT1B and 5-HT1D agonists, CP-93129 (CP= no effect up to 50) and sumatriptan (SUM = no effect up to 50). Pretreatment with ERG (0.1-0.3 mg/kg s.c.) and, surprisingly, SUM (20-50 mg/kg s.c.), but not CP, potentiated significantly the HS induced by submaximal doses (2.5-5 mg/kg) of 5-MeOT. Both direct and potentiated responses were inhibited by methiotepin (0.1-1 mg/kg s.c.).

Involvement of 5-HT1A, 5-HT1C and 5-HT2 receptors on HS induction was excluded previously 1 . CP inactivity indicates that 5-HT1B receptors are also not involved. Results obtained suggest that receptors mediating HS may be different to those recognized by SUM in bovine caudate and vascular tissue and that they can be positively modulated by a SUM-sensitive receptor.

579.8

ADAPTATION OF DOI-INDUCED EAR-SCRATCH RESPONSE FOLLOWING ACUTE AND REPEATED ADMINISTRATION OF DOI AND KETANSERIN.
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The 5-$HT_{2/1C}$ selective agonist DOI can produce both the head-twitch (HTR) and the ear-scratch response (ESR) in mice. Effects of withdrawal following acute and repeated administration of DOI and ketanserin on DOI-induced ESR was subject of this study.

In acute studies mice were treated with either a single injection of DOI (2.5 mg/kg) or ketanserin (1 mg/kg). In chronic studies different groups of mice were treated once daily with the cited doses of DOI (4 or 13 days) or ketanserin (5 days). Different groups of mice were challenged with DOI at different time intervals after cessation of acute or chronic treatments and the ESR was scored for 20 min following DOI injection. DOI administered 24h following its first injection reduced the ESR frequency by 80-97% but had no effect when the time lag between the first and second dose was greater than 72h. A single injection of ketanserin reduced (51%) the DOI-induced ESR 120h following its injection. Chronic DOI treatment reduced the ESR score by 80-97% throughout the treatment schedule. Following cessation of such treatment, the ESR score returned to control level in a time dependent manner. Repeated ketanserin treatment significantly reduced ESR score by 46% when tested 24-48h following cessation of antagonist treatment. These results suggest that serotonergic drugs may have the ability to change independently 5-HT receptor sensitivity and density.

579.9

EVIDENCE FOR INVOLVEMENT OF 5-HT_{1C} and 5-HT_2 RECEPTORS IN THE FOOD INTAKE SUPPRESSANT EFFECTS OF 1-(2,5-DIMETHOXY-4-IODOPHENYL)-2-AMINOPROPANE (DOI). James L. Hill, Charanjit S. Aulakh, Audrey Reid*, and Dennis L. Murphy. Lab. of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

We studied the effects of various 5-HT receptor subtype selective antagonists on DOI-induced decreases in food intake using the food-deprived paradigm in male Wistar rats. The effects of the antagonists when administered alone were also examined. Administration of DOI to rats produced dose-related decreases in one-hour food intake. Pretreatment with spiperone (5-HT_{1A}/5-HT_2/D_2 antagonist), propranolol or CGP361A (β-adrenoceptor antagonists that also have binding affinities for 5-HT_{1A} and 5-HT_{1B} sites) and MDL-72222 (5-HT_3 antagonist) did not attenuate DOI-induced suppression of food intake. In contrast, pretreatment with metergoline (5-HT_1/5-HT_2 antagonist) completely blocked whereas mesulergine, mianserin and ritanserin (5-HT_{1C}/5-HT_2 antagonists) partially blocked DOI's effect on food intake. On the other hand, pretreatment with MDL-72222 but not with m-chlorophenylpiperazine (m-CPP) significantly potentiated DOI-induced suppression of food intake. Furthermore, the food intake suppressant effects of various doses of DOI were found to be similar in the Fawn-Hooded (FH) rat strain as compared to the Wistar rat strain. These findings suggest that DOI-induced suppression of food intake is mediated by stimulation of both 5-HT_{1C} and 5-HT_2 receptors.

SEROTONIN: RECEPTOR MODULATION

580.1

5-HT_3 RECEPTORS ALTER THE REPETITIVE FIRING ACTIVITY IN VAGAL MOTONEURONS THROUGH MODULATION OF T TYPE CALCIUM CHANNELS. C.L. McNair* and M.S. Dekin, School of Biological Sciences, University of Kentucky, Lexington, KY 40506-0225.

In a previous study, we demonstrated that serotonin (5-HT) reduces spike frequency adaptation (SFA) and increases the post-burst hyperpolarization (PBH) observed in motoneurons from the dorsal motor nucleus (DMX) of the vagus nerve (McNair and Dekin, *Neurosci. Abstr.*, 17: 545.9, 1991). In this report, we have used a brainstem slice preparation (400 um thick) from adult guinea pigs to study the specific serotonergic receptor subtype responsible for this effect. Bath applied quipazine dimaleate (50 uM), an agonist for 5-HT_1 and 5-HT_2 receptors, did not effect either SFA or the PBH. In addition, serotonergic modulation of SFA and the PBH were not affected by the 5-HT_1 and 5-HT_2 antagonist cyproheptadine HCl (40 uM). 2-methyl-serotonin maleate (2-M-5-HT) (50 uM), a selective agonist for 5-HT_3 receptors, reduced SFA and increased the PBH with a similar magnitude and time course of action as 5-HT. Both the 5-HT and 2-M-5-HT reduction of SFA and augmentation of the PBH were effectively blocked by the selective 5-HT_3 antagonist MDL 72222 (30 uM). We have also attempted to further characterize the ionic mechanism underlying the action of 5-HT. Addition of 200 uM Ni^{++} to the bath had no effect on SFA or the PBH in control solutions but did block the effects of 5-HT and 2-M-5-HT. In contrast, 200 uM Cd^{++} in the bath completely eliminated SFA and the PBH. In the presence of both 5-HT and Cd^{++}, SFA and the PBH returned. These data suggest that 5-HT modulates the repetitive firing activity of vagal motoneurons through a 5-HT_3 receptor subtype acting on a Ni^{++} sensitive conductance such as T-type calcium channels. (Supported by NIH grants HL40369, HL02314, and RR07114).

580.2

MDL 100,907, A POTENT AND SELECTIVE 5-HT_2 ANTAGONIST WITH AN ATYPICAL NEUROLEPTIC PROFILE. S.M. Sorensen*, C.J. Schmidt, T. M. Humphreys, and J.H. Kehne Marion Merrell Dow Research Inst., Cincinnati, OH 45215

Progress toward understanding the role of the 5-HT_2 receptor in the therapy for schizophrenia has been hampered by the lack of agents with high selectivity for this receptor. Here we report on MDL 100,907 (R(+)-a-(2,3-dimethoxyphenyl)-1-[2-(4-fluoro-phenylethyl)]-4-piperidine-methanol), an extremely potent 5-HT_2 antagonist (Ki=0.4 nM) with a 300 fold or greater selectivity for the 5-HT_2 receptor over the 5-HT_{1c}, D_2 and other receptors (Carr et al., 1990). In order to characterize the contribution of 5-HT_2 antagonism to antipsychotic activity, we compared MDL 100,907 with haloperidol (HAL) and clozapine (CLOZ) in behavioral, electrophysiological, and neurochemical models predictive of antipsychotic potential and extrapyramidal side effect liability. Only MDL 100,907 blocked amphetamine (AMP)-stimulated locomotion in mice without antagonizing apomorphine-stimulated climbing. In acute recordings from A10 and A9 dopamine (DA) neurons, MDL 100,907 and CLOZ blocked the AMP-induced slowing of A10 neurons but caused only small increases in the number of spontaneously active DA neurons. Chronically, MDL 100,907 and CLOZ selectively reduced the number of active A10 neurons. HAL reduced these in both A9 and A10 brain regions. HAL produced marked changes in DOPAC and HVA in the A9 and A10 regions but MDL 100,907 and CLOZ had no significant effect on DA turnover. Following pretreatment with amfonelic acid (AFA), HAL caused massive increases in DA turnover whereas CLOZ or MDL 100,907 produced no such increase. The results indicate that MDL 100,907 has a profile which is similar to the atypical antipsychotic CLOZ. Furthermore, since MDL 100,907 is an extremely selective 5-HT_2 antagonist with no measurable D_2 affinity , the data suggest that MDL 100,907's 5-HT_2 antagonism may be sufficient to explain its antipsychotic potential.

580.3

IN VIVO STUDIES ON THE REGULATION OF DOPAMINE RELEASE BY THE 5-HT_2 RECEPTOR ANTAGONIST, MDL 100,907. C.J.Schmidt*, C.K.Sullivan, V.L.Taylor and G.M. Fadayel. Marion Merrell Dow Research Institute, Cincinnati, OH 45215

In vivo microdialysis in awake-freely moving rats was used to study the influence of 5-HT_2 receptor blockade on the release of dopamine produced by the amphetamine analogue, 3,4-methylenedioxymethamphetamine (MDMA). Peripheral administration of the selective 5-HT_2 antagonist, MDL 100,907 (1mg/kg, s.c.) reduced the increase in extracellular concentrations of striatal dopamine produced by MDMA (20 mg/kg, s.c.) by 46% ($P<0.05$). This occurred in the absence of any effect of the antagonist on basal dopamine efflux. To determine the location of the 5-HT_2 receptors involved in this effect, MDL 100,907 was infused into the striatum via the dialysis probe at 0.1 and 1.0 μM. Direct infusion of the antagonist resulted a concentration-dependent reduction in the efflux of dopamine produced by peripheral administration of MDMA (57% at 1.0 μM, $P<0.05$). In contrast, superfusion of striatal slices with 1.0 μM MDL 100,907 had no effect on the MDMA-induced release of preloaded [^{3}H]dopamine. Subsequently, rats were implanted with dialysis probes in both the substantia nigra and striatum to allow infusion of MDL 100,907 directly into the cell body region while measuring dopamine efflux in the striatum. Under these conditions, 1.0 μM MDL 100,907 was without effect on MDMA-induced dopamine release. These results suggest that striatal 5-HT_2 receptors can regulate the increase in dopaminergic activity produced by indirect agonists such as MDMA.

580.4

α_{2A}-HETEROCEPTORS AND 5-HT_{1B} AUTORECEPTORS MEDIATE THE OXYMETAZOLINE-INDUCED SUPPRESSION OF RAT VENTRAL HIPPOCAMPAL 5-HT RELEASE *IN VIVO*. *S. Hjorth* and R. Tao, Department of Pharmacology, University of Göteborg, Medicinareg. 7, S-413 90 Göteborg, SWEDEN*

There is evidence that both 5-HT_{1B} autoreceptors and α_2-heteroceptors participate in the control of transmitter release from 5-HT nerve terminals *in vivo* (Hjorth & Tao, EJP **209**: 249, 1991; Tao & Hjorth, NSAP **345**: 137, 1992). However, the subtype of α_2-adrenoceptor involved in this action has not been defined. We have used the α_{2A}-adrenoceptor selective agonist oxymetazoline (OXY) to further characterize the mechanism(s) regulating 5-HT release *in vivo*. The studies were carried out by means of *in vivo* microdialysis (probes placed in the ventral hippocampus) in chloral hydrate-anaesthetized male S-D rats (250-350 g). Drugs were introduced via the perfusion medium after a control period of 2-3h (to establish stable baseline 5-HT output). OXY (0.3-10 μM) concentration-dependently suppressed the 5-HT output (max. drop ≈ 40-50%). The effect of OXY (10 μM) was abolished by co-perfusion with the 5-HT_{1B} receptor blocker methiothepin (1 μM) plus the non-selective α_2-adrenoceptor blocker idazoxan (10 μM), but at best partly antagonized by either drug given alone. The α_{2B}-adrenoceptor antagonist prazosin (10 μM) did not affect the OXY response. The OXY-induced reduction of the 5-HT release in rat ventral hippocampus *in vivo* appears to be due to the combined 5-HT_{1B} and α_2-adrenoceptor agonistic properties of the compound, thus supporting the idea that there is a functional synergism between 5-HT- and NA-mediated control of 5-HT release. In this regard, the results of the agonist-antagonist interaction experiments suggest that the α_2-adrenoceptors involved are α_{2A}-heteroceptors situated on the 5-HT neuronal terminals.

580.5

CHRONIC ADMINISTRATION OF THE 5-HT_{1A} RECEPTOR AGONIST 8-OH-DPAT PRODUCES DESENSITIZATION OF 5-HT_{1A} AUTORECEPTORS. I. Lucki* and D. S. Kreiss. Depts. of Psychiatry and Pharmacology, Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

The function of 5-HT_{1A} autoreceptors was examined by measuring the ability of the 5-HT1A receptor agonist 8-OH-DPAT to reduce the release of serotonin (5-HT) in the striatum and the hippocampus using *in vivo* microdialysis. 5-HT release was measured in the ventral caudate nucleus and the ventral hippocampus of rats maintained under chloral hydrate anesthesia. Acute systemic administration of 8-OH-DPAT (1.0 mg/kg, s.c.) reduced the release of 5-HT in both the striatum and the hippocampus. Chronic treatment with 8-OH-DPAT (1.0 mg/kg, s.c.) for 7 days attenuated the effect of an acute challenge dose of 8-OH-DPAT in the striatum. Treatment with 8-OH-DPAT for 1 day did not significantly alter the effects of the agonist challenge on striatal 5-HT release. In contrast, the inhibition of 5-HT release in the hippocampus by 8-OH-DPAT was not altered by chronic treatment with the 5-HT_{1A} receptor agonist for 7 days. These results indicate that *in vivo* microdialysis can be used to study the effects of activation of 5-HT_{1A} autoreceptors on 5-HT release and the regulation of 5-HT release by chronic administration of psychoactive drugs. Moreover, this study indicates that the regulation of 5-HT release in different brain regions by 5-HT_{1A} autoreceptors may be selectively sensitive to regulation by chronic administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT.

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580.6

EFFECTS OF BMY 14802 ON RAT SOCIAL INTERACTION: EVIDENCE FOR 5-HT_{1A}-MEDIATED ANXIOLYTIC ACTIVITY.

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The potential anxiolytic efficacy of BMY 14802 was determined in a modified rat social-interaction paradigm. Unfamiliar pairs of male rats were placed into a novel high-light arena. Locomotor activity was recorded concurrently with observation of socially-interactive behaviors (e.g., mutual grooming and sniffing, following). BMY 14802 (0.025-1.0 mg/kg, i.p.) produced dose-dependent increases in social exploration with minimal effect on locomotor activity. Increases were comparable to those produced by buspirone (0.025-1.0 mg/kg, i.p.). In rats undergoing withdrawal following cessation of subchronic diazepam treatment (10.0 mg/kg, i.p., *bid*, 7 days), buspirone (0.5 mg/kg, i.p.) failed to increase time spent in social interaction, whereas BMY 14802 (0.1 mg/kg, i.p.) and ondansetron (0.1 mg/kg, i.p.) retained their anxiolytic-like effects. In rats treated subchronically with diazapam, flumazenil (10.0 mg/kg, i.p.) decreased social interaction without affecting locomotor activity, indicative of an anxiogenic-like action. BMY 14802 (0.1 mg/kg, i.p.) increased time spent in social exploration under conditions of precipitated diazepam withdrawal. *In vivo* microdialysis was used to study the effects of BMY 14802 on neural activity. BMY 14802 (5.0-20.0 mg/kg, i.p.) and buspirone (1.0-5.0 mg/kg, i.p.) produced dose-related decreases in extracellular 5-HT levels in the hippocampus of awake rats. The decrease in 5-HT produced by BMY 14802 (10.0 mg/kg, i.p.) was blocked by infusion of 1.0 mM (-)pindolol into the dorsal raphe nucleus. These data indicate that BMY 14802 produces anxiolytic-like effects in the rat that are maintained under conditions of diazepam withdrawal; these effects may arise from weak partial agonist actions at 5-HT_{1A} receptors.

580.7

IMMUNOCHEMICAL CHARACTERIZATION OF RECOMBINANT MOUSE TRYPTOPHAN HYDROXYLASE EXPRESSED IN E. COLI.

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Tryptophan hydroxylase (TPH) is the first and rate-limiting enzyme in serotonin biosynthesis. In order to produce the large quantity of purified TPH for the purpose of biochemical characterization and antibody production, recombinant DNA technology was used. In the present study a mouse cDNA containing the full coding region for TPH was cloned by the polymerase chain reaction, using poly A^+ RNA prepared from mouse dorsal raphe nuclei and oligonucleotide primers. The final construct was expressed in an expression plasmid under control of the tac promoter. Cultures of E.coli host strain MC 1061 were grown overnight in a liquid medium containing ampicillin, induced with 2 mM IPTG and pelleted by centrifugation. Large quantities of enzymatically active recombinant TPH were expressed in a highly insoluble form. However, more than 95% of total enzyme activity could be solubilized by lysis and repeated sonication. A band corresponding to TPH protein was eluted from SDS-polyacrylamide gels and extensively dialyzed to remove SDS. TPH protein eluted from the gel was injected subcutaneously into rabbits to raise specific antibodies. The magnitude of specific enzyme activity of recombinant TPH was similar to that of purified native enzyme. Western blot analysis indicated that the antibody directed against recombinant TPH recognized not only TPH but also tyrosine hydroxylase (TH) and phenylalanine hydroxylase (PH). Immunochemical titration data showed that the antiserum immunoprecipitated both TPH and TH proteins in a dosage-dependent manner. These data demonstrate that these methods can be used to produce large quantities of enzymatically active TPH. Supported by MH44043.

580.8

A 5-HT_{1D} AGONIST FOR THE TREATMENT OF MIGRAINE: PHARMACOLOGICAL STUDIES WITH MDL 100,687.

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The classification of 5-HT_1 receptors into various subtypes tantalizingly suggests specific targets for drug therapy. Yet, with the exception of 5-HT_{1A} receptors in the treatment of anxiety, the designation of a "disease for every subtype" remains unrealized. Even sumatriptan (SUM), which is effective in abolishing migraine attacks, is often described as acting at "5-HT_1-like" sites. In the present study, MDL 100,687 (MDL; 6-[[2-(5-hydroxy-1H-indol-3-yl)ethyl]amino]-N-[4-(trifluoromethyl)phenyl]-hexanamide, monohydrochloride), a novel compound targeted for migraine, was characterized in various assays of 5-HT_{1D} specificity and function.

MDL bound to 5-HT_{1D} sites in bovine caudate membranes with an IC_{50} of 34 nM compared to 81 nM for SUM. In guinea pig cortical slices, MDL inhibited K^+-induced release of [^{3}H]-5-HT by activating terminal 5-HT_{1D} autoreceptors; IC_{50} for MDL was 123 nM and for SUM, 1002 nM. MDL suppressed forskolin-stimulated adenylate cyclase in guinea pig substantia nigra with an IC_{50} of 398 nM vs. 1910 nM for SUM. In functional tests relevant to the treatment of migraine, both MDL and SUM reduced cat carotid arterial blood passing through arteriovenous anastomoses (MDL, 66% decrease at 1 mg/kg i.v.; SUM, 48%) and MDL dose-dependently constricted human pial arteries (pD_2 = 7.14 for MDL and 5-HT). The results indicate that MDL is clearly a 5-HT_{1D} agonist; its relative effectiveness in migraine awaits clinical trials.

580.9

DIRECT SEQUENCE AND SSCP ANALYSIS OF THE HUMAN TRYPTOPHAN HYDROXYLASE GENE.

J. S. Ellison, D. A. Nielsen, C. H. McDonald, L. Akhtar, M. Linnoila, and D. Goldman*. Lab. of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD 20892.

Tryptophan hydroxylase (TPH) is rate-limiting for the synthesis of serotonin, a neurotransmitter whose function may be perturbed in several neuropsychiatric diseases with a genetic component. The human cDNA sequence and mouse genomic DNA sequence, including intron/exon boundaries, were utilized to prepare primers for the PCR amplification and automated sequencing of the TPH gene from human genomic DNA. The exon portions of ten overlapping PCR fragments were sequenced and also compared by the single strand conformational polymorphism (SSCP) method. Intron 7 was sequenced also and the nature of a polymorphism detected by the SSCP method and located within this intron was determined. For SSCP analysis for mutations, DNA segments ranging from 121 to 3310 bp in length were amplified with fluorescent dye-labelled primers and the larger fragments were enzymatically digested prior to nondenaturing electrophoresis. Fragment mobility variants were argon laser detected using an ABI 373A Sequencer. Templates for PCR and sequencing were derived from individuals low in CSF 5-hydroxyindoleacetic acid, an index of serotonin turnover, from individuals with behaviors correlated with altered serotonin function (including alcoholics, murderers, firestarters, and suicide attempters), and from normal individuals.

580.10

REGULATION OF 5-HT_2 RECEPTOR mRNA LEVELS BY 5-HT_2 RECEPTOR AGONISTS AND ANTAGONISTS IN CULTURED CEREBELLAR NEURONS.

J. Akiyoshi*, C. Hough and D.-M. Chuang. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892

We have studied effects of 5-HT_2 receptor agonists (5-HT, DOI) and antagonists (mianserin, ketanserin) on 5-HT_2 receptor mRNA expression in cerebellar granule cells cultured in vitro for 8 days. Pretreatment with 5-HT or DOI induced a rapid desensitization of 5-HT_2 receptor-mediated PI response and a subsequent increase of ^{3}H-ketanserin binding to 5-HT_2 receptors in crude membranes and intact cells. The increase in 5-HT_2 receptor binding sites was dependent on the concentration of the prestimulating agonist and associated with an increase in both the B_{max} and K_d value of ^{3}H-ketanserin binding. Moreover, the up-regulation was temporally correlated with an increase of 5-HT_2 receptor mRNA level (from1-24 hr after treatment). Total RNA and mRNA for m_3-muscarinic receptors and ß-actin were not significantly affected by 5-HT or DOI pretreatment. Conversely, preexposure to the 5-HT_2 antagonists mianserin and ketanserin induced a time-dependent decrease of 5-HT-induced response but a concurrent loss of 5-HT_2 receptor binding sites. At least in the case of cells preexposed to mianserin, 5-HT_2 receptor down-regulation was accompanied by a marked loss of 5-HT_2 receptor mRNA. Thus in cerebellar granule cells, the number of 5-HT_2 receptors and 5-HT_2 receptor mRNA level are regulated by 5-HT_2 receptor agonists and antagonists in an unusual manner.

580.11

DIFFERENTIAL REGULATION OF SEROTONIN RELEASE BY THE DORSAL AND MEDIAL RAPHE NUCLEI. D.S. Kreiss* and I. Lucki. Institute of Neurological Sciences, Depts. of Psychiatry and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

The regulation of serotonin (5-HT) release by somatodendritic 5-HT_{1A} autoreceptors was examined using *in vivo* microdialysis. 5-HT release was measured in the ventral caudate nucleus and the ventral hippocampus of rats maintained under chloral hydrate anesthesia. Systemic administration of 8-OH-DPAT (1.0 mg/kg, i.p.) completely reduced the release of 5-HT in both the striatum and the hippocampus. Infusion of 8-OH-DPAT (3.2 µg in .25 µl over 5 min) into the dorsal raphe nucleus completely inhibited 5-HT release in the striatum, but did not alter 5-HT release in the hippocampus. Conversely, infusion of 8-OH-DPAT into the medial raphe nucleus reduced 5-HT release in the hippocampus, but not in the striatum. Simultaneous microinjection of the antagonist (-)propranolol (5.6 µg in .25 µl over 5 min) into the dorsal raphe nucleus with systemic administration of 8-OH-DPAT (1.0 mg/kg, i.p.) completely blocked the inhibition of 5-HT release in the striatum without affecting the inhibition of release in the hippocampus. Conversely, simultaneous microinjection of propranolol into the medial raphe nucleus with systemic 8-OH-DPAT blocked 5-HT release from being inhibited in the hippocampus, but not in the striatum. These results indicate that 5-HT release in certain areas of the striatum and the hippocampus are differentially regulated by 5-HT_{1A} autoreceptors of the dorsal and medial raphe nuclei.

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580.12

CP-110,330, A DIMETHYLTRYPTAMINE DERIVATIVE WITH HIGH BINDING AFFINITY FOR 5-HT_1 RECEPTORS AND DOPAMINE UPTAKE INHIBITING ACTIVITY. B.K. Koe*, L.A. Lebel, C.B. Fox, and J.T. Nowakowski. Central Research Div., Pfizer Inc, Groton, CT 06340

The discovery of serotonin receptor (5-HT_1) subtypes and the introduction of therapeutic agents with a serotonergic mechanism of action (serotonin reuptake inhibitors as antidepressants; 5-HT_{1A} agonists as anxiolytics and antidepressants; a 5-HT_{1D} agonist as antimigraine agent) have kindled interest in novel 5-HT_1 agonists. We describe a new N,N-dimethyltryptamine (DMT) derivative, 5-(5-anilinomethyl-2-thiazolyl)-DMT (CP-110,330), that exhibits potent binding affinity (IC_{50} in parenthesis) for 5-HT_{1A} (3.0 nM), 5-HT_{1B} (6.6 nM), and 5-HT_{1D} (3.8 nM) receptors with weak binding to 5-HT_{1C} (1.0 µM) or 5-HT_2 (14 µM in [^{3}H]ketanserin; 0.55 µM in [^{125}I]DOI) receptors. Agonist (5-HT_{1B}) character of CP-110,330 is suggested by a Gpp(NH)p shift to lower affinity in [^{125}I]iodocyanopindolol binding. In addition, CP-110,330 shows marked inhibition of DA uptake (IC_{50} 0.11 µM; cf. mazindol, IC_{50} 0.14 µM). The high binding affinity for 5-HT_1 sites is readily rationalized, because CP-110,330 is a C5-substituted DMT. The DA uptake blocking activity, however, is more difficult to explain. The conformation of CP-110,330 may allow the terminal phenyl ring and N of the aminoethyl sidechain to approximate a phenethylamine-like or a phenylbutylamine-like configuration spatially, thus permitting binding to the DA uptake site (IC_{50} 0.6 nM in [^{3}H]BTCP). The S.A.R. of some close analogs appears to be consistent with this possibility.

580.13

ANTIDEPRESSANT-LIKE ACTIVITY OF 8-OH-DPAT IS MEDIATED BY POST-SYNAPTICALLY LOCATED 5-HT_{1A} RECEPTORS. A. Singh* and I. Lucki. Depts. of Psychiatry and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

This study examined whether the antidepressant-like behavioral effects of the 5-HT_{1A} agonist 8-OH-DPAT, in the forced swimming test, are mediated by pre- or post-synaptically located 5-HT_{1A} receptors. 5-HT neurones were destroyed using 5,7-DHT (200 µg, icv) which resulted in a 90% reduction of 5-HT concentrations in the cerebral cortex and hippocampus. Forced swimming sessions were conducted 10-19 days following surgery. Sham- and 5,7-DHT-lesioned rats received three subcutaneous injections of 8-OH-DPAT (0.125-0.5 mg/kg) at time points corresponding to 0.5, 19 and 23 hours after the 15 min pretest swim session. Behavioral immobility was measured during a 5 min test session, which occurred one hour after the last injection.

8-OH-DPAT produced an identical dose-dependent reduction of immobility time in the sham- and 5,7-DHT-lesioned rats. The apparent absence of a shift to the left in the 5,7-DHT-treated group suggests that these post-synaptically located 5-HT_{1A} receptors are not sensitized. In contrast, the post-synaptically located 5-HT_{1A} receptors which mediate the 5-HT behavioral syndrome demonstrated a 3-fold shift to the left of the dose-response curve in the same group of rats. The results of this study confirm that the antidepressant-like effects of 8-OH-DPAT are mediated by post-synaptic 5-HT_{1A} receptors which are regulated differently from those mediating the 5-HT behavioral syndrome.

Supported by USPHS grants MH 36262 and MH 48125.

580.14

DIFFERENTIAL REGULATION OF RELEASE OF ENDOGENOUS 5-HT AND ^{3}H-5-HT BY NERVE TERMINAL AUTORECEPTORS. W.A. Wolf, R.E. Arthur Jr., L.D. Alphs and D.M. Kuhn*. Labs of Neurochemistry and Molecular Pharmacology, Dept. of Psychiatry, Wayne State University School of Medicine and Lafayette Clinic, Detroit, MI 48207.

Neuronal serotonin (5-HT) release is modulated, in part, through activation of nerve terminal autoreceptors (of the 5-HT_{1B} type in rat). This conclusion is based, in large part, on brain slice superfusion studies in which the release of ^{3}H from slices prelabelled with ^{3}H-5-HT is measured. However, such release may not totally reflect 5-HT release in vivo. Brain dialysis experiments can measure the regulation of endogenous 5-HT release in vivo. However the lack of sufficiently selective 5-HT_{1B} agonists, and the uncertainty of the drug concentrations achieved after systemic or even local application of such agonists can raise uncertainties as to the mechanism underlying their effects. The present work represents a comparison of the effects elicited by 5-HT_{1B} agonists on release of endogenous 5-HT versus ^{3}H-5-HT from superfused slices of rat striatum (400 uM thick). Striatal slices which were pre-loaded with ^{3}H-5-HT (70 nM for 30 min at 37°C in the presence of 10 uM nomifensine) released 0.88 ± 0.08 % of tissue ^{3}H upon depolarization (20 mM K^+, 4 min) at S1 and 0.73 ± 0.09 % of tissue ^{3}H at S2 (S2/S1 = 0.82 ± 0.08). The 5-HT_1 agonist RU 24969 (100 nM), reduced ^{3}H release at S2 to 0.34 ± 0.03 % of tissue ^{3}H (S2/S1 = 0.40 ± 0.02). In contrast, RU 24969 (100 nM) had no effect on release of endogenous 5-HT. Under control conditions 5-HT release at S2 was 0.275 ± 0.015 pmoles (2.06 ± 0.13 % of tissue 5-HT; S2/S1 = 0.89 + 0.01). In the presence of RU 24969 (100 nM) 5-HT release was 0.281 ± 0.007 pmoles (2.15 ± 0.20 % of tissue 5-HT; S2/S1 = 0.88 ± 0.02). These results suggest that although RU 24969 can inhibit release of ^{3}H-5-HT, release of endogenous 5-HT is not significantly altered. The effects of other 5-HT_{1B} agonists, stimulation parameters (electrical vs. K^+) on endogenous 5-HT and ^{3}H-5-HT release will be presented.

580.15

INTERACTIONS BETWEEN 5-HT_{1A} AND 5-HT_2 RECEPTOR ACTIVATION IN VIVO. L.G. Kirby*, J.G. Hensler & A. Frazer. Institute of Neuroscience, Depts. of Psychiatry & Pharmacol., Univ. of Penna. School of Medicine, DVA Med. Center, Philadelphia, PA 19104.

Previous studies by others have shown that either acute or chronic treatment of rats with 5-HT_{1A} agonists decrease a 5-HT_2 receptor-mediated behavior, quipazine-induced head shakes. We have studied further interactions between 5-HT_{1A} and 5-HT_2 receptor activation in vivo. Administration of the 5-HT_2 receptor agonist DOI (1 mg/kg, i.p., q.d.) to rats for 8 days resulted in an attenuation of the hypothermic response induced by the 5-HT_{1A} agonist DPAT (0.05 mg/kg, s.c.), measured 48 hrs after cessation of drug treatment. Chronic administration of the 5-HT_{1A} agonist gepirone (40 mg/kg/day, s.c.) by osmotic minipump also resulted in an attenuation of the hypothermic response induced by DPAT, which was not accompanied by changes in ^{3}H-DPAT binding. Chronic administration of gepirone to rats also decreased head shake behavior elicited by DOI (1 mg/kg, i.p.) measured 24 hrs after terminating gepirone treatment. Acute pretreatment of rats with gepirone (3 mg/kg, s.c.) 15 min prior to DOI administration (2 mg/kg, s.c.) reduced the number of head shakes elicited by DOI, but this effect was not seen in rats pretreated with gepirone 6 hrs and 24 hrs prior to injection of DOI. Chronic treatment with either 5-HT_{1A} or 5-HT_2 receptor agonists can inhibit a 5-HT_{1A} receptor-mediated response, whereas chronic treatment with 5-HT_{1A} agonists decrease a 5-HT_2 receptor-mediated behavior. To understand the mechanisms underlying these receptor interactions, it will be useful to develop model systems to study this in vitro. (Supported by research funds from the VA and USPHS grant MH 29094).

580.16

DIFFERENTIAL REGULATION OF 5-HT_{1A}-MEDIATED RESPONSES. P.A. Scott* & A. Frazer. Dept. of Psychiat., Univ. of Pa. Sch. of Med. and Vet. Affairs Med. Ctr., Phila. PA 19104.

Studies of the 5-HT_{1A} receptor are of interest because agonists for this subtype display both anxiolytic and antidepressant (AD) properties clinically. Some responses elicited by activation of this receptor show subsensitivity after chronic administration of some ADs or 5-HT_{1A} agonists. In the rat, 5-HT_{1A} receptor activation causes hypothermia and the 5-HT syndrome (flat body posture, forepaw treading, side-to-side body movement, and resting tremor). The effects of several 5-HT_{1A} agonists administered s.c. (gepirone; 8-OH-DPAT; (+) S-20499) on both hypothermia and the 5-HT syndrome were studied. All 3 drugs were much more potent in causing hypothermia than in eliciting the syndrome. The same maximal drop in body temperature (ca 2.5° C) was elicited by all three drugs with DPAT being the most potent (ED_{50} = 0.05mg/kg), followed by gepirone (1.8mg/kg) and (+) S-20499 (16mg/kg). The syndrome was reliably observed only at doses of 3-4mg/kg DPAT; doses up to 100 mg/kg of gepirone or (+) S-20499 did not produce any syndrome behavior. The effect of 14 day treatment of rats with either (+) S-20499 (24mg/kg/day; osmotic minipump) or the AD sertraline (5mg/kg, bid, i.p.) on either DPAT-induced hypothermia (E_{max} dose of 2.0mg/kg) or the syndrome (4mg/kg) was measured 18-24 hours after drug administration ceased. Both (+) S-20499 and sertraline completely blocked the syndrome. Chronic (+) S-20499 treatment significantly attenuated hypothermia produced by DPAT whereas chronic sertraline treatment did not. Differential regulatory influences may be exerted on 5-HT_{1A}-mediated responses by sertraline or novel anxiolytic/AD drugs depending, perhaps, on the anatomical location involved in producing the response and/or the presence of receptor reserve associated with particular responses. (Supported by Research Funds from the VA and USPHS Grants MH 29094, MH 48125 and IRI Servier).

581.1

HUMAN BRAIN KYNURENINE AMINOTRANSFERASE I: PURIFICATION AND CHARACTERISTICS. [1]H. Baran*, [2]E. Okuno, [2]R. Kido and [1]R. Schwarcz. [1]Maryland Psych. Res. Ctr., Baltimore, MD 21228 and [2]Wakayama Med. Coll., Wakayama, Japan.

Two kynurenine aminotransferases (KAT I and KAT II) are capable of producing the neuroinhibitory brain metabolite kynurenic acid from L-kynurenine in human brain tissue. We have now purified KAT I to homogeneity and characterized its catalytic properties. The enzyme was purified approximately 2,000-fold with a yield of 2%. Assessed by polyacrylamide gel electrophoresis, KAT I migrated toward the anode as a single protein with a mobility of 0.5. The pure enzyme is a dimer consisting of two identical 57 kDa subunits. Among oxo acid tested, KAT I showed highest activity with 2-oxoisocaproate. Using this co-substrate, kinetic analyses revealed an apparent K_m of 1.8 mM for L-kynurenine. KAT I activity was potently inhibited by glutamate, phenylalanine and tryptophan. Anti-KAT I antibodies were produced and partially purified. Subsequent Ouchterlony double diffusion, immunotitration and immunoblotting analyses confirmed that KAT I is distinct from other known kynurenine aminotransferases. Taken together, pure human KAT I and its antibody can be expected to serve as valuable tools in future studies of kynurenic acid production in the human brain under physiological and pathological conditions (cf. Jauch et al., this meeting).

This work was supported by USPHS grant NS 28236.

581.2

PURIFICATION AND CHARACTERIZATION OF SOLUBLE GLUTAMATE DECARBOXYLASE FROM PORCINE BRAIN. J. Bao*, B. Nathan, and J.-Y. Wu. Dept. of Physiology & Cell Biology, Univ. of Kansas, Lawrence, KS 66045-2106

Soluble L-glutamate decarboxylase (GAD) was isolated from porcine brain by homogenzing the brain tissue in water at 4°C. GAD was first purified by DEAE-cellulose and hydroxylapatite chromatography. Two peaks of GAD activity were obtained on hydroxylapatite column. The major activity peak, referred to as soluble GADI (sGADI), was further purified by a combination of gel filtration column, preparative nondenaturing 7.5% and 5-25% gradient polyacrylamide gel electrophoresis (PAGE). The purified preparation showed a single protein band on nondenaturing 5-25% gradient PAGE, which coincided with GAD activity. The molecular weight of native sGADI was calculated to be 123±1.0 KD from the nondenaturing gradient PAGE and 131± 1.0 KD from Sephadex G-200 column. The purified sGADI preparation was dissociated into two protein bands of 64±1.0 KD and 59±1.0 KD on SDS-PAGE, suggesting that sGADI is either a heterodimer composed of 64 KD and 59 KD subunits or a homodimer composed of either 64 KD or 59 KD subunits. Polyclonal antibodies were produced by immunizing rabbits with ~150 µg purified sGADI preparation. The specificity of anti-GAD serum was established by immunodiffusion, immuno-precipitation and Western blotting. Similar studies for the other forms of soluble GAD are in progress. [Supported by grants NS 20978 (NIH) and BNS-8820581 (NSF)].

581.3

PURIFICATION AND CHARACTERIZATION OF MEMBRANE ASSOCIATED GLUTAMATE DECARBOXYLASE FROM PORCINE BRAIN. B. Nathan*, J. Bao, and J.-Y. Wu. Dept. of Physiology & Cell Biology, Univ. of Kansas, Lawrence, KS 66045-2106

Several lines of evidence point to the conclusion that multiple forms of L-glutamate decarboxylase (GAD), the synthetic enzyme for GABA, are present in mammalian brain. Here, we describe purification and characterization of a membrane associated GAD, referred to as mGAD, from porcine brain. The purification involved solubilization of mGAD with 0.5% Triton X-100, followed by column chromatography on anion exchanger, DE-52. Three mGAD activity peaks were obtained, one of which was further purified to homogeneity by additional column chromatographies on ACA-34, hydroxylapatite, Sephadex G-200 and preparative SDS-PAGE. The molecular weight of native mGAD was calculated to be 125 ±10 kDa from gel filtration. The molecular weights of the probable subunits identified in analytical SDS-PAGE were found to be 71 & 74 kDa. Hence, the enzyme thus purified contains either a single form of mGAD which is a heterodimer of 71 and 74 kDa; or two forms of mGAD, one a homodimer of 71 kDa and the other a homodimer of 74 kDa. These results suggest that the mGAD thus obtained is different from soluble GAD in its molecular structure (see Abstract in this volume, Bao, J. et al.), but similar to soluble GAD in its kinetic properties, as well as in its sensitivity towards heat treatment and various GAD inhibitors like AOAA, PCMB and 3-MPA. The physiological role of mGAD in the brain remains to be determined.

581.4

THE GLUTAMINE CONTENT OF ASTROCYTES: REGULATION BY pH, cAMP AND HYDROCORTISONE. N. Brookes*. Dept. of Pharmacol. & Exptl. Therap., Univ. of Maryland School of Medicine, Baltimore, MD 21201.

A change in extracellular pH from 7.4 to 7.8 caused a >3-fold increase in the free glutamine content (Gln_i) of mouse cerebral astrocytes that were incubated with glutamate (Glu) and NH_4^+ (Brookes, J. Neurochem., 1992, in press). This effect of pH does not appear to result from increased free NH_3 concentration or from changes in transport of Glu or Gln. Does the mechanism involve regulation of glutamine synthetase (GS) activity by pH_i? To examine the effect of induction of GS activity on Gln_i and on the response of Gln_i to pH, astrocyte cultures were pretreated with dibutyryl cAMP (dbcAMP, 0.25 mM in serum-free MEM, changed twice during 6 d) or with hydrocortisone (HC, 1 μM in MEM supplemented with 5% fetal calf serum for 2 d). Then the cultures were preincubated to deplete them of free amino acids [1 h at 35°C in HEPES-Tris-buffered salts solution (HTB), pH 7.4], and then incubated for 30 min with 0.1 mM Glu and 0.1 mM NH_4^+ in HTB at pH 7.4 or 7.8. Values of Gln_i (measured by reversed phase HPLC with precolumn dabsylation) were as follows:

Cultures	Gln_i, nmol.mg^{-1}protein ± sem (n) pH 7.4	pH 7.8
Untreated	15.5 ± 1.1	54.4 ± 4.3 (6)
dbcAMP-treated	42.2 ± 14.5	104.1 ± 19.3 (3)
HC-treated	77.8 ± 3.7	228.9 ± 38.8 (3)

Clearance of Glu from the solution was 26-39% at 30 min. The results show that induction of GS activity markedly increased Gln_i, but that the pH effect remained large. This effect of pH may underlie the increased level of brain glutamine observed in hyperammonemia. (Supported by USPHS grant ES03928).

581.5

MDMA AND PCA INCREASE 5-HT LEVELS EXTRACELLULARLY IN CULTURED RAPHE CELLS GROWN IN SERUM FREE MEDIA: POTENTIATED BY DEPRENYL AND DEPOLARIZATION AND ATTENUATED BY RESERPINE AND NIMODIPINE. X.F.Gu* and E.C.Azmitia Dept. of Biology, New York University, Washington Square East, New York, N.Y. 10003

A novel serum-free microculture system coupled with HPLC-EC was developed in order to investigate the direct effects of 3,4-methylenedioxymethamphetamine (MDMA) and p-chloroamphetamine (PCA) on fetal raphe neurons. MDMA (10^{-5}M) and PCA (10^{-5}M) increased media 5-HT (20-40 nmoles; PCA > MDMA) as compared to non-detectable 5-HT levels in control cultures. After 48 hours of exposure to MDMA, 5-HT levels in the media increased from nmole range to µmole range. Exposure of the cultured cells to deprenyl (10^{-7}M), a monamine oxidase B inhibitor localized inside serotonergic neurons, increased the media 5-HT levels induced by MDMA by 30%. Intracellular storage of 5-HT was diminished by reserpine (10^{-6}M) and the MDMA or PCA induced increase in media 5-HT levels was attenuated by 17% or 47% respectively. Furthermore, MDMA and PCA-induced release of 5-HT could be blocked by fluoxetine at 10^{-8}M. The MDMA induced release appears to be both Ca^{++} dependent and independent since it was augmented by KCl at 50mM and attenuated by nimodipine at 10^{-8}M (an L-type Ca^{++}-channel blocker). Supported by NIDA contract# 271-90-7403.

581.6

ACCUMULATION OF [^{3}H]-(+)MDMA INTO RAT BRAIN SYNAPTOSOMES AND ASTROCYTES.

J.C. Poblete[1], X. Zhang[2], P.M. Whitaker-Azmitia[2] and E.C. Azmitia[1].

1. Dept. of Biology, New York University, NY, NY 10003, and 2. Dept. of Psychiatry, SUNY-Stony Brook, Stony Brook, NY 11794.

Previous studies have shown that 3,4-methylenedioxymethamphetamine (MDMA or ecstasy) inhibits [^{3}H]-5HT uptake and stimulates Ca^{++}-independent release of [^{3}H]-5HT. These actions of MDMA are mediated by the serotonergic high affinity uptake site. It has been suggested that the mechanism by which MDMA releases 5-HT is via the exchange diffusion mechanism, ie. MDMA is exchanged for 5-HT from the cytoplasmic pool. Thus, MDMA is accumulated into the terminal from which 5-HT is released.

Our present study characterizes the accumulation of [^{3}H]-(+)MDMA (0.568 Ci/mmol) into rat brain synaptosomes (P2) and astrocytes. Saturation analysis (0.2-20uM) for the accumulation of [^{3}H]-(+)MDMA into synaptosomes indicated an apparent Km of 3uM and a Vmax of 80 pmol/mg protein/2min. Sonication produced a 40% decrease in [^{3}H]-(+)MDMA (5uM) accumulation, while [^{3}H]-5HT (50nM) uptake was reduced by 70%. The accumulation of 5uM [^{3}H]-(+)MDMA was not significantly decreased by 100uM ouabain, while [^{3}H]-5HT uptake was diminished by 70%. Long-term degeneration of serotonergic terminals with a single dose of 10mg/kg p-chloroamphetamine (PCA) did not alter the accumulation of 5uM [^{3}H]-(+)MDMA, while it decreased the [^{3}H]-5HT uptake by 40%. This result suggests that [^{3}H]-(+)MDMA accumulation is not into 5-HT terminals.

Our results in culture indicate that [^{3}H]-(+)MDMA is accumulated into rat brain astrocytes. Saturation analysis of [^{3}H]-(+)MDMA accumulation into astrocytes exhibit an apparent Km of 6 uM and a Vmax 65 pmol/mg protein /2min, and has regional specificity. (Research Supported by NIDA contract # 271-90-7403)

581.7

SELECTIVITY OF VARIOUS SUBSTITUTED AMPHETAMINES FOR MAO-A.

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PCA, methamphetamine (METH), fenfluoramine (FEN) and MDMA are substituted amphetamines that release serotonin from serotonergic terminals. PCA,FEN and MDMA have been linked to serotonin neuropathology. We have previously shown that MDMA inhibits MAO-A but not MAO-B activity. MAO-A catabolizes serotonin with a higher affinity than does MAO-B; serotonergic cells contain mostly MAO-B. Dopamine is metabolized with equal affinity by both MAO subtypes. The selectivity of PCA, METH and FEN for MAO subtype was examined in the brain stem and hippocampal rat brain homogenates. Selectivity for MAO-B was determined by pre-treating homogenates with 100 nM clorgyline, a specific MAO-A inhibitor, for 10 mins., 37° C. Amphetamines were added at final concentrations of 10 $^{-4}$M-10^{-7}M, 10 mins, 37°C. ^{3}H-DA at 5 uM was added as substrate; homogenates were incubated for 30 min., 37°C. MAO-A activity was determined with 5 uM ^{3}H-5-HT as substrate. Radiolabelled metabolites of both reactions were measured by scintillation counting.

We found no significant effects by any of the amphetamines tested on MAO-B activity. MAO-A was significantly inhibited with PCA>>METH>FEN, with IC_{50}s of 1uM, 25 uM and >100uM respectively. In cultured serotonergic cells treated with 10 uM MDMA, the development of the uptake of ^{3}H-5-HT was inhibited by 43% ($p<0.001$). This MDMA inhibitory effect was potentiated in cultures that were treated with clorgyline (10nM-100nM) (58% inhibition, $p<0.01$). These results suggest that inhibition of MAO-A may contribute to the neurotoxic properties of these drugs. This work was supported by NIDA contract #271-87-8144.

581.8

EXPRESSION OF CATECHOL-O-METHYLTRANSFERASE (COMT) IN RAT BRAIN AND PERIPHERAL TISSUES. T.Karhunen*, C.Tilgmann, I. Julkunen, I. Ulmanen and P. Panula. Dept. Anatomy, Univ. Helsinki, Orion Pharmaceuticals, Espoo, and National Public Health Institute, Helsinki, Finland.

Antisera against rat recombinant COMT were raised in guinea pigs to reveal the distribution of COMT in rat tissues. Immunoprecipitation of in vitro-synthesized membrane-bound and soluble COMT proteins indicated that both forms were effectively recognized by the antisera. Western blotting analysis showed that both forms were detected in rat kidney, liver, brain, stomach and adrenal.

Indirect immunofluorescence technique showed specific immunoreactivity in the ependymal cells lining cerebral ventricles, plexus choroideus, median eminence, Bergmann glia of the cerebellum, and pituicytes of the posterior pituitary. Cells lining the intermediate lobe were intensely immunoreactive. Hepatocytes, epithelial cells in the stomach and duodenum, muscle layer of the gut, islet cells of the pancreas, and kidney tubules were also immunoreactive. Preadsorption of the antisera with recombinant rat COMT protein abolished all staining, and preimmune serum did not stain any structures.

The results suggest that COMT is a widespread enzyme both in the brain and peripheral organs.

581.9

CHRONIC COCAINE AND HALOPERIDOL ELEVATE 3-METHOXYTYRAMINE IN THE ANTERIOR PITUITARY OF THE MALE RAT. S. J. Chrapusta, F. Karoum, M. F. Egan and R. J. Wyatt*. Neuropsychiatry Branch, NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032, U.S.A.

According to previous reports, changes in tuberoinfundibular dopamine neuron (TIDA) activity do not correlate with the anterior pituitary dopamine (DA) content. Since the TIDA terminals do not form classical synapses and are juxtaposed to blood vessels, it is also unclear whether 3-methoxytyramine (3MT; a DA metabolite used as an indirect index of DA release) can reflect activity of these neurons.

We measured 3MT in the anterior pituitary of male rats using gas chromatography/mass spectrometry with negative chemical ionization. The animals were treated chronically or acutely with 0.4 mg/kg of haloperidol (HAL) (3 weeks of daily ip injections of HAL followed by HAL or vehicle (VEH) injection 1 hour before sacrifice, or 3 weeks of daily ip VEH injections followed by HAL or VEH injection 1 hr before sacrifice), or cocaine (10 mg/kg twice daily for 1 week followed by 1 week withdrawal versus twice daily injections of VEH followed by 1 week withdrawal). An injection of monoamine oxidase inhibitor pargyline (75 mg/kg, ip) was given to the rats 0, 10 or 20 min before sacrifice by microwave brain irradiation.

Increased 3MT accumulation (133-142% of control, $p<0.001$) was observed in the rats treated chronically and/or acutely with HAL. Chronic cocaine treatment significantly elevated steady-state 3MT levels (164% of control, $p<0.05$) and tended to increase 3MT rate of formation (160% of control). These findings indicate that anterior pituitary 3MT - in contrast to DA - may be a useful index of TIDA neuronal activity.

581.10

Uptake and stimulus-dependant release of [32]P-labelled Vasopressin RNA by hypothalamic primary cell cultures

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We recently showed that magnocellular hypothalamic neurons in Brattleboro rat can accumulate, transport and translate exogenous vasopressin (VP) mRNA. To investigate the mechanisms of RNA uptake, transport and secretion, we incubated primary cultures of dissociated fetal (ED 17) rat hypothalamus with [32]P-labelled VP cRNA. Autoradiograms revealed that a distinct portion of cells, mostly neurons, accumulates RNA in perikarya and processes. This group of cells includes VP immunoreactive perikarya but is not limited to this cell type. We could further demonstrate that depolarization of the cells with 50 mM KCl increased the amount of radioactivity released into the culture medium, indicating a stimulus dependant secretion of VP RNA. This effect was in part Ca^{++} dependent. Stimulation of the cultures with 10^{-6} M of either synthetic Arg-vasopressin or oxytocin resulted in release of incorporated exogenous VP RNA, but 1mM glutamate did not facilitate any release. Our results indicate that a stimulus dependant secretion of certain RNAs might be a common physiological feature of hypothalamic neurons, thus providing for a novel way of interneuronal communication.

581.11

Coexistence of vasopressin and oxytocin in rat magnocellular neurons upon intrahypothalamic injections with vasopressin RNA. G.F. Jirikowski*, D. Maciejewski-Lenoir, F.E. Bloom. Dept. of Neuropharmacology, Scripps Res. Inst., La Jolla, CA 92037

In previous studies we demonstrated that magnocellular hypothalamic neurons in the homozygous Brattleboro rat are capable of accumulating, transporting and translating exogenous vasopressin (VP) RNA, resulting in a temporary correction of diabetes insipidus. In the present study intact adult Wistar rats were stereotaxically injected into the hypothalamo neurohypophysial tract with [32]P-labelled VP cRNA. After a survival time of 18h, radiolabelling could be observed in most of the VP immunostained neurons. In addition a fraction of the oxytocin (OT) cells as well as in the posterior lobe were labelled, indicating that VP cRNA was taken up and transported anterogradely as well as retrogradely by both VP and OT cells. Immunocytochemistry of consecutive semithin hypothalamus sections revealed that in RNA injected animals about 15% of the OT immunoreactive perikarya contained VP. This indicates that OT neurons can be recruited into translation of exogenous VP RNA. In preliminary experiments, RNA binding sites were localized on cryostat sections of rat hypothalamus by autoradiography after in vitro incubation. Radiolabelled VP RNA is intensely bound by fiber tracts, but was absent in the magnocellular nuclei. The present findings indicate that uptake, axonal transport and translation of VP cRNA by magnocellular hypothalamic neurons is not restricted to the genedeficient Brattleboro rat but may represent a feature of the hypothalamic neurons generally. Exchange of RNAs between neurons could allow for the transitory coexistence of otherwise unrelated neuropeptides.

581.12

METABOLIC AND BIOCHEMICAL EFFECTS OF DIFFERENT GROWTH CONDITIONS IN THE PHEOCHROMACYTOMA CELL LINE, PC12. T. Flanagan*, M. Palmatier, T. Wright, W. Bell, M. Lavoie. CytoTherapeutics, Inc., Providence, RI 02906

The PC12 cell line is derived from a rat pheochromacytoma. PC12 cells grow better at high cell densities rather than low cell densities and under these conditions metabolize glucose quickly . We examined the effect of plating density and acute or sustained glucose depletion of >90% of initial medium level on the growth rate, viability, media levels of lactate and glucose, and dopamine storage in these cells.

Sustained (>3 days), but not acute (<1 day), glucose depletion decreased the growth rate of PC12 cells. Unexpectedly, the viability was unaffected by either sustained or transient glucose depletion. Under normal growth conditions, lactate is produced by PC12 cells and media levels rise. However, under sustained glucose depletion, media levels of lactate fall and lactate is consumed by PC12 cells. Consumption of lactate develops slowly as PC12 cells which were acutely glucose starved did not consume lactate, suggestive of a shift in the metabolic state of the cells. This apparent shift was reflected in changes in the storage of dopamine. The amount of stored dopamine was five-fold less per cell after sustained glucose depletion than in PC12 cells grown under optimal conditions.

A five-fold difference in plating densities, 3 $X10^{5}$/ml vs 6 $X10^{4}$/ml, with no depletion of medium glucose, did not affect the growth rate, viability, or the amount of stored dopamine in PC12 cells at the end of a 9 day culture period.

Taken together, these results indicate that rapidly developing changes in culture conditions can lead to shifts in metabolic pathways in PC12 cells. Differences in the internal metabolism of separate cultures of PC12 cells plated from a common cell suspension correlate with quantitative differences in secretory product stores. Thus, closely monitored growth conditions are important in predicting the behavior of PC12 cells.

581.13

DIFFUSIONAL PROPERTIES OF DOPAMINE IN A RAT MODEL OF PARKINSON'S DISEASE AS MEASURED BY MULTIPLE IN VIVO ELECTROCHEMICAL DETECTOR ARRAYS. C.G. van Horne*, J. Hudson, B.J. Hoffer, and G.A. Gerhardt. Departments of Pharmacology and Psychiatry and The Rocky Mountain Center for Sensor Technology, University of Colorado Health Science Center, Denver, Colorado 80262.

Parkinson's disease (PD) is characterized by the loss of dopaminergic neurons in the substantia nigra with a subsequent depletion of dopamine (DA) and DA nerve terminals, which contain a high-affinity DA uptake system, in the striatum. Although current treatments include DA replacement strategies, the fate of DA in the extracellular space is poorly understood. The goal of this study was to determine the temporal and spatial diffusional properties of locally applied DA in the 6-hydroxydopamine (6-OHDA) lesioned striatum as compared to the intact non-lesioned striatum and two control brain regions, the cerebral and cerebellar cortices, which are naturally devoid of DA nerve terminals. Male rats were unilaterally lesioned with 6-OHDA to remove the nigro-striatal DA-ergic pathway. Rats with greater than 95% depletions of striatal DA, determined by apomorphine rotational analysis, and non-lesioned rats were studied. DA (200 uM) was pressure ejected locally and detected by utilizing high-speed in vivo chronoamperometry with 4 Nafion coated carbon-fiber electrodes (30 um tip diameter) assembled in a linear array. Distances from the first electrode to the DA source and between each subsequent electrode measured between 200 and 300 um. Results showed that increasing volumes of DA were required to consistently detect signals at 200-300 um in the different brain regions and were as follows: 10-13 nl in the cerebral and cerebellar cortices, 50 nl in the 6-OHDA lesioned striatum, and 110-250 nl in the non-lesioned striatum. With respect to changes in the concentration of DA over time and distance, i.e. clearance, the intact striatum showed the greatest capacity for clearing extracellular DA, with a clearance time (the time required for a signal to decrease from 80 to 40 % of maximal amplitude) of 10-20 seconds and a 90-96% decrease in signal amplitude over 200-300 um. Lower capacities for DA clearance were found for the cerebral (30-70 seconds and an 80-90% decrease over 200-300 um) and cerebellar (60-120 seconds and a 70-80% decrease over 200-300um) cortices. The lesioned striatum showed the lowest capacity for DA clearance (120-200 seconds and a 40-70% decrease over 200-300 um). Thus DA appears to diffuse for greater distances and is cleared more slowly in brain regions which lack DA fiber systems. (Supported by USPHS grants AG06434, NS09199, and AG00441). (NSF BNS-911030)

581.14

OVEREXPRESSION OF GAP-43 IN AtT20 CELLS. C. Gamby, R. G. Allen* and L. Baizer, R. S. Dow Neurological Sciences Institute, Good Samaritan Hospital and Medical Center and C.R.O.E.T., Oregon Health Sciences University, Portland, Oregon 97209.

GAP-43 is a membrane bound phosphoprotein expressed primarily in neurons. While its precise function remains to be determined, it is concentrated in the growth cone, where it may be involved in the membrane addition associated with axonal growth. GAP-43 may also play a role in neurotransmitter and hormone release by facilitating the fusion of secretory vesicles with the plasma membrane. We have investigated the role of GAP-43 in secretion of adrenocorticotrophic hormone (ACTH) by a pituitary-derived neuroendocrine cell line (AtT20).

Western blot analysis demonstrates high levels of GAP-43 expression in AtT20/D16-16 cells. Rat or chicken cDNAs for GAP-43 under the control of the RSV promoter were transfected into AtT20/D16-16 cells and expression of exogenous GAP-43 was tested by RNase protection and western blot analyses. Cell lines over-expressing both rat and chicken GAP-43 have been isolated; none exhibit significant changes in cellular morphology. Spontaneous release of ACTH (measured by radioimmunoassay) by the clones overexpressing chicken or rat GAP-43 is similar to that in control cells, but the CRF-stimulated release is reduced by 30 to 40 %. Supported by NIH #NS26806.

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS: ANATOMY

582.1

VARIABILITY IN THE CYTOARCHITECTURAL PATTERN OF THE BODY REPRESENTATION WITHIN THE PRIMARY SOMATOSENSORY CORTICAL AREA OF ADULT CATS. S.S. Leclerc*, C. Avendaño, I. Salimi, R.S. Waters and R.W. Dykes. Centre de recherche en sciences neurologiques, Dept. Physiologie, Faculté de médecine, Univ. de Montréal, P.Q., Canada, H3C 3J7.

Cat primary somatosensory cortex classically includes four cytoarchitectonic subdivisions, areas 3a, 3b, 1 and 2 (Hassler and Muhs-Clement, '64) and each band appears to be functionally distinct. Anatomical and electrophysiological mapping techniques used to correlate somatotopic and functional organization within more than fifteen cats revealed important idiosyncrasies in the size and distribution of pyramidal cells within layer V of area 3b as observed from Nissl-stained material. In most cases there appeared to be significant changes along the lateral-to-medial axis in the number and size of large pyramidal cells associated with major body parts within area 3b. Layer V pyramidal cells located in the hindlimb representation appeared larger and more intensely stained than those observed in the forelimb representation. This pattern was not gradual but periodic and interrupted. In some occasions, large pyramidal cells within layer V formed a continuous band spanning across all primary somatosensory cytoarchitectonic areas. However, in other cases, only a few scattered large pyramidal cells were observed throughout the entire medial-to-lateral extent of the body representation within area 3b. Layer IV thickness was also found to vary within area 3b in the same animals. Confirmation of some cytoarchitectonic borders were obtained using AChE histochemistry. The pattern of large pyramidal cells in layer V of cat primary somatosensory cortex appears to be an idiosyncratic feature and cannot be used alone as a factor for the determination of cytoarchitectonic borders. It has yet to be determined if these variations have any functional implications in the processing of sensory information. Support by DGCyT fellowship to C.A. and MRC grant MA 8700.

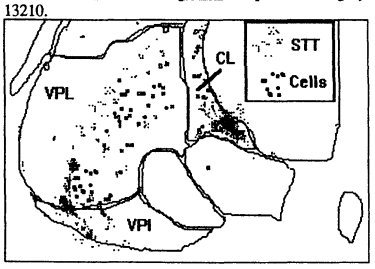
582.2

ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL PROPERTIES OF LAYER 6 CELLS IN THE RAT NEOCORTEX. J.F.M. van Brederode* and G. L. Snyder. Dept. of Biol. Structure, Univ.of Washington, Seattle, WA 98195.

In vitro studies performed in brain slices have shown that cortical neurons differ in their intrinsic membrane properties. We used a combination of intracellular recording and dye-filling to study the electrophysiological and morphological characteristics of layer 6 cells in the rat sensorimotor cortex, which have received little attention. Anatomical reconstruction of filled cells showed them to be a morphologically diverse group consisting of regularly- and irregularly-oriented pyramidal cells and spiny nonpyramidal cells. Regular layer 6 pyramidal cells had either long, straight or short, sinuous apical dendrites, and differed in the extent of their axonal arborizations. Irregularly-oriented pyramidal cells consisted of sideways or inverted pyramidal cells of variable size and morphology, often characterized by U-turn axons. Spiny nonpyramidal cells included bitufted and multipolar cell types that differed in soma size and extent and orientation of their dendritic trees and axonal arborizations. Intracellular stimulation revealed that this morphological diversity was mirrored by a similar electrophysiological diversity. Most layer 6 cells were capable of firing trains of action potentials characterized by an initial doublet or triplet followed by a train of single spikes (phasic-tonic or PT mode). The majority of layer 6 cells could fire either single spikes only (tonic or T mode) with low strength input or in PT mode with stronger stimulation. A minority were either PT mode-only or T mode-only cells. The size and sequence of spike afterpotentials during slow repetitive firing was highly variable among layer 6 cells. Hyperpolarizing inward rectification was common and hyperpolarizing sag was present in most cells although the amount was variable. We were unable to establish a clear relationship between cell morphology and firing properties among layer 6 cells, possibly due to the large variety of cell types found in this layer. This work was supported by NIH grants EY 01208, EY 04536, EY 07031, and RR 00166.

582.3

SPINOTHALAMOCORTICAL INPUT IS NONPREFERENTIALLY DISTRIBUTED TO SUPERFICIAL AND DEEP CORTICAL LAYERS. T. Shi, J. Tessier, R.T. Stevens*, and A.V. Apkarian. Dept. Neurosurgery, SUNY HSC, Syracuse, NY 13210.



The VPL, VPI and CL in monkeys are a major connection between the spinothalamic tract (STT) and the primary somatosensory cortex (SI; Gingold et al. JCN 1991). Rausell and Jones (J Nsci 1991) recently suggested that nociceptive information preferentially accesses the superficial layers of SI. We studied this question in 1 macaque and 3 squirrel monkeys. DY was deposited on the surface of the mapped hand SI and WGA-HRP was injected in the contralateral C6-T1. Thalamic DY-labeled cells located within 100um of STT terminals were considered overlapping and thus having STT inputs. Despite smaller numbers (10%of the total SI projection), surface injections produced a pattern of label no different than that of complete SI injections either in locations, proximity to STT terminals or proportion of overlap from each thalamic nucleus. On an average 100um section, 49 of 148 (33%) SI surface projecting cells had STT inputs, as compared to 507 of 1695 (30%) of total SI projecting cells. The result suggests that the STT afferent can be relayed into layer I of SI by VPL, VPI and CL but they are not preferential pathways.

582.4

DISTRIBUTION OF $GABA_A$ RECEPTORS IN CAT SOMATOSENSORY AND MOTOR CORTEX. J. Li and H.D. Schwark*. Department of Biological Sciences, University of North Texas, Denton, TX 76203.

Rapidly-adapting (RA) and slowly-adapting (SA) submodality-specific regions have been described in area 3b of cat primary somatosensory (SI) cortex (Sretavan & Dykes '83). Compared to SA regions, a higher proportion of neurons in RA regions are sensitive to locally applied bicuculline (Dykes et al. '84), suggesting differences in the organization of GABAergic systems between these two regions.

To determine whether $GABA_A$ receptor densities might correlate with the segregation of submodalities, we have examined the distribution of [^{3}H]muscimol binding sites in film autoradiographs of parasagittal sections through SI. Reliability of patterns was determined in series of 4-6 closely spaced sections. Density histograms were used to measure the distribution of [^{3}H]muscimol binding along the vertical extent of cortical columns in areas 2, 1, 3b, 3a, 4 and 6.

Although initial visual inspection of muscimol binding in area 3b failed to reveal density differences related to submodality segregation, the issue is presently being addressed by quantitative image analysis. Average binding density in areas 3b, 3a, 4 and 6 was approximately 18% higher than in areas 1 and 2. Within each area, the highest levels were in layers I and II (average values 170-390 fmoles/mg). Below layer II, the levels of binding decreased with depth, except that in most of the density histograms through SI, upper layer IV showed a peak in binding density. Supported by NIH grant NS25729.

582.5

THE ORGANIZATION OF CORTICOCORTICAL AND CALLOSAL PROJECTION NEURONS AND CALBINDIN-IMMUNOREACTIVE NEURONS IN THE RAT SECOND SOMATOSENSORY CORTEX. K. A. Baskerville*, P. Herron, and H. T. Chang. Dept. of Anatomy and Neurobiology, The Univ. of Tennessee, Memphis, Col. of Medicine, Memphis, TN 38163

We investigated the spatial distributions and collateralization of corticocortical and callosal projection neurons in the rat second somatosensory cortex (SII) and investigated whether these projection neurons were immunoreactive for calbindin. In double-labeling studies, retrograde tracers were injected into electrophysiologically-identified homotopic areas of ipsilateral primary somatosensory cortex (SI) and motor cortex (MI), contralateral SI and SII, or SI of both hemispheres. In some animals, the SII representation area was mapped to determine the congruence of retrogradely labeled corticocortical and callosal neurons in the lateral SI-suprarhinal area with the SII somatotopic map. Injections into homotopic areas of contralateral SI and SII, ipsilateral SI and MI, or SI of both hemispheres resulted in two overlapping populations of neurons in appropriate homotopic areas of SII. Very few double-labeled cells were found. Neurons immunoreactive for calbindin were located principally in the same laminae as corticocortical and callosal neurons in SII; however, very few corticocortical or callosal neurons were immunoreactive for calbindin. These results suggest that corticocortical and callosal neurons in SII with collaterals to two or more homotopic areas are relatively rare and that those immunoreactive for calbindin are even rarer. Given their laminar proximities to corticocortical and callosal neurons, calbindin-containing neurons may be the targets of reciprocal corticocortical and callosal axonal terminals in the SII cortex. (Supported by NIH grant AG05944 and The Center for Neuroscience of The Univ. of Tennessee, Memphis).

582.6

DIFFERENTIAL EXPRESSION OF GAD AND CAM II KINASE-α mRNA DURING DEVELOPMENT OF THE MOUSE SOMATOSENSORY CORTEX. J.L. Massengill, M.M. Huntsman and E.G. Jones* Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717

In situ hybridization with specific riboprobes was used to examine differential expression of 67 kDa GAD and CAM II kinase-α mRNA during development of the mouse somatosensory cortex. Animals were particularly examined during the first postnatal week when correlative physiological studies (Agmon and O'Dowd, J. Neurophysiol., in press) indicate that the normal GABA-mediated disynaptic inhibitory thalamocortical response is maturing. CAM II kinase-α is expressed in cortical excitatory neurons and absent from GABA neurons. GAD and CAM II kinase-α are expressed early in cortical neurons but preliminary findings suggest that expression of GAD lags behind that of CAM II kinase-α during the critical postnatal period and this may correlate with the earlier maturation of the monosynaptic excitatory thalamocortical response. Supported by NIH grant NS21377.

582.7

COLUMNS IN RAT SOMATOSENSORY CORTEX SEEN WITH DEOXYGLUCOSE AUTORADIOGRAPHY. *S. M. Feldman* and L. L. Brown†*. *Center for Neural Science, New York University, New York, NY 10003 and †Department of Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.

Deoxyglucose autoradiography was used to examine cortex during somatosensory stimulation applied to forelimb (FL; n=10), hindlimb (HL; n=5), trunk (TR; n=5) or FL+HL (n=5) in awake rats. A plexiglass restraining device permitted automated stroking by a nylon bristle (0.5 or 2.5g @3-4/sec). The path of the bristle was up to 2.0 cm within the receptive field of individual cells (Chapin & Lin, 1984). Radiolabelled deoxyglucose was injected iv 10 min after onset of a 55-min period of stimulation, after which rats were sacrificed and brains were prepared for autoradiography. Controls (n=5) received no stimulation.

Stimulation of FL, HL or TR activated cortex in regions expected on the basis of electrophysiological studies. Regions of activation had columnar shapes, when viewed in coronal sections, and were most prominent in Layers III, IV, Va and Vb. At 0.5g, FL and HL columns were 0.2-0.4 mm in diameter mediolaterally, and extended 0.3-1.0 mm anteroposteriorly. At 2.5g, the mediolateral extent was 0.4-0.8 mm. Also at 2.5g, TR columns extended 0.6-0.8 mm mediolaterally and 0.4-0.5 mm anteroposteriorly. Comparison of the autoradiograms with stained alternate sections revealed that columns were confined to regions defined by the granular strips in Layer IV, regions also sparsely invested with large Layer Vb cells. Deoxyglucose activation was not seen in perigranular regions. TR and HL were represented bilaterally in granular cortex; FL representation was contralateral only. The results confirm, in conscious animals, electrophysiological localization of cutaneous activity, and permit visualization of neural activity in all cortical layers.

582.8

SPECIALIZED VASCULARIZATION OF THE RAT SOMATIC SENSORY CORTEX. D.Zheng, D. Riddle and D. Purves*. Dept. of Neurobiology, Duke Univ. Med. Center, Durham, NC 27710

We have analyzed vascular density across the primary somatic sensory cortex in the rat to obtain a picture of differential metabolic demands, and, by inference, of differential electrical activity in this region. Adult Sprague Dawley rats were anesthetized, and perfused through the heart with saline followed by 10% glycerol and 20ml of ink (Rapidograph Universal 3080). The cerebral cortices were removed, flattened, and frozen at -20°C. Serial tangential cryostat sections were stained for succinic dehydrogenase, and the complete map of SI was reconstructed as previously described (Riddle et al, 1992 J. Neurosci. in press). Blood vessel density was measured using a computerized image analysis system. Capillary density was greatest in barrels and barrel-like structures of the major somatic representations (whisker pad, anterior snout, lower jaw, forepaw and hindpaw). The vascularization was least in the interbarrel regions, where the density averaged 40% less than that within the barrels; an intermediate density was found in the dysgranular cortex within and around SI. These findings, which are consistent with earlier observations in the monkey visual cortex (Zheng et al, 1991 J. Neurosci. 11:2622), indicate systematically different metabolic demands in various regions of the rat somatosensory cortex. Because the majority of energy consumption in the adult cortex is devoted to maintaining the ion gradients needed for neural signaling, these differences presumably reflect different average levels of electrical activity across SI.

582.9

HETEROGENEOUS DISTRIBUTION OF $GABA_A$ RECEPTOR COMPLEX IN RAT SOMATOSENSORY CORTEX (SMI). P.W. Land*[1], N.A. Reddy[1] and A.L. de Blas[2]. [1]Neurobiol., Anat. and Cell Sci., Univ. of Pittsburgh, Pittsburgh, PA 15261 and [2]Div. Mol. Biol. and Biochem., UMKC, Kansas City, MO 64110, USA.

Monoclonal antibody 62-3G1 was used to study distribution of $GABA_A$ receptor complex ($GABA_AR$) in rat SmI. This antibody recognizes the β_2 and β_3 subunits of $GABA_AR$ (de Blas, et al., 1988). $GABA_AR$ immunostaining occurs on neuronal somata and throughout the neuropil of all cortical laminae. Immunostaining is most intense in laminae IV and VI while laminae I - III and Va are moderately stained. Lightest staining occurs in Vb. Thus, except for the pattern observed in lamina Vb, $GABA_AR$ distribution parallels both the arrangement of GABA containing terminals and the intensity of cytochrome oxidase (CO) activity described previously for rat SmI.

Interestingly, $GABA_AR$ immunostaining is not uniform within lamina IV. The CO-dark hollows of vibrissae-related barrels and barrel-like structures representing distal extremities are intensely immunoreactive. By contrast, barrel septa and surrounding dysgranular cortex stain lightly. These data indicate that regional variations in $GABA_AR$ expression in the cortex reflect 1) density of GABAergic input, and 2) overall level of neuronal activity.
(Supported by National Science Foundation Grant IBN 91-10731).

582.10

GLUTAMATE AND ASPARTATE IMMUNOREACTIVITY IN TERMINALS OF THALAMOCORTICAL FIBERS V.N. Kharazia*, A. Rustioni and R.J. Weinberg. Dept. of Cell Biology & Anatomy, University of North Carolina, Chapel Hill, NC 27599.

Pharmacological and biochemical studies suggest that thalamocortical afferents use an excitatory amino acid transmitter. We have combined anterograde transport of HRP with postembedding immunocytochemistry to study the content of glutamate, aspartate, and GABA in terminals of thalamocortical fibers within rat somatosensory cortex (SI). WGA-HRP aminocaproate (E-Y labs, 2% in 2% DMSO) was injected into the thalamus of anesthetized rats. Injections were targeted at VPL, but also spread into other nearby thalamic regions. After 48 h survival, animals were perfused with 2.5% glutaraldehyde, 0.5-1% paraformaldehyde, and 0.2% picric acid in phosphate buffer pH 7.4. Brains were postfixed in the same fixative 2-8 h. Vibratome sections of somatosensory cortex were cut and reacted for peroxidase histochemistry using a tungstate/TMB method. Thin sections from plastic-embedded wafers were mounted on nickel mesh grids and processed for EM postembedding immunocytochemistry for glutamate, aspartate and GABA, using primary antisera from Arnel.

Thalamocortical terminals in SI (identified by crystals of reaction product) contained low levels of GABA, and significantly higher levels of glutamate than did dendrites, glia, or nearby synaptic terminals identified by their high concentration of GABA staining as likely to be GABAergic. Thalamocortical terminals also contained more aspartate than did GABA-positive terminals; however, dendrites and neuronal somata contained more aspartate than either class of terminal. These results support the hypothesis that thalamocortical synapses use glutamate as neurotransmitter, and raise the possibility that some may also use aspartate.

582.11

AXOSOMATIC SYNAPSES ONTO INTRINSICALLY BURSTING NEURONS IN RAT SmI (BARREL) CORTEX.

E.L. WHITE*, Y. AMITAI and M. J. GUTNICK, FACULTY OF HEALTH SCI., BEN-GURION UNIV., BEER SHEVA, ISRAEL.

Recording under threshold conditions for synchronization shows that most regular spiking cells have strong and dominant inhibitory potentials, while intrinsically bursting cells show little evidence of inhibition (Chagnac-Amitai and Connors, 1989). This study is part of a broader effort to determine if differences in inhibitory responses are reflected in the numbers or in the spatial distribution of symmetrical, presumed inhibitory synapses. Our approach is to use intracellular recording to classify neurons by their intrinsic firing properties, and to label them using HRP or biocytin. Then the neurons are processed for EM and serial thin sectioned. The somata and proximal dendrites are reconstructed and the distribution of their synapses displayed, using a newly developed system for capturing video images directly from the electron microscope and for making 3-D reconstructions from them. Two somata of intrinsic bursters were calculated to have surface areas of 541 μm2 and 855 μm2. These cells had 54 and 86 synapses respectively, that is, one axosomatic synapse per 10 μm2 of somatic surface area. The results of ongoing studies will determine whether this ratio of synapses to somatic area holds for other bursting neurons and whether it is shared by regular spiking neurons. NIH 20149, Israel Acad. of Sciences 236/90.

582.12

MORPHOLOGY OF ELECTROPHYSIOLOGICALLY IDENTIFIED NEURONS IN ADULT RAT NEOCORTEX.

R. Schröder* and H.J. Luhmann. Institute of Neurophysiology, University of Cologne, D-5000 Cologne 41, FRG.

We were interested in the morphological and electrophysiological properties of single supragranular cells in primary somatosensory cortex of mature rats. Intracellularly recorded neurons were analyzed *in vitro* in their intrinsic membrane properties and their synaptic inputs. After functional characterization neurons were labelled by intracellular injection of biocytin. Out of 85 stained cells, 33 neurons with a relatively complete axonal and dendritic arborization pattern were selected for detailed graphical reconstruction and subsequent morphometrical analysis. Resting membrane potential V_m was -81.2 ± 4.6 mV (mean ± SEM) and neuronal input resistance R_N was 38.9 ± 4.6 MΩ. All cells, including four sparsely spiny neurons, were classified as regular spiking cells with a prominent inhibitory synaptic input, consisting of a biphasic IPSP. Somatic area ranged from 96 to 744 μm^2 and did not correlate with R_N. The horizontal extent of the basal dendritic field (310 μm) was significantly (*t* test, $p<0.01$) larger than the lateral spread of the apical dendritic field in layer I / upper layer II (207 μm), indicating that synaptic inputs are integrated over a larger spatial domain in more proximal regions. Descending axons could be traced up to 1.4 mm from the soma and total length of intracortical axon collaterals ranged up to 6 mm.

We were unable to detect any significant correlation between the morphological and electrophysiological properties tested, suggesting that supragranular regular spiking cells represent a functionally homogeneous group.

Supported by SFB 194/B4 and a grant from Ministerium für Wissenschaft und Forschung in NRW (HJL).

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS: PHYSIOLOGY

583.1

CENTRAL EFFECTS OF HISTAMINE ON THE STATISTICAL FEATURES OF UNIT ACTIVITIES IN SOMATOSENSORY CORTEX OF CATS.

Yang Tsau, Yu-Zhong Yao, Gan-Quan Liu, Pei-Xi Chen, Lawrence B Cohen*. Dept. of Physiol., Sun Yat-Sen U. of Med. Sci., Guangzhou, P.R.O.China.

We studied the effects of histamine (HA) on unit activities in the somatosensory cortex of cats. Microiontophoresis and t-test of mean value of inter-spike intervals (MISIs) and normalized power spectrum density function (NPSDF) methods were used. We found that: 1) HA (0.5M) both raised (n=14) and reduced (n=6) MISIs in the recorded neurons (n=20), suggesting HA has both inhibitory and excitatory effects on the cortical neurons. 2) Cimetidine (0.1M) excited the neurons slightly and blocked the inhibitory action of HA (n=10), suggesting the inhibitory effect of HA is via the H2 receptor. Diphenhydramine (0.1M) inhibited neurons and did not block the inhibitory action of HA (n=9), suggesting the excitatory effect is via the H1 receptor. 3) HA reduced both the peak frequency and value of NPSDF ($p<0.01$, n=20) and induced a 'post-inhibitory rebound' of the peak value with the peak frequency remaining low after iontophoresis, suggesting HA modulates the cortical rhythm and has after-effects.

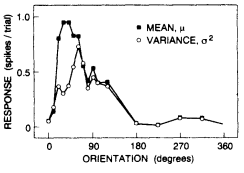
583.2

SPIKE STATISTICS AND RECEPTIVE FIELD PROPERTIES OF NEURONS IN PRIMARY SOMATOSENSORY CORTEX OF RAT.

D. Kleinfeld* and R. A. Stepnoski. AT&T Bell Labs., Murray Hill, NJ 07974.

Neurons within the barrel field of rat S1 cortex encode features of the deflection of a vibrissa (Simons, J. Neurophys. 41, 1978). Here we report on the variability of this code in response to multiple presentations of a stimulus. Our measurements consisted of single unit recordings from adult Sprague-Dawley rats anesthetized with urethane. Each stimulus trial consisted of displacing a vibrissa from its natural position to a new position and, after a brief rest, returning the vibrissa to its natural position. The new positions were chosen at random from a set of up to 24 different combinations of orientation, maximum angular displacement (~ 5° to 10°) and angular velocity (~ 50°/s to 500°/s). Stimuli, chosen at random from the set, were presented at 1 s intervals and the entire set was presented up to 200 times. We observed: (1) The mean number, μ, of spikes elicited in units at the depth of layer 4 was relatively low, $\lesssim 2$ spikes per trial, even for apparently optimal stimuli. The trial-to-trial variance in the number of spikes, σ^2, was equal or significantly less than its mean, *i.e.*, $\sigma^2 \leq \mu$ (Fig.). (2) Units at the depth of layers 2/3 fired multiple spikes per trial and exhibited a large, nonstationary variance. (3) Units with similar orientation preference appear to be spatially clustered within a barrel.

The low variance in layer 4 units is reminiscent of that found in peripheral pathways and, notably, contrasts with the large variance, $\sigma^2 \simeq (2-4)\mu$, for units in cat and monkey V1 cortex (*e.g.*, Tolhurst *et al.*, Vision Res. 23, 1983). The implication of the above observations for population coding is under investigation.



583.3

TEMPORAL INTEGRATION AND TEMPORAL TRANSFER PROPERTIES OF RAT SOMATOSENSORY CORTICAL NEURONS.

K. Selpien and H.R. Dinse. Dept. Neuroinformatic, Theoret. Biol. Ruhr-Univ. Bochum, RUB, D-4630 Bochum, FRG. (Spon: ENA)

We studied the temporal transfer characteristics (TTC) of cortical neurons recorded in the hindpaw representaion of SI in Urethane anesthetized rats. Tactile stimuli (TS) of 8 ms duration were applied by use of a computer-controlled mechanical stimulator as small skin indentations. Neuron responses were quantitatively analysed based on PSTHs. We varied systematically interstimulus intervals (ISI) between 20 and 200 ms and the number of stimuli (NS) in each train (2 to 6). Betweeen each train was a pause of 3 sec. By this we were able to investigate the transition from transient to steady state conditions. In the double-click condition, i.e. using two stimuli, we found the often described TTC with low cut offs at ISIs between 20 and 80 ms. However, this TTC was not replicated when the ISI effect to the third or higher TS was tested. Accordingly, the overall responses depended not only on ISI, but in addition on the number of the stimulus and its ISI condition. Further analysis revealed that amplitude measures of peak height yielded only partial insight in neuron reponse properties. Inhibition seen for short ISIs appeared to be uncorrelated with the preceeding response. This leads to the observation that inhibitory action is restricted to given numbers of TS revealing strong sequencing effects. The results are discussed in respect to modulation of neuron responses under natural conditions that are characterized by severe temporal constraints.

Supported by the DFG

583.4

LAMINAR ANALYSIS OF THALAMOCORTICAL INTERACTIONS IN SOMATOSENSORY SYSTEM OF RATS.

K.D. Alloway*, M.J. Johnson, and M.B. Wallace. Dept. of Neuroscience & Anatomy, M.S. Hershey Medical Center, Penn State University, Hershey, PA.

Thalamocortical neurons terminate within cortical layers IIIb and IV where they synapse upon the soma and proximal dendrites of local neurons, and on distal dendrites extending from neurons located in supra- or infragranular cortical layers. In view of differences in thalamocortical inputs to cortical layers and in interlaminar connections, this study used electrophysiological techniques to detect a laminar sequence of sensory activation.

Single neuron responses to computer-controlled tactile stimulation were simultaneously recorded in the ventrobasal thalamus and somatosensory cortex of halothane-anesthetized rats. Hairy and glabrous skin RFs were activated by either air puffs or mechanical indentations, respectively. Response magnitude and latency analyses clearly indicate that thalamocortical inputs initiate responses in cortical layers IIIb and IV. Response magnitude is next highest in layers II, III, and lowest in layers V, IV. By contrast, response latency was shorter in layers V, VI than in layers II, III. Cross-correlation analyses of laminar differences in thalamocortical connection strength are currently in progress. These preliminary results provide evidence in support of activity proceeding along serial and parallel circuits within a cortical column. Supported by NIH grant NS29363 and PSU RIG430-22.

583.5

CROSS CORRELATION STUDIES OF INTRACORTICAL NEURONAL CONNECTIONS IN RAT SOMATOSENSORY CORTEX. S.C. Silbert, T.A. Woolsey* & H. Burton, Dept. Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

We simultaneously identified the responses of several single neurons in the posteromedial barrel subfield. Two independently controlled multibarrel iontophoretic electrodes were positioned in neighboring whisker representations. Anesthesia with chloral hydrate, supplemented with N_2O_2 and halothane, was stable for many hours. On-line spike waveform analysis, perievent time histograms, and cross-correlograms guided identification of related cell pairs. Iontophoresis onto cortical neurons and controlled whisker stimulation enabled characterization of the transmitters used by physiologically connected cells. Several cell pairs, with and without shared whisker inputs, had correlated activity. These studies show whether cortical neurons receive common or direct interbarrel synaptic connections and which transmitters mediate these interactions. (Supported by NINDS 22012)

583.6

RESPONSES OF CAT SI RAPIDLY ADAPTING NEURONS TO CONTROLLED MECHANICAL STIMULATION. H. Esteky*, M.J. Pettit and H.D. Schwark. Department of Biological Sciences, University of North Texas, Denton, TX 76203.

Cutaneous rapidly-adapting first order fibers of many mammals have been classified by their responses to the velocity and displacement components of controlled stimuli (Burgess and Perl, 1973). We have used constant-velocity ramp stimuli and a modification of the Burgess and Perl criteria (1973) to study single neurons in the forelimb region of primary somatosensory cortex of anesthetized cats.

Thirty-five of 140 units responded well to single probe stimuli and were analyzed in detail. G_1/F_1 units (8/35) responded to ramp stimuli with 1-3 action potentials at the beginning of the ramp and none during the rest of the ramp. This group had little spontaneous activity and required relatively high ramp velocities. G_2/F_2 units (9/35) responded throughout the ramp. They had higher spontaneous activity and responded to lower ramp velocities than G_1/F_1 units. G_{int}/F_{int} units (14/35) showed intermediate properties: an initial brief response was followed, after a period of low or no activity, by a response during the remainder of the ramp. Five units had responses which were combinations of these patterns, suggesting convergence between groups.

The instantaneous firing frequency of all units generally increased with increasing ramp velocities. The average firing frequency of G_1/F_1 and G_{int}/F_{int} units also increased in a graded manner with ramp velocity, whereas the response of G_2/F_2 units was discontinuous. These results suggest that rapidly-adapting cells in SI cortex which respond to punctate ramp stimuli have response patterns similar to those of first order afferents. Supported by NIH grant NS25729.

583.7

RECEPTIVE FIELD AND RESPONSE PROPERTIES OF SIV SOMATOSENSORY NEURONS. H.R. Clemo* and B.E. Stein. Dept. of Physiology, Med. Coll. VA, VA Commonwealth Univ., Richmond,, VA 23298.

The somatosensory region (SIV) of the anterior ectosylvian sulcus (AES) is an important source of inputs to the superior colliculus (SC) a structure involved in orientation and localization behaviors. SIV also shares reciprocal connections with somatosensory cortical regions, SI and SII, involved with tactile perception and discrimination. However, it is not known whether the properties of SIV neurons are similar to those in SI and SII or to those in the SC. Therefore, this investigation compared the response properties of SIV neurons to those of the SC, SI and SII.

The receptive fields and responses of SIV somatosensory neurons (n=22) in cats (n=3) were examined. All neurons were rapidly-adapting and activated by low threshold mechanoreceptors. While the majority required high velocity stimulation and were broadly tuned to different directions of stimuli, few exhibited directional selectivity or spatial summation, and none showed spatial inhibition. Most exhibited medium to large-sized receptive fields that contained areas ('best areas') where a significantly higher response could be elicited than elsewhere in the receptive field.

The receptive field size, rapidly-adapting nature, and velocity sensitivity of SIV neurons are features more similar to those observed in the SC than SI or SII.

Supported by BNS 8719234 and EY 05554.

583.8

OPTIC NERVE EXCITATION OF PERICRUCIATE CORTEX IN CAT. A.L. Towe* and Y. Gahery. Dept. of Physiol. and Biophys., Univ. of Wash. Sch. of Med., Seattle, WA 98195

Single neurons were recorded on both sides of the cruciate sulcus in chloralose-anesthetized cats. About 24% of the cutaneous small-field neurons and all of the wide-field neurons were excited by input over each optic tract. In addition, about 7.4% of the neurons failed to respond to skin stimulation, but were excited by optic chiasm input. Response latencies to optic chiasm stimulation fell into four distinct groups. Timing relations suggested the first surge arrived via superior colliculus and the third via visual cortex; the routes for the second and fourth surges remain unidentified. These was no indication that these separate surges converged onto the same neurons. Input via the contralateral optic tract evoked activity at a higher threshold, produced fewer spikes per discharge, and has a longer latency than did the ipsilateral optic tract. The difference in response latencies was largest for neurons responding in the first surge, and decreased progressively through the later surges. The mean latency of response to optic chiasm input was the same as that to contralateral forepaw stimulation, though optic chiasm input evoked more spikes per response. It is suggested that the visual input to pericruciate cortex serves to modulate on-going cortical output and thereby modulates the behavior of the animal. (Supported by USPHS grant NS00396)

583.9

DISTRIBUTION OF PROPRIOCEPTIVE INPUT TO PERICRUCIATE CORTEX OF AWAKE CATS. Jefferson C. Slimp* and Arnold L. Towe. Dept of Rehabilitation Medicine and Dept of Physiology and Biophysics, Univ. of Wash. Sch. of Med., Seattle, Wa 98195.

A previous report (Exp.Neur., 107:78-96) concluded that the data from three restricted but well sampled regions of pericruciate cortex of awake cats failed to support any model of organization that emphasized local, bounded regions within which all neuron response properties are the same. The data reported here, taken from a larger extent of pericruciate cortex in four cats, confirms this conclusion. Focusing only on proprioceptive neurons (ignoring the interspersed cutaneous neurons), most tracks contained a mixture of modalities: joint, muscle/tendon/joint, and active (during movement but not driveable). Many neurons responded to movement or two or more joints. About half of the neurons recorded simultaneously or sequentially showed reciprocal sensitivities (e.g., flexion/extension or adduction/abduction). However, given a neuron with one pattern of response, the probability that the next neuron would show that same pattern was equal to that expected by chance, i.e. the distribution of input was random. Tracks in which many elbow-sensitive and wrist-sensitive neurons were found yielded similar results, although there was a slight tendency for elbow and wrist neurons to clump. Neurons responsive to movement of two joints showed no preferred relationships: e.g., there were equal numbers of elbow flexion/wrist flexion as elbow flexion/wrist extension, etc. These findings suggest that restricted areas of cerebral cortex receive input from more diverse and scattered sources than had previously been thought. Even somatotopy was rather more fuzzy than precise. Neighboring neurons with reciprocal response patterns behaved as though they mutually inhibit one another; they could serve to code joint position through their differential action. (This work was supported by USPHS Grants NS00396 and NS05082.)

583.10

SCALP POTENTIAL TOPOGRAPHIES EVOKED BY SURAL NERVE STIMULATION IN MAN. R. Dowman[*1], T.M. Darcey[2] [1]Dept. Psychol., Clarkson University, Potsdam N.Y. 13699, [2]Dartmouth Med. Sch. and V.A. Med. Ctr., White River Jct. VT 05009.

Potentials evoked by sural nerve stimulation were recorded from 30 scalp locations in 15 healthy humans. Eight different stimulus levels were given. The 3 lowest levels were innocuous and the 3 highest were noxious, as defined by physiological criteria.

The 512 ms epoch following the evoking stimulus could be separated into 6 stable periods (SP), i.e., consecutive time points where the patterns were the same. For each SP the onset and offset latencies and the topographic patterns were comparable across subjects. The topographies for each of the first 5 SPs (59-88 ms, 93-117 ms, 135-156 ms, 180-219 ms, and 225-275 ms, respectively) were stable across all stimulus levels, suggesting that the sources (neural generators) underlying a given SP were the same at noxious and innocuous levels. The topographies for SP6 (287-325 ms) were stable across noxious but not innocuous levels, suggesting that the sources underlying SP6 are different at noxious vs. innocuous levels. We are presently analyzing these data using dipole source modeling to test hypotheses about the location of the sources generating these potentials and to quantify the source amplitude and duration. This analysis will help determine whether these putative sources behave similarly to neurons involved in innocuous and noxious somatosensory cortical processes identified in animal studies.

583.11

SOMATOTOPIC ORGANIZATION OF SNOUT REPRESENTATION IN THE CORTEX OF THE SWINE REVEALED BY SOMATIC MAGNETIC EVOKED FIELD. Y. C. Okada*, H. Nowak and C. Xu. Center for MEG, V A Med. Center 87131 and Depts. Neurol. & Physiol., Univ. New Mexico Sch. of Med., Albuquerque, NM 87018.

A high-resolution magnetometry was used to determine the representation of snout in the cerebral cortex of the juvenile farm swine (3-6 weeks old, 5-10 kg). Each of five locations on the snout was transcutaneously stimulated with an array of concentric bipolar electrodes (0.3 ms, 6 mA) attached to an isolated stimulator to produce activity in the cerebral cortex of the swine anesthetized with ketamine (10 mg/kg/hr, i.p.) and xylazine (4 mg/kg/hr, i.p.). The somatic magnetic evoked field (MEF) associated with the neuronal activity was measured on a plane 1.2 mm above the apex of the intact dura mater with a 4-channel superconducting magnetometer with a passband of 0.3 Hz to 1 kHz, after removing the scalp and the dorsal half of the skull.The stimulation of each site produced an MEF detectable on single epochs. The topography of averaged MEF normal to the measurement plane indicated that the cortical tissue active at 15 and 19 ms after stimulation was located systematically around the contralateral coronal gyrus, confirming the electrophysiological work of Craner (Ph.D. Thesis, East Carolina Univ., 1988). The lateral, top, midline and bottom regions of the snout were located in the medial, posterior, lateral and anterior portions, respectively, of the snout area. Some areas of the snout were represented in two sulci on each side of the gyrus. Together with the field potential data, these results indicate the existence of a somatotopically organized projection of the snout in the coronal gyrus and in the surrounding sulci.

Supported by Dept. of Vet. Affairs, NSF grant DIR8820556 and NIH grant RO1-NS21149.

583.12

LOCALIZING DIPOLES OF SOMATOSENSORY EVOKED POTENTIALS IN MONKEY BRAIN RECONSTRACTION. H. Nishijo*, N. Hayashi[1], T.Ono, S. Endo[1], T. Musha[2], and S. Homma[3] Depts. Physiol. & [1]Neurosurg., Fac. Med.,Toyama Med. & Pharmaceu. Univ., Toyama 930-01, [2]Brain Function Lab., Kawasaki 213, and [3]Dept. Physiol., Meiji College of Oriental Med., Kyoto 629-03, Japan.

We developed dipole localization (dipole tracing, DT) by a boundary element method, using a 3-dimensional reconstructed model of the cerebral cortex. The DT was used to investigate dipoles of somatosensory evoked potentials (SEPs) produced by electrical stimulation of the median nerve. Potentials were recorded with 21-27 electrodes placed on the dura in 3 anesthetized monkeys. The estimated dipoles were stereotaxically superimposed on magnetic resonance images and specimens of brain.

The results indicated generators with 7 ms latency located in the contralateral thalamus, and in the posterior wall of the central sulcus, area 3b of the somatosensory cortex with the 9-11 ms latency range. Generators were also located in the anterior parietal cortex, areas 1 and 2, with latencies of 12-13 ms; and in the posterior parietal cortex, area 5, with latencies of 15-20 ms. Locations and latencies of these generators were confirmed by recording multiple unit activity from the sensorimotor cortex, and area 5 of the same monkeys investigated by SEPs. It was concluded that DT could accurately localize electric dipoles, and that successive generators of SEPs by median nerve stimulation are located in the thalamus, and cortical area 3b, areas 1 and 2, and area 5.

583.13

DIFFERENTIATION BETWEEN MAGNETIC SIGNALS FROM HUMAN SI AND SII CORTICES. R. Hari*, M. Hämäläinen and J. Karhu. Low Temp. Lab., Helsinki Univ. Technol., 02150 Espoo, Finland.

Peaks around 100 ms in the somatosensory evoked magnetic field (SEF) have been thought to originate at the second somatosensory cortex SII. We confirmed this with 4 different methods, analysing SEFs recorded from healthy humans with a 24-channel planar SQUID gradiometer.

(1) Comparison with functional landmarks: Field patterns to auditory and ipsilateral median nerve stimulation were modeled with equivalent current dipoles (ECDs). ECDs for both SEFs and auditory evoked magnetic fields (AEFs) were close to the Sylvian fissure, with the ECDs for SEFs situated slightly higher than those for AEFs. (2) Comparison of the ECD locations with individual anatomy, derived from MR images, suggested activation of the upper and lower lips of the Sylvian fissure during SEFs and AEFs, correspondingly. (3) Time-varying two-dipole model with fixed source locations satisfactorily accounted for the pattern of SEFs to contralateral median nerve stimulation. One dipole waveform corresponded to activation of hand SI and the other to SII area; the sources were about 4.5 cm apart and had different orientations and time dependencies. (4) Very similar results were obtained with a MUSIC algorithm which automatically determines the number and locations of time-varying dipole sources.

We conclude that it is possible to reliably differentiate between neuromagnetic responses arising from the human SI and SII cortices.

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS: THALAMUS

584.1

EXPRESSION OF CALCIUM BINDING PROTEINS IN ADULT RAT THALAMUS FOLLOWING CORTICAL LESIONS. V. Rema, M.E. Diamond, L. Van Eldik† and F.F. Ebner* Institute for Developmental Neuroscience and Department of Pharmacology†, Vanderbilt Univ. Nashville, TN 37203.

To test the hypothesis that thalamic cell survival after cortical damage depends on increased expression of calcium binding proteins, we used immunocytochemistry to estimate the levels of three calcium binding proteins (calbindin, parvalbumin, calmodulin) and S100ß at 5, 7 & 14 days following subtotal aspiration lesions of rat SI (barrelfield) cortex. At 5 days Nissl changes and reduced levels of calbindin were visible in the thalamus ipsilateral to the lesion, whereas neuronal changes in parvalbumin and calmodulin expression were not evident. However, a marked increase in astrocytic parvalbumin staining was seen in the posterior nucleus. By 7 days calbindin expression increased on the lesion side. Parvalbumin expression was also elevated in VPM, but most of the reaction product appeared to be in processes arising from the reticular nucleus. All thalamic neurons are normally calmodulin positive, but those labeled in the area affected by the lesion appeared smaller, paler and fewer in number. At 14 days, chromatolytic neurons were swollen, pale, contained vacuolated cytoplasm or were disintegrating with only ghosts remaining. These neurons stained lightly for calbindin and calmodulin, but outside the reactive zone both neurons and glia showed greatly increased parvalbumin expression. Glial cells expressed increasing levels of S100ß protein during the period studied. Our results suggest that in the thalamus there is a progressive increase in the intensity of staining for all four proteins examined during the first two weeks after cortical injury. Most of the increase can be attributed to glial cells, which show dramatically increased staining over time for the calcium binding proteins tested. (supported by NIH grant #NS13031)

584.2

AREAL DISTRIBUTION OF CORTICOTHALAMIC PROJECTION NEURONS TO THE POSTERIOR THALAMIC COMPLEX IN RAT SOMATOSENSORY CORTEX. Karen E. Good* and H. P. Killackey, Department of Psychobiology, University of California, Irvine, 92717-4550.

We previously reported in rats, cortical projection neurons to the Ventral Posterior Nucleus (VP) and to the medial division of the Posterior Thalamic Complex (PoM) have different laminar origins; cortico-VP projections arise from neurons in superficial layer VI (VIa), whereas cortico-PoM projections arise from neurons in deep layer VI (VIb) and layer V. Thalamocortical afferents from VP and PoM to the posteromedial barrel subfield (PMBSF) are distributed in a complementary areal fashion (Koralek et.al., 88; Lu and Lin, 85). Furthermore, corticothalamic neurons whose target is VP have an areal distribution reflecting the pattern of layer IV cell aggregations in the PMBSF (Chmielowska et.al., 89). This study examines if the distribution pattern of cortico-PoM neurons is complementary to that of cortico-VP neurons.

Stereotaxic pressure injections of WGA-HRP or fluorescently labeled beads were confined to PoM. Flattened hemispheres were sectioned at 30-40 um, and the tissue processed for cytochrome oxidase. Preliminary results indicate that retrogradely labeled cortico-PoM neurons are not distributed uniformly. In preliminary analysis of tangential sections, we observed a restricted pattern of labeled cortico-PoM neurons in layer VI. In one case, labeled cells in three consecutive sections at 40 um had a similar pattern of distribution (920-1080 um from pial surface). In layer VI, labeled cells were aligned in one of three parallel bands which appear to correspond to the septae of the PMBSF. Each of these three bands was 60-150 um wide and 1000 to 1500 um long, extending in an anterolateral to posteromedial fashion. Sections were stained with bisbenzamide revealing a uniform laminar cytoarchitecture, thus potential artifactual discontinuities in these sections are unlikely. In addition, labeled cells were found in surrounding areas corresponding to dysgranular cortex. We tentatively conclude that cortico-PoM projection neurons are distinct in both areal and laminar origins from cortico-VP projection neurons.

584.3

ELECTRON MICROSCOPIC ANALYSIS OF CORTICAL INPUTS TO THE THALAMIC RETICULAR NUCLEUS (TRN) IN THE MONKEY. A.M. Williamson*, D.D. Ralston, and H.J. Ralston, III. Dept. of Anatomy and the W.M. Keck Foundation Center for Integrative Neuroscience, U.C.S.F., San Francisco, Ca. 94143.

The TRN is composed of a thin layer of GABA-ergic cells surrounding the lateral and rostral thalamus, and lying medial to the internal capsule. Its function is not completely understood, but appears to integrate ascending information from the thalamus, several brainstem nuclei and collateral innervation from corticothalamic inputs. We examined the input from the somatosensory cortex to determine the nature of the synaptic contacts formed in TRN, and to describe the placement of those contacts on TRN neurons.

To label the cortical inputs into TRN, we injected small amounts (<0.2 µl) of 4% WGA-HRP in the somatosensory cortex of *M. fascicularis*, allowed the animals to survive 2-4 days, perfused with mixed aldehydes, and prepared tissue for electron microscopic visualization of the transported WGA-HRP.

Results of these experiments show that cortical projections to the TRN end as synaptic terminal profiles similar to those described for cortical projections to thalamic sensory nuclei. The terminals are small, contain round vesicles, and form asymmetric contacts with small caliber dendrites in TRN. We have also observed in single section what appear to be dendro-dendritic contacts between TRN neurons. We conclude that cortical projections to TRN neurons can activate these GABA-ergic cells, some of which contact one another, to result in feed forward inhibitory mechanisms. Supported by NS-23347 and NS-21445.

584.4

QUALITATIVE ASSESSMENT OF TACTILE RESPONSES IN ALERT MONKEY VENTRAL POSTERIOR NUCLEI. Susan Warren* and Jennifer E. Taylor. Dept. of Anatomy, University of Mississippi Medical Ctr., 2500 North State Street, Jackson MS 39216-4505.

The response properties of ventral posterior (VP) neurons were characterized in the alert monkey by application of a variety of static and moving tactile stimuli. Data were obtained from a total of 75 penetrations in both thalami of an adult, female rhesus macaque. Utilizing tungsten microelectrodes (20-50 MegOhms) yoked with extracellular, chronic recording techniques, 724 single neurons and multiple neuron clusters were recorded. Most cells were spontaneously active, some firing multispike bursts (3-8 spikes/burst). Single VP cells displayed either rapidly adapting (RA) or slowly adapting (SA) responses, or, less frequently, an SA response with an Off discharge in response to application of punctate stimuli. Neurons responsive to motion, some of which displayed a direction preference, were located on the digits and palm or the face. Six cells with receptive fields on the hand were tested with a moving bar pattern on the OPTACON. Fifty percent of the cells tested responded. All tested cells displayed a slowly adapting discharge to static pressure and 5 of the six responded to brushing over the cell's receptive field. Von frey force threshold for OPTACON responsive neurons was 676 mg, that of the unresponsive cells was 1.2 gm. Low frequency stimuli (20 Hz) appeared more effective in driving the OPTACON responsive VP neurons than did high frequency stimuli (100 Hz). These preliminary results suggest that ventral posterior neurons encode specific features of a tactile stimulus including motion, direction and temporal features of a moving bar pattern. Supported by NINDS Grant NS27996.

584.5

PHYSIOLOGICAL MAPS OF RETROGRADELY LABELED SI-PROJECTING NEURONS IN THE SOMATOSENSORY THALAMUS OF THE RAT. P. Herron*. Anatomy and Neurobiology, The Univ. of Tennessee, Memphis, Col. of Medicine, Memphis, TN 38163

This study examined the somatotopic organization and modality of inputs to thalamocortical neurons in the somatosensory thalamus retrogradely labeled by injections of retrograde tracers into the forepaw representation area of the primary somatosensory area (SIf). Conventional extracellular electrophysiological mapping techniques were utilized. Injections of retrograde tracers were made into SIf before or after the somatosensory thalamus was electrophysiologically mapped.

Most retrogradely labeled neurons were located in the ventroposterior lateral nucleus (VPL), rostral and medial region of the posterior complex (POm), and the central lateral nucleus of the intralaminar complex. We recorded from labeled neurons in VPL and POm. Combined retrograde labeling and mapping results show that SIf-projecting neurons in VPL: 1) were overwhelming activated by low-threshold mechanical stimulation of small receptive fields on the glabrous skin of the forepaw, 2) had responses that slowly adapted to maintained stimulation, and 3) were somatotopically organized. Retrogradely labeled neurons in POm were not driven as readily as those in VPL; however, when driven, they were typically activated by intermediate- to high-threshold stimulation.

We conclude that SIf-projecting thalamocortical neurons in VPL receive primarily low-threshold, slowly adapting inputs from the cutaneous surface of the forepaw. Given their rostral location within VPL, neurons that project to the SIf digits area do not overlap with those that project to the SII forepaw representation area. (Supported by The Center for Neuroscience of The Univ. of Tennessee, Memphis).

584.6

PHYSIOLOGICAL STUDIES OF THALAMOCORTICAL RHYTHMS, RECORDED *IN VITRO* IN A BRAIN SLICE PREPARATION.
Douglas A. Coulter* Department of Neurology Medical College of Virginia, Richmond, VA 23298

The mouse thalamocortical slice, developed by Agmon and Connors (*Neuroscience 41 (1991): 365*), has been employed to study physiological properties of spontaneously occurring and stimulus-evoked thalamocortical rhythms *in vitro*, under conditions where rigorous control of the extracellular environment is possible, and where high resolution current- and voltage-clamp recordings are feasible. This slice maintains the reciprocal connections between somatosensory thalamus and cortex, by slicing the brain at a particular angle.

Stimulation in the ventrobasal complex of the thalamus (VB) elicited orthodromic activation of restricted areas of somatosensory (SS) cortex, which consisted of mono- and polysynaptic E- and IPSPs. In deep cortical layers, stronger VB stimulation could also evoke antidromic activation of a proportion of cortical neurons. Similarly, stimulation of SS cortex elicited orthodromic EPSPs and antidromic activation of VB neurons. In 60% of preparations, at least one slice exhibited spontaneous, low-frequency oscillations, in the frequency range of 3-15 Hz. These oscillations were restricted to SS cortex and VB, and did not occur in slices cut so as to destroy the reciprocal connections between these areas. In paired field recordings, tight coupling between areas of SS cortex and VB was evident. Extracellular single-unit recordings in VB and SS cortex detected spontaneously active neurons firing bursts of spikes coinciding with the thalamocortical rhythms. Intracellular recordings in middle SS cortical layers revealed spontaneously active neurons firing in the range of 10 Hz, generated by underlying bursts of EPSPs.

This slice preparation may prove valuable in exploration of cellular and pharmacological factors important in generation, maintenance, and termination of normal and pathological thalamocortical rhythms.

584.7

CORTICOFUGAL INFLUENCE OF SI CORTEX ON VENTRAL POSTERIOR THALAMIC NEURONS IN THE SQUIRREL MONKEY. T. J. Morrow*, J.A. Kiritsy-Roy and K.L. Casey., Depts. of Neurology and Physiology, University of Michigan and VAMC, Ann Arbor, MI 48105.

We recently reported that the somatosensory responsiveness of ventral posterior (VP) thalamic neurons is modulated during changes in arousal in awake monkey (*J. Neurophysiol.*, 67: 305-317, 1992). Shifts in arousal are often accompanied by marked changes in cortical activity as evidenced in the EEG. Accordingly, we wished to investigate how changes in corticofugal activity might alter the somatosensory processing in primate thalamus.

We studied the influence of corticofugal activity on the responses of VP thalamic neurons to repetitive somatic stimuli in the lightly anesthetized squirrel monkeys by suppressing primary somatosensory (SI) cortical activity with topically applied magnesium sulfate. The effect of $MgSO_4$ was confined to SI and the immediately adjacent cortex and suppressed both spontaneous EEG activity and the cortical somatosensory evoked response. Suppression of SI increased the number of somatically evoked spikes discharged by all VP neurons studied. Three neurons exhibited a marked increase in receptive field size during SI deactivation, including one wide dynamic range cell which responded to both noxious pinch and to CO_2 laser stimulation within its RF. Cortical suppression produced no consistent changes in spontaneous activity. **We conclude that, in the anesthetized monkey, SI corticofugal activity serves primarily to inhibit the sensory transmission of ventral posterior thalamic neurons.** This contrasts with our previous work in rat (*J. Neurosci.*, 5: 2871-2978, 1985), which showed SI activity to primarily facilitate VP activation.

584.8

EXCITATORY POSTSYNAPTIC POTENTIALS (EPSPs) EVOKED IN NUCLEUS RETICULARIS BY MINIMAL STIMULATION OF THALAMIC RELAY NUCLEI. S. Radpour, J. Deuchars and A.M. Thomson (SPON: Brain Research Association) Dept. Physiology, Royal Free Hospital School of Medicine, London NW3 2PF, UK.

Nucleus reticularis (RTN), the major source of inhibition in rat thalamus, receives major inputs from cortex via internal capsule (IC) and from thalamic relay neurones. This study compared EPSPs evoked from IC with those evoked by micro-stimulation of relay areas, recorded intracellularly *in vitro* from RTN neurones identified electrophysiologically and morphologically. Both types of EPSPs were fast (width at half amplitude 6.9±2.8, IC and 5.1±1.1 ms, relay) at around -80mV. Although the peak of the EPSP often exhibited a conventional voltage relation, EPSPs increased in duration with depolarization (De Curtis et al). Both EPSPs fluctuated in amplitude, the relay input dramatically, with a peak standard deviation time course of between 30 and 80% of peak EPSP amplitude. Relay EPSPs exhibited profound paired pulse facilitation, but declined in average amplitude with maintained stimulation at 1Hz. Both stimuli could evoke variable and long latency bursts of EPSPs whose time course resembled those of evoked relay EPSPs. These may result from activation of slow spikes and burst firing in presynaptic relay neurones within the slice.

De Curtis M. Spreafico R. Avanzini G.(1989) Neuroscience 33:275-83

584.9

SENSORY AND OSCILLATORY PROPERTIES OF SIMULTANEOUSLY RECORDED MULTI-SINGLE UNITS IN THE THALAMIC RETICULAR NUCLEUS OF THE RAT. T.M. Fisher*, M.A.L. Nicolelis and J.K. Chapin. Department of Physiology and Biophysics, Hahnemann University, Philadelphia, PA 19102.

The thalamic reticular nucleus (RT) is thought to control sensory transmission through the ventral posterior (VP) thalamus, in particular by controlling the oscillatory state of the thalamocortical network. It is also believed that RT neurons are mutually inhibitory. Computer models employing such competitive inhibition phenomena are known to exhibit spontaneous and evoked state-switching activity. The aim of this study was to determine whether such state changes can be seen in the spatio-temporal patterns of activity across neuronal populations in the RT and VP. For this multi-single neurons were simultaneously recorded through chronically implanted electrodes in the face-whisker representations in the RT and VP thalamic nuclei in awake and anesthetized rats. In the pentobarbital anesthetized condition, 8-10 Hz spontaneous oscillatory activity was very prominent. Cross-correlations between simultaneously recorded RT neurons revealed both in-phase and 180° out-of-phase synchronization. Both patterns were often seen between the same neurons, spontaneously switching between in and out of phase oscillations. These RT neurons also exhibited a distinctive series of responses to peripheral stimulation, including: 1) an initial excitation, of 5-15 ms latency and persisting up to 60 ms post-stimulus, 2) a strong inhibition, lasting up to 120-160 ms post-stimulus, and 3) a series of 7-10 Hz oscillatory bursts, which persisted up to 3-4 sec after the stimulus. These bursts were also somewhat synchronous, though they often switched from an in-phase to an out-of phase pattern. Increasing the stimulus frequency from 0.2 Hz to 1.0 Hz resulted in a 180° phase advance, in which most neurons suddenly began their bursting pattern about 60 ms earlier. To conclude, the RT-VP thalamic network does not show simple synchronization, but instead exhibits complex dynamical state changes. Since these phase switching phenomena are quantal, they are reminiscent of the sudden frequency bifurcations characteristic of nonlinear dynamical systems, especially those containing coupled oscillators. *Supported by grants NS23722, AFOSR-90-0266, and AA06965 to JKC.*

584.10

MULTIVARIATE STATISTICAL TECHNIQUES ALLOW CHARACTERIZATION OF DISTRIBUTED POPULATION CODES IN SIMULTANEOUSLY RECORDED NEURONAL ENSEMBLES. Bacala, L.*, Nicolelis, M.A.L., and Chapin, J.K., Dept. Elect. Engin., U. of Pennsylvania, and Dept. Physiol/Biophysics, Hahnemann U., Philadelphia, PA.

In recent investigations we have routinely obtained simultaneous extracellular single neuron recordings from up to 24 single neurons in the somatosensory thalamus and/or cortex system of awake, behaving rats. Here we describe use of multivariate statistical techniques to characterize the information and population codes contained within such multi- single neuron ensembles. Discriminant analysis (DA) was used to determine how well a population of simultaneously recorded neurons can discriminate a sensory stimulus. Data from 10-23 neurons recorded during repetitive sensory stimulation were used to generate a matrix of discriminant functions which quantitate the predictive value of each neuron. Though single neurons by themselves were found to be poor predictors of whether a certain stimulus had occurred, this predictability improved greatly when increasing numbers of neurons were used for the discrimination. Furthermore, most of these neurons were found to contribute to several types of discriminations. Principal components analysis (PCA) was used to extract the factors which underly activity in neuronal populations. For this, multi-single neuronal discharge recorded over a range of stimulus conditions was time integrated and used to generate a correlation matrix. This matrix was rotated to generate a series of principal components, i.e. weighted combinations of the original variables (neurons). Each of these reconstructed components was generally found to reveal, in a statistically improved fashion, definable factors underlying activity within the neuronal populations, e.g. different aspects of sensory responses, movements or network oscillations. Similar analyses performed on data obtained from computer models of neuronal networks verified that PCA yields a spectral decomposition of the activity within the network. This optimally summarizes the network's functional organization. In conclusion, both DA and PCA provided evidence for a distributed representation of information within the recorded neuronal populations. *Supported by grants NS23722, AFOSR-90-0266, and AA06965 to JKC.*

584.11

DISTRIBUTED PROCESSING OF SOMATIC INFORMATION BY NETWORKS OF THALAMIC CELLS INDUCES TIME-DEPENDENT SHIFTS OF THEIR RECEPTIVE FIELDS Miguel A. L. Nicolelis , Rick C.S. Lin, John K. Chapin. Department of Physiology and Biophysics, Hahnemann University Philadelphia, PA 19102.

Previous recordings in anesthetized animals have suggested that the thalamus contains a detailed somatotopic map of the cutaneous periphery formed by extremely small neuronal receptive fields (RFs). However, very few data are available in awake animals to validate these propositions. We approached these issues through chronic simultaneous recordings of the extracellular activity of up to 24 statistically characterized single neurons per animal in awake adult rats. Overall, 132 neurons located in the ventral posterior (VP) complex of the thalamus were recorded in 10 adult hooded rats in which 8 to 16 50 um microwires were chronically implanted. Sensory stimulation (5-10 degrees, 1Hz, 100 ms) of several single facial whiskers per animal were produced by a computer controlled probe. VP neurons responded vigorously to the stimulation of multiple whiskers at different latencies (mean 6 ms) defining extremely large RFs (covering circular areas of 2-5 whiskers in diameter). Stimulation of single whiskers produced both early and late responses, and the temporal pattern of single cell responses changed when different whiskers were stimulated. In particular, many neurons exhibited time-dependent spatial shifts of their RF center over the 50 ms following the stimulus. We have found that such thalamic RFs are better described by complex and dynamic temporo-spatial surfaces. Thus, for each given stimulus thalamic networks produced a unique temporo-spatial populational response formed by weighted contributions from its neurons. Local subcutaneous injections of lidocaine induced rapid changes in latency, enhancement of secondary responses, and enlargement of VP cell RFs. These results indicate that in behaving animals thalamic maps are rather dynamic representations in which networks of neurons process somatic information through highly distributed time-dependent mapping functions. These may provide the thalamus considerable plastic potential even in adult animals. Supported by grants NS23722, AFOSR-90-0266 AND AA06965 to JKC.

584.12

SOMATOSENSORY STIMULATION SUPPRESS 8-12 Hz OSCILLATIONS IN THE VENTRAL POSTERIOR COMPLEX OF AWAKE RATS AS PREDICTED BY A COMPUTER MODEL Amitabha Gupta , Miguel A. L. Nicolelis, and John K. Chapin. Department of Physiology and Biophysics, Hahnemann University Philadelphia, PA 19102.

Thalamic neurons exhibit oscillations which can be clearly correlated with different states of the sleep cycle. However, very little is known about the physiology of oscillations in the range of 7-14 Hz observed in awake animals. To address this we have simultaneously recorded the extracellular activity of up to 24 statistically characterized single thalamic neurons per animal in each of 5 awake rats. Oscillations in the range of 8-12 Hz normally occurred in the ventral posterior complex of awake rats which were not carrying out either active sensory or motor tasks. These oscillations, which resembled sleep spindling and the slow "somatosensory rhythm" described in cats, occurred upon cessation of whisker or paw movement. These oscillations could be promptly disrupted by cutaneous stimulation. Long duration recordings revealed that thalamic neurons dynamically switch from a tonic firing mode (around 7 Hz) to a bursting mode (8-12 Hz). Similar oscillatory behavior was then reproduced in a large scale computer model of the rat somatosensory system which contained multiple brainstem, thalamic, and cortical compartments but no individual pacemaker elements. In this model background oscillations in the spindling frequency range were ubiquous and were disrupted only by sensory volleys that crossed a certain threshold. Upon terminating the stimulus the somatosensory network naturally evolved to a bursting mode. Introduction in the model of more realistic thalamic cells containing a Ca^{++} dependent K^+ current and a low-threshold (T) Ca^{++} current did not alter the main observation but demonstrated that modulatory influences on the T current could damp the background thalamic oscillation. These results suggest that in the awake behaving rat VP neurons dynamically switch from a tonic to a bursting mode and that this latter may be responsible for gating the flow of sensory information conveyed by the thalamus to cortex. *Supported by NS23722, AFOSR-90-0266 and AA06965 to JKC.*

584.13

CIRCUIT OSCILLATIONS IN A NEURONAL NETWORK MODEL OF THE SOMATOSENSORY SYSTEM. J.P. Utz* and J.K. Chapin. Department of Physiology, Hahnemann University School of Medicine, Philadel phia, PA, 19102-1192.

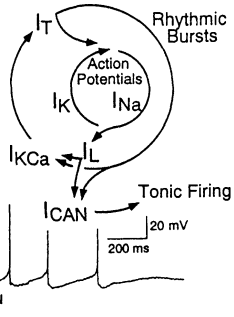
As a tool for investigating the mechanisms underlying spontaneous oscillations and sensory response properties in the rat somatosensory thalamocortical system a computer model was developed. This model consists of four layers, each containing a 30x30 sheet of neuron-like elements. The layers are connected in a pattern which resembles, in a simplified fashion, the axonal connections between the ventral basal thalamus (VB), the nucleus reticularis thalami (nRT), the SI cortical layer VI (CTX), and a layer of inhibitory cortical interneurons (INH). Each "neuron" incorporates several biologically realistic features, including: 1) a membrane potential (Vm) calculated from membrane conductances and Nernst potentials for several ion currents (Na^+, K^+, and Cl^-), 2) a membrane leak conductance, 3) a membrane capacitance which produces membrane potential time decays, 4) a voltage-sensitive spike-triggering threshold, and 5) axonal conduction delays. State variables (eg., conductances and Vm) are re-calculated every ms of simulation time. The VB is driven by a 30x30 array of randomly-timed input fibres, with a bar of increased activity serving as a focal stimulus. Each connection fans out in a 5x5 array. This model may exhibit either steady- state random firing, or distinctive spatiotemporal oscillations depending on the values of certain parameters. For example, oscillations may be suddenly induced by: 1) increasing the strength of the corticothalamic excitatory feedback, 2) decreasing the strength of a medium duration afterhyperpolarization (mAHP; τ = 40 ms), and 3) decreasing the inhibitory feedback from the nRT to the VB. *Supported by grants NS23722, AA06965 and AFOSR-90-0266 to JKC.*

584.14

IONIC BASIS OF OSCILLATION AND 30-60 HZ FIRING IN THE THALAMIC RETICULAR NUCLEUS (NRT), A MAMMALIAN PACEMAKER. T. Bal and DAMcCormick*, Sec. Neurobiology, Yale Univ. Sch. Med., New Haven, CT 06510

During slow wave sleep, the nRt synchronizes rhythmic thalamo-cortical activity and contributes to the generation of spindle waves. The ionic mechanisms of rhythmic and tonic firing in nRt cells were investigated with standard *in vitro* slice techniques.

We found that endogenous rhythmic bursting of nRt cells is mainly due to the interaction of a low threshold Ca++ spike (I_T), a Ca++ activated K^+ current (I_{KCa}) and a Ca++ activated non-specific cation current (I_{CAN}) according to the following scheme.



Maximal block of a leak potassium current by 5HT resulted in 30-60 Hz tonic firing owing to the inactivation of I_T and the activation of a persistent sodium current.

584.15

A NEW TOOL TO CHARACTERIZE ONGOING NEURONAL DISCHARGE: COUNTING STATISTICS OF f^{-b}-FLUCTUATIONS. K.-D. KNIFFKI, M.K.C. MENGEL and C. VAHLE-HINZ*. Physiologisches Institut, Universität Würzburg, Röntgenring 9, W-8700 Würzburg, Germany.

Temporal fluctuations in natural phenomena in the absence of intentional stimulation are not always the consequence of statistically independent random events. It has been shown that temporal fluctuations found in phenomena as different as membrane currents, earthquakes, intensity of sunspots, heart beat or breathing activity can be characterized by their power spectrum density S(f). In all these different systems S(f) is decaying as f^{-b} at low frequencies with $b \leq 1$. This behaviour of S(f) is called "f^{-b}-noise". Usually S(f) is obtained by Fast Fourier Transformation (FFT). To avoid the well known problems in using FFT for neuronal spike trains, a new simple method (Meesmann, Grüneis, Flachenecker, Kniffki, submitted) is introduced to analyse the low frequency part of S(f) of the recorded action potentials.

The series of recorded action potentials is considered to be a point process described as $y(t) = \Sigma\delta(t-t_i)$, whereby $\delta(t-t_i)$ represents Dirac's delta function, and t_i is the time of occurrence of a particular action potential within the spike train. The entire observation time was divided into counting windows Δt and the variance of counts $Var[N(\Delta t)]$ was determined. This was repeated for different lengths of Δt. If $Var[N(\Delta t)] \sim (\Delta t)^{1+b}$, the power spectral density $S_y(f)$ scales as $S_y(f) \sim f^b$ with $b \leq 1$. In thalamic neurones tested so far, the variance-time curve $Var[N(\Delta t)]$ was proportional to $(\Delta t)^{1+b}$ with a mean $b = 0.6$, thus indicating that the neuronal discharge exhibited f^{-b}-fluctuations. The applied method reliably discriminates these fluctuations from a pure random process, in which case $b \equiv 0$.

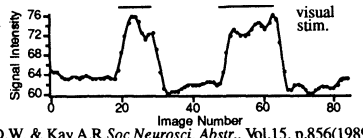
We speculate that the basic mechanisms underlying neuronal activity in thalamic sensory systems are expressions of a "self-organized critical state", as introduced by Bak, Tang and Wiesenfeld (Phys. Rev. Lett. 59: 381, 1987).

VISUAL CORTEX: BRAIN IMAGING TECHNIQUES

585.1

FUNCTIONAL BRAIN MAPPING IN HUMANS USING MRI: INTRINSIC SIGNAL CHANGES ACCOMPANYING SENSORY STIMULATION. S.Ogawa♦*, D.W. Tank♦, T.M. Lee♦, R. Menon+, J.M. Ellermann+, S.G. Kim+, H. Merkle+, and K. Ugurbil.+ ♦AT&T Bell Labs, Murray Hill, NJ and +U. of Minn. Med. Sch., Minneapolis, MN.

We have previously shown that changes in venous blood oxygenation produce Blood Oxygenation Level Dependent (BOLD) changes in water proton magnetic resonance signals in mammalian brain (1-3). We demonstrated that the effect is produced by a magnetic susceptibility change in venous blood vessels produced by changes in the concentration of the paramagnetic molecule deoxyhemoglobin and suggested that BOLD contrast imaging could be used for human functional brain mapping. We have now demonstrated that visual stimulation produces an easily detectable (5-20%) transient increase in the intensity of water proton magnetic resonance signals in human primary visual cortex in gradient echo images obtained at 4T field strength.The accompanying figure shows the time course of change in signal intensity produced in a region of primary visual cortex by two periods of a patterned flash binocular visual stimulus.The change is consistent with stimulus-produced neural activity producing an increase in venous blood oxygenation. With the present 4T imaging system, we can follow changes with a temporal resolution of ~3 sec and with 1.6x3.3x10 mm image voxel size. Images have been assembled into real-time movies that show the temporal and spatial changes. The sensory stimulation produced changes in BOLD signal intensity are quite robust and suggest the possibility of functional brain mapping of higher cognitive operations.



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585.2

MAGNETOENCEPHALOGRAPHIC IDENTIFICATION OF EXTRASTRIATE VISUAL AREAS IN MAN Edward L. Maclin Ph.D.[1,2*], Stephen E. Robinson Ph.D.[1,2], Jeanne E. Knight M.S.[1,3], Kim Paulson[1] 1) Center for MEG, Veterans Medical Center, 2), Dept of Neurology, Univ. New Mexico 3) Dept of Psychology, UNM, Albuquerque NM, 87108.

Sets of stimuli and procedures have been developed for differentially activating primary and secondary visual cortical areas, and using the associated magnetic field responses to localize the activated regions on the subject's MRI. Achromatic or isoluminant colored gratings are first presented in a fixed position, and then abruptly begin drifting. Both pattern appearance and motion onset evoke magnetic responses. The response to motion onset localizes to the region of the temporo-parieto-occipital pit. This response is much smaller for isoluminant stimuli than for achromatic stimuli, as expected for the so called 'motion area' or cortical region V5. Even simple achromatic spot stimuli produce responses from multiple brain regions, and both multiple dipole and distributed current models have been developed to analyze the complex evoked field patterns. We have studied one patient with a scotoma due to a stroke in primary (striate) visual cortex who appears to have no response from primary visual cortex, but who exhibits a robust response over parietal cortex, in the region which appears to subserve motion detection in control subjects.

Supported by the Department of Veterans Affairs

585.3

FUNCTIONAL MRI STUDIES OF HUMAN VISION ON A CLINICAL IMAGER. J.S. George[2], J. Sanders[1], J.W. Belliveau[3], J.D.Lewine[2], A. Caprihan[4], C. J. Aine[2], D. van Hulsteyne[2], E. Maclin[1], and C.C. Wood[2*] 1. Albuquerque VA Medical Center; 2. Los Alamos National Laboratory; 3. Massachusetts General Hospital; 4. Lovelace Medical Foundation. During the past decade, Magnetic Resonance Imaging (MRI) has become the method of choice for imaging the anatomy of the human brain. Recently, Belliveau and colleagues (Science, 254: 621-768,1991) have reported the use of echo planar magnetic resonance imaging (EPI) to image patterns of neural activity. By imaging the passage of a bolus of injected paramagnetic contrast agent through the cerebral microvasculature with and without visual stimulation they estimated changes in regional blood volume associated with neural activation. The MGH group has also shown that intrinsic hemodynamic changes (in the absence of injected contrast agents) can serve as the basis of functional MR Imaging. Here, we report functional MR imaging without the use of contrast agents, and without the extensive hardware modifications required for EPI. Experiments were conducted on a conventional clinical MR Imager (1.5 T Siemens) using a 15 cm diameter surface coil. Computer generated visual stimuli (annular checkerboards subtending ~10° in the lower hemifield, with contrast reversals at 8 Hz) were projected onto a translucent screen over the bore of the magnet using an LCD video projector. Subjects viewed the images in a 45° mirror attached to eyeglass frames. A sequence of T2 weighted gradient echo images (TR=38 ms,TE=26 ms, flip=40°) were obtained at 2.48 s intervals. A single slice (8 mm thick) was aligned along the calcarine fissure in a horizontal oblique, or sagittal plane lateral to the midline. A sequence of images (9-11) was obtained with no stimulus, and during visual stimulation. The cycle was repeated 3 to 6 times. Summed images from the stimulus-off condition (N=6,18,or 36) were subtracted from summed stimulus-on images, disclosing regions of focal activation. Horizontal oblique images showed activation along the midline near the occipital pole and along the lateral rim of occipital cortex. In the sagittal slice, activation was seen near the expected locations of V1, V2 and possibly V3. Another active region was observed near the parietal occipital sulcus on the superior surface of the cerebrum. These locations are consistent with sources observed in neuromagnetic studies of the human visual response.

585.4

EVIDENCE OF RETINOTOPIC ORGANIZATION IN EXTRASTRIATE REGIONS OF HUMAN VISUAL CORTEX: NEUROMAGNETIC MEASURES. C.J. Aine*, S. Supek, E.R. Flynn and D. Ranken. Biophysics Group. Los Alamos National Laboratory. MS M715. Los Alamos, NM 87545.

Studies of human visual cortex have shown evidence of retinotopic organization of striate cortex using a variety of methods (e.g., ERPs, MEG, PET). Using MEG measurements, we have identified two additional cortical regions (occipital-temporal and occipital-parietal) that show evidence of retinotopic organization. Seven circular sinusoids (Targets) were presented sequentially to the lower right quadrant of the visual field at 3°, 4°, 6.5° and 9° beneath the horizontal meridian (horizontal alignment) and to the right of the vertical meridian (vertical alignment). Stimulus sizes ranged from .4°-1.0°. Stimulus duration was 266 msec and the rate of presentation was 1.1-1.8 Hz.

Neuromagnetic responses were recorded from 12-13 placements of a 7-sensor array in each of two subjects. Multiple-dipole models were applied to neuromagnetic field maps constructed at 5 msec intervals and source locations were transformed into MRI coordinates. Striate activity was evident throughout the 80-130 msec sequence. A second source was evident (onsetting at ~100 msec) in occipital-temporal regions whose retinotopy can be characterized as follows: 1) for the vertical alignment, source locations shifted in a medial and dorsal direction as a function of eccentricity; and 2) for the horizontal alignment, source locations shifted in a medial and anterior direction. A third source in occipital-parietal regions, evoked by peripheral stimulus locations in the vertical alignment (6.5° and 9°), also showed evidence of retinotopic organization. The occipital-parietal source was more dorsal (in the parietal-occipital sulcus) for the most eccentric placement. The trends noted here for stimuli in the vertical alignment have been noted in a previous study using 2-dimensional, difference of gaussians stimuli (Supek and Aine, 1992).

585.5

AREA V5 OF HUMAN VISUAL CORTEX IDENTIFIED IN INDIVIDUALS USING PET AND MAGNETIC RESONANCE IMAGING. I D G Watson, S Shipp, R S J Frackowiak* and S Zeki. MRC Cyclotron Unit, Hammersmith Hospital, London W12 0HS and Dept of Anatomy, University College, London WC1E 6BT.

We have previously used positron emission tomography (PET) to find the visual motion centre (V5) by averaging groups of subjects. Now, we report findings from individual subjects scanned with a Siemens-CTI 953B PET camera, with its sensitivity enhanced by removing the collimating septa, to allow collection of data in 3D mode. Images of regional cerebral blood flow (rCBF) were obtained by using intravenous infusions of $H_2{}^{15}O$. 12 subjects viewed computer displays of moving or static checkerboard patterns. Each subject had 6 scans in each of the 2 conditions. Statistical parametric maps (SPMs) were then used to indicate areas of the brain in which significant rCBF changes took place in response to visual motion. We also obtained high resolution magnetic resonance imaging (MRI) scans for all subjects and then coregistered individual PET SPMs onto the respective MR images. Thus we could relate the sites of individual statistically significant activations to specific gyri and sulci of the individual brains.

SPMs revealed bilateral activation of the area previously defined as V5 in all subjects. It was located ventrolaterally in the anterior part of the occipital lobe. The rCBF increase induced in V5 ranged from 2.4 % to 8.6 %. On normalization to Talairach stereotactic co-ordinates the locations of the most significant foci of activation at V5 varied within a range of 24 mm for either hemisphere. In addition to V5, our stimuli activated sites in the cuneus and the lingual gyrus, and these may correspond with the superior and inferior divisions of monkey V2/V3.

585.6

PET STUDY OF STEREOPSIS. A. Ptito*, R. Zatorre, M. Petrides, S. Frey, B. Alivisatos and A. Evans. Montreal Neurol. Inst. McGill University, Montreal, Quebec H3A 2B4.

To map the areas of the brain involved in global stereopsis, regional cerebral blood flow was measured by means of positron emission tomography(H_2O^{15} bolus technique) in nine right-handed male adults under three conditions of testing: 1) DEPTH: a random dot stereogram depicting either a vertical or a horizontal bar at 100% binocular correlation. 2) SHAPE WITHOUT DEPTH: a 2-D vertical or horizontal black outline of a bar drawn on a background of random dots. 3) NEUTRAL: random dots without form or depth. Increases in blood flow due to depth perception were assessed by substracting activation in the shape condition from that in the depth condition. Results showed activation on the border of areas 17 and 18 in the right cerebral hemisphere and in areas 18 in the left cerebral hemisphere. When the neutral condition was substracted from the depth condition a focus was obtained only in the right cerebral hemisphere again along the border of areas 17 and 18. These data are concordant with evidence of a right hemisphere superiority for stereopsis, and they indicate that the analysis and processing of unambiguous stereoscopic information high in binocular correlation begins early in posterior visual areas of that hemisphere.

585.7

STIMULUS SPECIFIC INCREASE OF OXIDATIVE METABOLISM IN VISUAL CORTEX S. Marrett*, H. Fujita, H. Kuwabara, Y. Yasuhara, L. Ribiero, E. Meyer, A.C. Evans, A. Gjedde, Montreal Neurological Institute, 3801 University St., Montréal H3A 2B4, Québec.

The mechanisms that mediate the coupling of neuronal activity to blood flow and oxidative metabolism have been the subject of controversy. Previous PET studies have demonstrated an apparent uncoupling of blood flow and oxygen metabolism in both somatosensory and visual cortex (Fox et al, 1988.) The reported changes in oxygen metabolism (5-7%) have been considerably smaller than those reported for blood flow and glucose consumption (25%-50%). Using a newly developed method for measuring oxygen consumption using a single bolus inhalation of $[^{15}O]O_2$ (Ohta et al, 1992) with 3 minutes of multi-frame data acquisition, we confirmed the lack of a coupling of blood flow to oxygen metabolism in somatosensory cortex (Fujita et al, submitted). We hypothesised that a specific visual stimulus would be able to modulate oxidative metabolism in visual cortex, since primate V1 has a heterogeneous distribution of cytochrome oxidase (blobs) that has been correlated with glucose uptake and stimulus variables in the macaque (Tootell et al, 1988).

Measurements of oxygen metabolism and blood flow in normal volunteers were made using separate bolus $[^{15}O]O_2$ and $[^{15}O]H_2O$ PET scans. Subjects had the right eye covered and fixated a cross on a computer monitor with the left eye. During the activation scans a semi-annular reversing contrast stimulus was presented to the left visual field of the left eye at a retinal eccentricity of about 15°. Correlative MRI scans were obtained from all subjects. A focal increase of oxygen consumption (≈30%) was measured in primary visual cortex. This result is consistent with a stimulus specific modulation of oxidative metabolism in visual cortex.

585.8

DEPENDENCE OF VISUALLY EVOKED MAGNETIC FIELDS ON INTERSTIMULUS INTERVAL (ISI). R.J. Ilmoniemi,*S.P. Ahlfors, J. Numminen, and K. Portin. Low Temperature Laboratory, Helsinki University of Technology, 02150 Espoo, Finland.

To develop rapid stimulation sequences for the systematic mapping of the visual field, we studied how the visually evoked magnetic field depends on the stimulus repetition rate.

Black-and-white checkerboard patterns were shown at intervals of 0.25 to 8 sec for 200 ms (100 ms for the 250-ms ISI) in one 45° sector of an annulus lying between 1.7° and 3.7° from the center of the visual field. The magnetic field over the occipital cortex was recorded with a multi-channel SQUID magnetometer. The amplitude of the averaged response 100 ms after the stimulus appearance was smallest for rapidly presented stimuli, grew until an ISI of about 2 seconds, and decreased for longer ISIs. The characteristic time constant of the amplitude rise, obtained from a fit to an exponential, was typically 1 second; it was several seconds for the decrease. The optimal presentation rate for a single stimulus location appears to be 1/sec; for faster rates, the quicker accumulation of responses cannot compensate for the loss of signal.

In the second experiment, the stimuli were presented to different octants of the visual field in pseudorandom order. When the total repetition rate was 4 stimuli per second with one octant repeating itself once a second, the response for this octant was nearly identical with the response obtained when this octant was stimulated separately once per second. It therefore appears that the functions of the visual cortex can be mapped effectively by presenting stimuli in rapid succession in alternating locations of the visual field.

VISUAL BEHAVIOR: CLINICAL STUDIES

586.1

SPATIAL VISUAL FUNCTION LOSS IN ACCIDENTAL HUMAN LASER EXPOSURE. H. Zwick, D.A. Gagliano, J. Gunzenhauser, D.O. Robbins* Letterman Army Institute of Research, San Francisco, CA 94129 and Ohio Wesleyan University, Delaware, OH 43015.

In previous experiments (Zwick et al, Neurosci, 1989) to simulate laser effects on human visual performance, we utilized laser induced deficits obtained in rhesus contrast sensitivity to alter the spatial frequency content of complex images used in human detection and recognition studies. In this paper five human laser accident cases are reported where the spatial characteristics of the relative scotoma have been characterized. Human laser ocular accident injuries suggest the presence of both absolute and relative regions of the scotoma. Visual acuity loss exceeds 20/200 with peripheral retinal fibrosis. Contrast sensitivity measurements reveal high as well as mid to low spatial frequency contrast sensitivity loss relative to non-exposed eyes. Visual threshold field loss may exceed 30 degrees where mid to low spatial frequency sensitivity loss is extensive. Retinal fibrosis, traction, and photoreceptor alteration are suspected in mediating the spatial vision degradation of the relative scotoma. Our results suggest that future perceptual simulations of such retinal injuries should incorporate the complex representations of both the relative and absolute spatial aspects of the induced scotoma into the simulated image.

586.2

RESIDUAL VISUAL FUNCTIONS IN THE BLIND FIELD OF A HEMIANOPIC PATIENT. C.M. Wessinger*, R. Fendrich and M.S. Gazzaniga. Center for Neurobiology, Univ. of California, Davis CA 95616

Previously, we reported the existence of a small island of residual visual function in the blind field of a hemianopic patient, CLT. In a forced choice task, CLT reliably detects a 1 deg black stimulus flashed within this island. Above chance detection was also found along CLT's horizontal and vertical retinal meridians. We now report results of additional testing which further characterize residual visual functions in this patient's blind field.

We performed dense perimetry to map the boundaries of the isolated island within the scotoma. The results of this testing suggest that the island of sparing is primarily confined to an area not much larger than 1 deg. We found CLT could not identify the location of stimuli presented within the island of sparing. He was also unable to direct a saccade toward 1 deg stimuli flashed at this location. Nevertheless, he could discriminate between a diamond and a square (both 1 deg on a side) centered within the island of sparing. However, when we increased the size of the diamond and square to 2 deg on a side, CLT performed at chance. This suggests that discrimination of the smaller stimuli was based on form or edge detection within the island of sparing. Larger stimuli completely blanketed the island depriving the subject of this information.

We also investigated CLT's residual abilities at other locations within his blind field. Near the vertical meridian, CLT was able to direct a saccade toward flashed stimuli, but could not discriminate between a 1 deg diamond and square. On the other hand, he could discriminate between 2 deg diamonds and squares. This demonstrates a dissociation which is in direct contrast to that found at the island of sparing.

Supported by NIH/NINDS P01 NS17778-10 and the McDonnell-Pew Foundation.

586.3

DETECTION OF TEXTURE-AND MOTION-DEFINED BOUNDARIES BY NONRETARDED AND MENTALLY RETARDED ADULTS. Stephen Oross III* & Robert Fox. Department of Psychology, Vanderbilt University, Nashville, Tennessee 37240.

Forms defined solely by relative differences in binocular disparity or motion have proven to be difficult for most mildly mentally retarded adults (MMR) to either discriminate between or to detect. Because both binocular disparity and motion are processed preattentively by early components of the visual system, one conclusion supported by these findings is that the neural integrity of the visual system is compromised in MMR. This impairment, however, appears selective because sensitivity to other visual attributes (e.g., contrast) falls within normal limits. To explore the degree to which the impairment can be related to visual rather than response difficulties and to investigate sensitivities to another fundamental visual attribute we examined the ability of MMR and nonretarded adults to detect forms defined by texture and motion. Using signal detection methodology, subjects viewed a randomly textured and moving background and were instructed to indicate the presence or absence of a rectangular form. Texture differences were introduced by varying the grain size of the form relative to the background. Motion differences were introduced by varying the correlation with which the texture elements were displaced across temporal frames. Analyses of d' and response bias indicate that: (1) MMR encountered difficulty in veridically perceiving motion information, (2) both groups responded similarly to texture information, and (3) the impairment in perceiving motion noted in MMR cannot be simply ascribed to response difficulties. These results are discussed with respect to the possible genesis, extent, and impact of these selective impairments.

Supported by HD15051, HD27716, and EY00590

586.4

MOVEMENT IN ORTHOGONAL DIRECTIONS: DISCRIMINATION BY MENTALLY RETARDED AND NONRETARDED ADULTS. Sandy A. Shimp*, Robert Fox, & Stephen Oross III. Department of Psychology, Vanderbilt University, Nashville, Tennessee 37240.

Our prior research has demonstrated that the perception of motion-defined forms is impaired in mildly mentally retarded adults (MMR). The existence of perceptual deficits in MMR suggests underlying neural impairment. Interestingly, multiple sclerosis patients are impaired at perceiving motion-defined forms yet are able to veridically discriminate between different directions of motion. One explanation is that the perception of motion-defined forms and direction of motion involve different neural structures. The present study investigated the ability of MMR and nonretarded adults to discriminate between movement in orthogonal directions. Subjects were asked to discriminate between random-element kinematograms moving either up-down or left-right. The coherence of the motion in the kinematograms was manipulated by varying the correlation (ranging from 0 to 100%) with which the elements were displaced across temporal frames. Both MMR and nonretarded adults were essentially equivalent in their ability to discriminate between the directions. These results are similar to those found with multiple sclerosis patients and are consistent with the explanation relying upon different neural structures. Alternative hypotheses, however, are available that can account for these results. One considers the signal/noise ratios in displays used to test the perception of motion-defined forms and directions of motion. Another postulates that differences between groups would be detected if smaller differences in direction were used. We are currently exploring the validity of these alternatives.

Supported by HD15051, HD27716, and EY00590

586.5

LACK OF VEP EVIDENCE FOR MAGNOCELLULAR DYSFUNCTION IN DYSLEXIA. J. Victor*, M. Conte, L. Burton, and R. D. Nass, Dept. of Neurology and Neuroscience, The New York Hospital - Cornell Medical Center, New York, NY 10021.

It was recently reported (Livingstone et al., PNAS, 1991) that five dyslexic individuals showed loss of the pattern-reversal visual evoked potential (VEP) under conditions designed to isolate the magnocellular pathway: high temporal frequency, low contrast, and low luminance. We measured contrast-reversal VEPs in 9 dyslexic individuals (ages 8 to 46) and in 11 controls (4 with learning disability but not dyslexia, 7 normals) of similar age. For the purpose of this study, dyslexics were defined as subjects of normal intelligence with a history of reading difficulty. We used steady-state conditions similar to those used by Livingstone et al. (16 Hz reversal rate, 2% contrast, 4 cd/m^2, 3 deg checks), and an analytical method (Victor and Mast, EEG J., 1991) which provided rigorous statistical criteria for detection of significant responses and significant differences between responses. Contrary to the findings of Livingstone et al., we found that VEPs were absent in most subjects not only in the dyslexic group, but also in the control group. At lower temporal frequencies (4 and 8 Hz reversal rate), higher luminance (59 cd/m^2) and higher contrast (20%), significant responses were more widely present in both groups, but the response amplitudes and phases did not differ across groups. We find no evidence for a low-contrast, low-luminance VEP abnormality associated with dyslexia.

Supported by EY7977 (JV).

586.6

PUPILLARY LIGHT REFLEX (PLR) AFTER OPEN LOOP RETINAL STIMULATION IN HUMANS. W.B. Pickworth*, J.S. Fosnaugh, J.D. Nichels and E.B. Bunker. NIDA, Addiction Research Center, Baltimore, MD 21224.

Pupil diameter (PD) and the PLR are popular, noninvasive measures of drug action. Ambient light level and an opaque patch over the contralateral eye significantly change prestimulus PD and PLR in closed loop experiments (Pickworth et al, Soc Neurosci Abs 17:1467, 1991). In the present study, ambient light and the stimuli were presented in Maxwellian view (open loop) to nine drug-free volunteers using a Pulse Medical Instruments pupillometer. The PLR was evoked at four levels of ambient light: with both eyes open, a patch over one eye. In both patch conditions, increased ambient light decreased PD from 6.4 to 4.2 mm and constriction velocity (CV) from 6.3 to 3.4 mm/sec. These results differ from the closed loop experiment where the patch doubled CV and increased PD by 50%. Under Maxwellian view binocular summation is eliminated suggesting that midbrain control of PD is diminished.

586.7

AUGMENTING AND REDUCING OF THE VISUAL EVOKED POTENTIAL IN ROMAN HIGH- AND LOW-AVOIDANCE RATS. J. Siegel* and D.F. Sieson. School of Life and Health Sciences, Univ. of Delaware, Newark, DE 19716 and P. Driscoll. Laboratory of Beharioral Biology, ETHZ, Zurich, Switzerland.

Human and cat high sensation seekers (SSs) tend to show increasing amplitudes (augmenting) of the P1 and N1 components of the visual evoked potential (VEP) to increasing intensities of light flash, whereas low SSs show VEP reducing. Roman high- (RHA/Verh) and Roman low-avoidance (RLA/Verh) rats, bred in Switzerland, have behavioral traits comparable to human and cat high and low SSs, respectively. RHA/Verh rats show greater exploration, activity, and aggression than do RLA/Verh rats. Ten male rats of each strain were anesthetized with chloral hydrate and maintained at a stable moderate anesthetic level. Fifty VEPs recorded from visual cortex at each of five flash intensities were computer averaged per rat. The slopes of P1 and P1-N1 amplitudes as a function of flash intensity were significantly greater in the RHA/Verh than the RLA/Verh rats. RHA/Verh rats were clear augmenters; RLA/Verh rats had almost flat amplitude-intensity functions.

This study demonstrates a rat model of SS-related augmenting and reducing that yields advantages of genetic homogeneity and a short generational time, and provides access to a wealth of behavioral data and experimental manipulations available for the rat. In addition, we show that this relationship has a heritable base and extends across species from human, cat, and rat. (Supported in part by ARO Contract DAAL 0388K0043)

586.8

FEASIBILITY OF AN INTRACORTICAL VISUAL PROSTHESIS FOR THE BLIND: I. DESCRIPTION OF AN EXPERIMENT. D.K. O'Rourke, M.J. Bak*, F.T. Hambrecht, C.V. Kufta, E.M. Schmidt, P. Vallabhanath. Surgical Neurology Branch (D.K.O'R, C.V.K), Lab. of Neural Control (M.J.B., E.M.S., P.V.), Neural Prosthesis Program (F.T.H.), NINDS, NIH, Bethesda, MD 20892.

We performed a study to investigate the feasibility of a visual prosthesis for the blind based on electrical intracortical microstimulation of the occipital cortex in a blind volunteer. A healthy, 42 year old woman with a 22 year history of bilateral complete blindness secondary to glaucoma was selected based on a rating system involving eight independent observers. The experimental and time-limited nature of this first study was explained in detail to this patient volunteer who gave her consent.

The surgical procedure was designed based primarily on the fragility of the electrodes and the complex anatomy of the occipital pole region. A strain relief system utilizing silicone tubes and flanges was devised to minimize movement of the electrodes. Parylene insulated iridium "hat-pin" microelectrodes (n=38) with attached gold leads were manually implanted 2mm deep in the visual cortex near the occipital pole. The leads exited the craniotomy site via separate contoured ramps in the skull and penetrated the scalp through four separate incisions.

The occipital cortex was stimulated in daily sessions over four months with occasional respites. Stimulation was never associated with pain or any other discomfort. The sensations produced were typically described as small spots of light varying in color, depth, position, and intensity. Percept qualities could be modulated by adjustment of stimulation parameters, including current amplitude, frequency, pulse duration and train length. Breakages occurred in some electrode leads, which we feel can be prevented with future designs.

The leads and several microelectrodes were removed when testing was completed. There has been no significant morbidity associated with this study.

586.9

FEASIBILITY OF AN INTRACORTICAL VISUAL PROSTHESIS FOR THE BLIND: IV. SUMMARY AND FUTURE. F.T. Hambrecht*, M.J. Bak, C.V. Kufta, D. K. O'Rourke, E.M. Schmidt, and P. Vallabhanath. Neural Prosthesis Program (F.T.H.), Lab. of Neural Control (E.M.S, M.J.B., P.V.) Surgical Neurology Branch (C.V.K. & D.K.O'R.) NINDS, NIH, Bethesda, MD 20892

Using intracortical microstimulating electrodes, cortical phosphenes were produced at the lowest stimulus levels ever reported in humans (see preceding abstracts for details). Reliable and rapid recognition of visual images made up of groups of electrically elicited phosphenes is the most important goal yet to be achieved in developing a visual prosthesis for the blind. Breakage of leads in a patient-volunteer who had been blind 22 years limited the simultaneous activation of multiple intracortical microelectrodes and resulting phosphenes to six in a vertical row which could be recognized as a letter "I".

To further evaluate pattern recognition, a visual cortical implant system with over 200 intracortical microelectrodes arranged in pairs is being developed. In the next, more recently blinded patient-volunteer a percutaneous connector rigidly mounted to the skull is planned. Also a television camera and image processing electronics will be used to provide visual images in addition to computer generated patterns. In a definitive prosthesis a multichannel transcutaneous telemetry system will be needed.

586.10

FEASIBILITY OF AN INTRACORTICAL VISUAL PROSTHESIS FOR THE BLIND: II. VISUAL SENSATIONS PRODUCED BY CORTICAL STIMULATION. P. Vallabhanath*, M.J. Bak, F.T. Hambrecht, C.V. Kufta, D. K. O'Rourke, E.M. Schmidt, Lab. of Neural Control (M.J.B., E.M.S., P.V.), HHMI-NIH Research Scholar (P.V.), Neural Prosthesis Program (F.T.H.), Surgical Neurology Branch (C.V.K., D.K.O'R.), NINDS, NIH, Bethesda, MD 20892

Stimulation of 34 of 38 microelectrodes implanted in the area of the visual cortex of a blind patient volunteer (see preceding abstract for details) produced visual percepts (phosphenes). The phosphenes appeared as sensations of light ranging in size from pinpoint to a nickel held at arms length. At currents near threshold, phosphenes were reported to have colors such as violet or blue, while at higher stimulation currents they were reported as white or yellow .

Microelectrode impedance and threshold currents were monitored daily. Many electrodes had thresholds between 2μa and 20μa using cathodal first, biphasic stimulation (train length = 125ms, frequency = 200Hz, pulse width = 200μs). Threshold currents decreased with increasing pulse width, train length, and frequency within certain limits. Thresholds rose at frequencies below 100Hz, and train lengths below 250ms. At a given current, the phosphenes were perceived to be brighter and more distinct at a pulse width of 800μs than at 200μs or 400μs.

Sequential phosphenes produced by stimulation of the same electrode required an inter-train interval greater than 125ms. Electrodes were repetitively stimulated daily with only slight and temporary increase in thresholds. During repetitive stimulation, the phosphenes initially decreased in brightness (accomodation) and then either stabilized or extinguished.

586.11

FEASIBILITY OF AN INTRACORTICAL VISUAL PROSTHESIS FOR THE BLIND: III. MAPPING OF PHOSPHENE LOCATION. E.M. Schmidt*, M.J. Bak, F.T. Hambrecht, C.V. Kufta, D. K. O'Rourke and P. Vallabhanath. Lab. of Neural Control (E.M.S, M.J.B., P.V.) Neural Prosthesis Program (F.T.H.), Surgical Neurology Branch (C.V.K. & D.K.O'R.) NINDS, NIH, Bethesda, MD 20892.

Sequential and simultaneous stimulation of microelectrodes implanted in the area of the visual cortex of a blind patient-volunteer (see preceding abstracts for details) produced multiple phosphenes in the perceived visual field. Phosphene locations were mapped using subject descriptions, a dart board mapping procedure or a pair-wise computer mapping algorithm. All phosphenes were located in the left hemi-field with most near or above the horizontal meridian within 40 degrees of the fixation point. The apparent distance at which phosphenes appeared ranged from a few inches to the location of a "distant star". When multiple phosphenes were produced simultaneously they tended to look alike and appeared at the same depth. When the stimulating current was increased above threshold on some electrodes, a second phosphene appeared in close proximity to the first phosphene. Multiple phosphenes, produced with either single or multiple electrodes, maintained their relative positions as they moved with conjugate eye movements. The relative relationship of the phosphenes in visual space was roughly similar to the placement of the electrodes in the visual cortex.

The apparent spacial concentration of the phosphene map may be due to the anatomical placement of the intracortical electrodes, the length of blindness (22 years) or the type of blindness (glaucoma).

VISUAL BEHAVIOR: PSYCHOPHYSICS AND EYE MOVEMENTS

587.1

A DYNAMICAL MODEL FOR COMPUTING THE POSITION OF AN OBJECT FROM ITS RETINAL LOCATION AND EYE POSITION. A. Pouget, T. Albright* and T.J. Sejnowski. The Salk Institute, La Jolla, CA 92037.

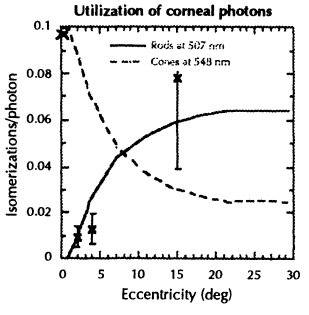
The position of a visual object with respect to the viewer, the egocentric position, can be computed by adding the retinal position of the object with the position of the eyes. An experiment performed by Matin et al. (Science, 248, 1965) suggests that humans use this simple algorithm when localizing visual objects. The task was to localize a point of light briefly flashed (<1 ms) on a screen during a saccade. The subjects could accurately localize the position of the flash in the absence of any other visual stimulus which suggests that the eye position was apparently available to the observer. Additional experiments, however, showed that humans tend to make a systematic localization error in the direction of the saccade (Mateef, Psych. Perc., 24, 1978). Furthermore, the size of the error was a function of the retinal position on which the flash impinged (O'Regan, Psych. Perc., 36, 1984). Thus, the perceived egocentric position of an object is not a static linear sum of the retinal position and eye position.

We have developed a biologically plausible model that computes egocentric position by dynamically combining the retinal position of an object with eye position. The model accurately locates an object when it is presented for a long duration (>100 ms) with the eyes at rest. However, for brief presentations while the eyes are moving, the model exhibits a pattern of errors identical to that reported in humans. Taken together with recent physiological and psychophysical results (Gauthier et al., Science, 249, 1990), our model suggests that the eye position is used for object localization. (Supported by the Howard Hughes Medical Institute).

587.2

THE QUANTUM EFFICIENCY OF HUMAN VISION: COMPARING PSYCHOPHYSICS & ANATOMY. Denis G. Pelli* Institute for Sensory Research, Syracuse University, Syracuse, NY 13244-5290.

Transduction efficiency (fraction of corneal photons that produce an isomerization) has received much attention since the time of Hecht, but is still subject to much uncertainty. Pelli (1990) introduced a new psychophysical technique that measures transuction efficiency by measuring the effects of visual noise masking. The X's show preliminary measurements indicating peak transduction efficiencies of 5-10% for both rods and cones. They agree with anatomical estimates shown as curves, which are computed from the known number of receptors and their optical densities and aperture sizes.



Pelli, D. G. (1990) The quantum efficiency of vision. In C. Blakemore (Eds.), *Vision: Coding and Efficiency* (pp. 3-24). Cambridge: Cambridge University Press.

587.3

A NETWORK MODEL OF DEPTH CUE INTEGRATION. M.B. Ladner* and D. Zipser. Department of Cognitive Science, UC San Diego, La Jolla, CA 92093-0515.

Many different cues give information about depth, but it is not known how these cues are integrated to form unified percepts. To investigate this, we trained neural network models with biologically realistic visual input to produce depth maps of the same objects used in psychophysical experiments on depth cue integration (Bulthoff & Mallot, *J. Optical Society of America, A5*:1749-1758, 1988). In the psychophysical study, subjects judged the depth of points on computer generated objects with various combinations of shading and stereo cues. Their study showed that perceived depth decreases with fewer cues. Our network model was trained to estimate the depth at the center of its binocular receptive field. The use of targets corresponding to psychophysically measurable quantities for network training ensures that network responses can be directly compared to human responses. Networks trained solely on cue-rich images show a systematic decrease in "perceived depth" to cue-impoverished images similar to that found in humans. Differences between human and network responses highlight local-global cue conflicts in depth perception. By analyzing how these model networks integrate different depth cues, hypotheses can be generated as to how this integration takes place in the brain. These results extend the neural systems identification paradigm (Zipser, *Neuroscience, 47*(4):853-862, 1992) to models that can be compared to psychophysical data.
This research was funded by NIMH Grant MH45271 and System Development Foundation Grant G359D to DZ.

587.4

INTERACTIVE ACTIVATION MODEL OF HIERARCHICAL PROCESSING OF VISUAL OBJECTS. H. E. Schendan* and G. Ganis. Neurosciences Graduate Office, 0608, UCSD, La Jolla CA, 92093; Dept. of Cognitive Science, 0515, UCSD, La Jolla CA, 92093.

A parallel distributed processing model similar to the interactive activation model of McClelland and Rumelhart (1986) is presented that accounts for some of the main findings from neuropsychological and psychophysical studies of hierarchical processing of visual objects. The input units of the network are feature detectors tuned to a range of different spatial frequencies and orientations. The second layer is composed of letter detectors and each detector receives feedforward projections from all input units for a particular spatial position. Weights coming from input units consistent with the configuration of that particular letter are positive, whereas weights corresponding to input units inconsistent with the configuration of that particular letter are negative. Lateral inhibitory connections between units in the second layer result in a winner-take-all configuration. How low and high spatial frequency filters may contribute to hierarchical processing is explored. Differential hemispheric rates of processing of high versus low spatial frequencies have been postulated to be the best explanation for global and local effects in the processing of hierarchical visual objects (Robertson and Rafal, 1991). The results of the simulations using the interactive activation model support this hypothesis.

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587.5

VISUAL AFTEREFFECTS IN THE MONKEY: THE JUDGEMENT OF VERTICAL AFTER ADAPTATION TO OBLIQUE CONTOURS.
M. A. Berkley*[1], R. Vogels, G. Sáry & G. A. Orban. Laboratorium voor Psycho-en-Neurophysiologie, Katholieke Universiteit Leuven, Belgium.

In humans, it has been shown that the subjective estimate of the orientation of a contour is shifted by several degrees from its real orientation after extended viewing of contours differing 5-50 degrees from the orientation of the contour to be judged. This phenomenon, called the tilt aftereffect (TAE), has frequently been used in studies attempting to define the neural substratum of contour perception. As a preliminary to making direct studies of neurons that might participate in the TAE, we trained a monkey with an implanted eye coil to fixate a central target (95% contrast sq. wave grating) and report the orientation of a subsequent, briefly presented (100ms) test target (20% contrast sq. wave grating of various orientations) by making a leftward saccade to indicate a counterclockwise (CCW) orientation and a rightward saccade to indicate a clockwise (CW) orientation. After learning the task, the fixation time at the start of each trial was gradually increased to 5 secs, and a 600 ms blank interval introduced before each test target presentation. The proportion of test orientations judged to be CW were used to construct psychometric functions and showed that the monkey's estimate of subjective vertical (orientation at which CW or CCW choices were 50%) was dependent upon the orientation of the grating viewed during the fixation interval. Fixation gratings tilted 15 degs. from vertical produced a shift of 1-1.5 degs in subjective vertical, while fixation gratings orientated vertically or horizontally produced no shift. The TAE observed was slightly smaller than that shown by human subjects tested under the same conditions. The presence of a TAE in monkeys rules out many cognitive explanations for the effect, and demonstrates the feasibility of quantitative investigations of perceptual effects in animals. [1]on sabbatical leave from Florida State Univ., Tallahassee, FL.

587.6

A NEW EFFECT IN COLOR AND SPATIAL VISION: VISIBILITY OF A TEST PATTERN IN A MASK IS BANDPASSED WITH VIEWING DISTANCE
R.L.P. Vimal and R. Pandey*, New England College of Optometry, Boston, MA

We observed that a masked pattern was often most visible at intermediate viewing distances. We examined this masking effect as a function of the mask's relative spatial frequency (SF) and relative orientation in both chromatic and achromatic modes. A spatially localized test target (e.g. 1 cpd at 80 cm with 30% contrast) and sinusoidal masks (2 cpd with 60% contrast) displayed on a Sony GDM1936 color monitor interfaced with ATVista Graphic System was used. The Red-Green (RG) channel was isolated by the minimum flicker equiluminant criterion and the hue cancellation technique. Two normal observers made magnitude estimations (ME) using ratings between 0 (test pattern was not seen) and 10 (test was most distinctly seen). We found bandpass ME visibility curves (ME rating vs. viewing distance) for both achromatic (reddish and greenish patterns inphase) and RG chromatic (patterns antiphase) modes for vertical test and masks oriented 14.5°, 45° and 90° from the vertical. The visibility of the test pattern increased as the orientation of the mask relative to the test increased at a given distance. Our data showed that the visual system has SF and orientation tuned mechanisms at suprathreshold contrasts (similar to threshold contrasts[1]) in both chromatic and achromatic modes. Low test pattern visibility suggested that the test was processed mostly by the same SF and orientation tuned mechanisms as the mask whereas higher pattern visibility indicated processing by different mechanisms. This masking effect, which is stronger in the chromatic mode, can be explained by SF and orientation tuned mechanisms, and by the apparent contrast of the mask relative to the test at different viewing distances.

1. Vimal and Pandey, Invest. Ophthalmol. Vis. Sci. (Suppl) 1992, 33, 704

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587.7

SPATIAL SUMMATION OF VISUAL INFLUENCE ON EGOCENTRIC LOCALIZATION: VARIATIONS WITH ECCENTRICITY. L. Matin* and W. Li, Psychology Dept., Columbia Univ., New York, NY 10027.

The elevation of a target set to appear at visually perceived eye level (VPEL) varies linearly with the pitch of a visual field that is complexly-structured or consists of only 1 or 2 vertical lines (Vis Res'89, JEPHPP'92).

The influence on VPEL of the pitch of 1 vertical line of variable length was measured at 2 eccentricities (ecc), 5° and 25°; slopes of VPEL/Pitch functions increased exponentially w/length with space constants of 5° and 13°, respectively. For the shortest line (3°) pitch was 33% more potent at 5° ecc, but this predominance decreased and reversed with longer lines so that at 64° length the more peripheral stimulus was 55% more potent. Max. VPEL/Pitch slopes were 0.36 and 0.55 at 5° and 25° ecc, respectively. However, VPEL is uniformly more sensitive to retinal orientation of the parafoveal line than the peripheral line.

The relative effectiveness for egocentric localization of short and long lines in the parafovea as compared to the periphery and the variation in space constant is in a similar relation to that found for visual acuity and for V1 receptive field size. However, the 13° space constants for VPEL are at least an order of magnitude larger than is appropriate for visual acuity or V1 receptive field size, but they do correspond to receptive field sizes reported in MT, MST, and 7a. (Supported by AFOSR 91-0146)

587.8

SPECIFICITY OF SELECTIVE VISUAL ATTENTION FOR ORIENTATION AND SPATIAL FREQUENCY. A.F. Rossi* and M.A. Paradiso. Center for Neural Science, Brown Univ., Prov., RI.

Most studies of visual attention concern the finding that when attention is drawn to one location in the visual field, performance at that location is enhanced. However, there are suggestions from both physiological and psychophysical experiments that there are also feature-specific aspects to selective attention. Our psychophysical experiments quantify the extent to which attention is specific to attributes of visual stimuli such as spatial frequency and orientation. A subject's attention is engaged by performing a temporal forced choice discrimination task with foveal stimuli consisting of Gabor patches. During the experiment a secondary task is performed in which the subject detects the presence of a grating briefly presented in the periphery shortly after the second Gabor target. In order that attention be kept on the primary discrimination task, the subject is prompted to indicate whether a grating is present on only one third of the trials. On only half of these detection trials is a grating actually present. We find that the hit rate for detection of the surround grating is highly dependent on its orientation and spatial frequency relative to the Gabor stimuli. The detection rate is considerably higher for gratings having the same frequency and orientation as the discrimination targets. Performance degrades as the grating frequency and orientation deviate from that of the targets and we have measured bandwidths for these attentional processes. Importantly, performance on the secondary task is selective for both orientation and spatial frequency even though the discrimination task only involves one of these attributes. These data provide quantitative measurements of the extent to which selective attention can be specific to featural attributes in addition to location.

587.9

EXPRESS SACCADES ELICITED DURING NATURAL FIXATIONS OF VISUAL SEARCH. M. A. Sommer* and P. H. Schiller, Department of Brain and Cognitive Sciences, Mass. Inst. of Tech., Cambridge, MA 02139.

Very short latency saccadic eye movements (≤105ms) are elicited from primates after extensive training on simple tasks, but do such "express" saccades occur during normal visual search? Highly- and barely-trained macaques with implanted scleral search coils were tested on a "Standard" task (fixation point stays on during presentation of target) in which the fix-spot could appear in one of nine positions (3x3 matrix). Foveation of this point caused a target to appear at one of four locations around it, after a target onset lag (50 to 150 ms). A direct saccade to this target location resulted in juice reward. A second "Free Fixation" task was identical except that fix-spots would appear at the other eight locations along with the one "real" fix-spot at the start of a trial; a monkey had to search over the 3x3 fix-spot array until it foveated this "real" fix-spot, initiating the remaining trial events. In this Free Fixation task, monkeys made saccades every 205 ±60ms for up to 10 sec. to search the array. A subject's ability to execute a direct saccade to a suddenly appearing target decreased with target onset lag, from 90% correct at 50 ms to 30% at 150 ms. Reaction times of correct saccades were 95 ±12ms (unimodal) for highly-trained and 98 ±20ms (often bimodal) for barely-trained monkeys. In contrast, reaction times in the Standard task were 120 ±23 and 130 ±26ms respectively. This suggests that express saccades are a natural means of foveating stimuli that suddenly appear during normal visual search.

587.10

VISUAL SEARCH STRATEGIES OF MONKEY AND MAN. M.R. Dürsteler* and R. von der Heydt, Department of Neurology, University Hospital Zürich, CH-8091 Zürich, Switzerland.

A rhesus monkey was trained on search tasks that produce either parallel or serial search in humans. In a display of 4, 8, or 16 bars he had to detect a bar that differed from the others by a unique color, orientation, or size, or only by a conjunction of two such features in different dimensions. A target combination was rewarded until the animal could detect it reliably, and then replaced by another combination. We found that the monkey could detect feature conjunctions in parallel that humans can find only by searching serially, e.g. color*orientation: 492ms-0.1ms/item for monkey vs. 622ms+ 12.6ms/item for human (reaction times for target-present trials). Signs of serial search were seen during learning phases; however, when performance reached 90% correct search functions became flat. Significantly, transfer to a new target conjunction required several hundred trials of retraining, even after several conjunctions had been learned successfully. For difficult discriminations (size difference 20%) the monkey did use serial search, but conjunctions were found as fast as features (size*orientation: 7.7ms/item, size: 6.5ms/item). We conclude that monkeys accomplish conjunction search differently from humans. Either they employ conjunction detectors that humans cannot use (cells with appropriate joint selectivity are known to exist in monkey visual cortex), or they effectively avoid searching serially by other strategies, similarly as humans can do under certain conditions.

587.11

TIME COURSE OF COVERT ORIENTATION IN A RHESUS MONKEY. M. Davidson, L.J. Thomas-Thrapp, E.A. Witte, M.I. Posner*, C.R. Collazo, Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Covert orienting of visual attention has been widely studied in humans. Following a peripheral visual cue, a target presented at the cued location is responded to more rapidly than when the target occurs at an uncued location (validity effect). When the cue predicts the target location the advantage for the cued side occurs within the first 100 msec following the cue and the validity effect is usually sustained throughout an interval of 1-2 seconds.

An adult female rhesus macaque was trained to do a covert target detection (CTD) task. During the CTD task a trial was initiated when the monkey depressed a bar and fixated a spot in the center of a video monitor. Fixation was checked by measuring eye movements with the scleral search coil method. Following a random delay interval, a peripheral cue was presented to one or both hemifields. Either 100, 400, or 700 msec after the cue a target was presented to either the left or right hemifield. In 57% (8/14) of the trials the cue was presented to the same hemifield as the target (valid condition). In 14% (2/14) of the trials the cue was presented to the opposite hemifield as the target (invalid condition). In 28% (4/14) of the trials a cue was presented to both hemifields (neutral condition). The monkey indicated target detection by releasing the bar.

The results showed a validity effect of 26 msec at a cue-target latency of 100 msec. The size of the validity effect remained at this value at 400 msec and appeared to show a small reversal at 700 msec. These results were quite similar to those found by Bowman et al. for a rhesus monkey trained and tested in a similar way. The data suggest that the orienting of attention to peripheral cues by these animals is similar to that found in humans. However, the monkey seems less influenced by target probabilities than our humans because she did not show evidence of maintaining attention to the cue at the 700 msec cue-target latency. This research was supported by the Pew Memorial Trust and James S. McDonnell Foundations.

587.12

BEHAVIORAL EVIDENCE OF THE FILLING-IN AT THE BLIND SPOT OF THE MONKEY. H. Komatsu* and S. Yamane, Neuroscience Section, Electrotechnical Lab., Tsukuba, Ibaraki, 305 Japan.

It is well known that when a human subject closes one eye, color and brightness fills in to the blind spot from the visual field surrounding it, and that he does not recognize the absence of the visual information at the blind spot. In the present experiments, three monkeys were tested if there is a behavioral indication of the filling-in at the blind spot. The location of the blind spot was determined from the failure of making saccades to a small visual target in a saccade task. The blind spots located on the horizontal meridian at about 14 to 15 deg ipsilaterally from the fixation point and had a vertically elongated ovoid shape with a size of 5 to 6 deg vertically, and 4 to 5 deg horizontally. Then filling-in at the blind spot was tested using a discrimination saccade task. In this task, when the monkey was fixating a central spot, a filled circle and an annulus with the same color and size were presented simultaneously to the right or to the left of the fixation point. The filled circle covered the region in the visual field corresponding to the blind spot of one eye and the edge of the blind spot of the opposite eye located between the outer and the inner circle of the annulus. Monkeys were rewarded if they made a saccade to the filled circle. When both eyes were open, or when one eye was closed and the filled circle was presented on the blind spot of the opened eye, monkeys performed well in a performance rate over 80% correct. However, when one eye was closed and the annulus was presented on the blind spot of the opened eye, the performance dropped to nearly chance level indicating that monkeys were unable to distinguish an annulus from a filled circle. These results suggest that the filling-in occurs at the blind spot of monkeys as is the case in human subjects.

AUDITORY, VESTIBULAR AND LATERAL-LINE HAIR CELLS

588.1

CHARACTERIZATION OF THE POTASSIUM CURRENTS IN TYPE I MAMMALIAN AND AVIAN SEMICIRCULAR CANAL HAIR CELLS. K.J. Rennie and M.J. Correia* Depts. of Otolaryngology and Physiology and Biophysics, UTMB, Galveston, TX 77555.

Type I vestibular hair cells differ in their ionic currents from type II hair cells. Isolated pigeon type I cells are known to have significantly lower input resistances than type II cells and a potassium current active above -80mV. We have used the perforated patch technique (amphotericin B) to make whole-cell recordings from type I hair cells, in order to further identify the currents. Cells were dissociated from the semicircular canals of gerbils and pigeons. Two potassium-selective conductances and a putative non-selective cation current modulated by acetylcholine (100μM), have been found so far. At potentials above -40mV, one of the potassium currents is first present. This current is sensitive to externally applied 100μM quinidine, 4mM barium and 37μM nifedipine, properties consistent with it being a calcium-activated potassium current. At potentials positive to -80mV the other potassium current is present and is blocked by externally applied cesium ions at 5mM (n = 10), but not by intracellularly applied cesium (140mM). Large inward tail currents (approx. 1nA) associated with this conductance appear to lead to cell membrane depolarization. This current is also sensitive to calcium, being reduced by externally applied nifedipine (37μM), 4mM barium, 1.2mM nickel and removal of external calcium. At the average zero-current potential in gerbil hair cells (-61mV), this current is 90% activated. Supported by NIH grant DC01273.

588.2

CROSSLINKING OF VANADATE-TRAPPED NUCLEOTIDES TO TWO MYOSIN CANDIDATES IN HAIR BUNDLES. P. G. Gillespie* and A. J. Hudspeth. Department of Cell Biology and Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX 75235-9039.

Our model for mechanoelectrical transduction by hair cells (Hudspeth, A. J. [1989] *Nature* **341:** 397-404) invokes an actin-based motor, perhaps a member of the myosin family, to account for adaptation of the receptor current. Because direct evidence for a hair-bundle myosin is lacking, we sought candidate molecules by asking which constituents of purified hair bundles bind nucleotides under circumstances characteristic of myosins.

Irradiation with ultraviolet light efficiently crosslinks underivatized nucleotides to many myosins (Maruta, H. and Korn, E. D. [1981] *J. Biol. Chem.* 256: 499-502). After exposure to [α-^{32}P]ATP or UTP, photocrosslinking labeled proteins of 120 and 230 kD in hair bundles isolated from the bullfrog's sacculus. Vanadate trapping experiments were consistent with their being myosins. After forming a complex with myosin and newly hydrolyzed ADP or UDP, vanadate dramatically decreases the dissociation rate of the bound nucleotide. We found that vanadate was required for tight binding of nucleotide to the 120 and 230 kD proteins. We could eliminate labeling of the two bundle proteins by simultaneous incubation with an excess of unlabeled ATP (but not AMP) or by preincubation with a mixture of ADP and vanadate. A divalent cation (Mg^{2+} or Mn^{2+} but not Ca^{2+}) was required for observation of labeled proteins. The myosin candidates were surprisingly resistant to solubilization by high- salt conditions and sonication. Consistent with this observation, we detected no biotinylated proteins in ATP extracts of isolated hair bundles. We surmise that the 120 and 230 kD proteins are myosins that are tightly attached to a cytoskeletal structure.

This research was supported by NIH grant DC00241.

588.3

DISPLACEMENT - CLAMP MEASUREMENT OF FORCES EXERTED BY GATING SPRINGS IN THE HAIR BUNDLE. F. Jaramillo* and A. J. Hudspeth. Department of Cell Biology and Neuroscience, University of Texas Southwestern Medical Center, Dallas, Texas 75235-9039.

The mechanically sensitive organelle of each hair cell in the auditory and vestibular systems is the hair bundle, a cluster of stereocilia protruding from the apical cellular surface. Deflection of a bundle acts through elastic elements, the gating springs, to open cation-permeable transduction channels atop the bundle. Using a displacement-clamp system on hair cells from the bullfrog's sacculus, we measured the forces that the gating springs exert upon the stereocilia. The position of a hair bundle was clamped with a flexible glass fiber, whose position was monitored with a photodiode imaging system and maintained by negative feedback. To estimate the force exerted at rest by the gating springs, we disrupted them by focal iontophoretic application of the calcium chelator, BAPTA. The ensuing force on the glass fiber indicated that the gating springs are under tension at rest, and exert a negatively directed force of 57 ± 34 pN (mean $\pm$ standard deviation, n = 11) on the hair bundle. Consistent with earlier results, disruption of the gating springs diminished the bundle's dynamic stiffness by at least 27%. We also quantified the mechanical effect on the hair bundle of gentamicin, a drug which blocks transduction channels in the open state. When the channels' gates were held ajar by iontophoretically applied gentamicin, the tension in the gating springs decreased, producing a force on the bundle of approximately 6 pN in the positive direction. This result, which indicates that a channel's gate swings by at least 2 nm, accords with the 4 nm estimate obtained from gating-compliance measurements. This research was supported by National Institutes of Health grant DC 00317.

588.4

VOLTAGE-DEPENDENT CURRENTS IN HAIR CELLS OF THE RAT UTRICLE. M. Saeki* and R.A. Eatock. Dept. of Physiology, University of Rochester, Rochester, NY 14642-8642.

Macroscopic currents were recorded from dissociated rat utricular hair cells using the whole-cell tight-seal patch-clamp technique. The average zero-current potential was -68 ± 3.5 mV (mean $\pm$ SEM, n=4). Hyperpolarizing steps from the holding potential of -61 mV elicited an inwardly rectifying current (I_{IR}) that was reversibly blocked by 5 mM external Cs, suggesting a K-selective current. The slope conductance from -80 to -120mV was 6 ± 1.5 nS (n=7). Depolarizing steps from -61mV elicited an outwardly rectifying current (I_o) that sometimes deviated from Ohmic relationship at positive potentials. Over the linear range, the slope conductance was 34 ± 25.7 nS (n=7). I_o always exceeded I_{IR} but the ratio was variable (7 ± 4.1, mean $\pm$ SD). Block of I_o by internal Cs showed that it is K-selective. The lack of N-shape in most current-voltage (I-V) curves suggests that Ca-dependent current is not predominant. I_o shows voltage-dependent recovery from inactivation below about -10 mV and was blocked 72 % by 1 mM 4-AP. With internal BAPTA to block any Ca-dependent current, tail currents following depolarizing steps could be fit by the sum of 2 exponentials (τ's of 0.6 and 9 ms). The slow component was 50% activated at -19 mV. These results are consistent with 2 outward K currents, possibly delayed rectifier and A current. A 50 ms pulse to -41 mV elicited a non-inactivating inward current. With internal Cs to block K current, depolarizing steps positive to -40 mV elicited inward currents that peaked at 0 mV. These are likely to be L-type Ca current.

588.5

The Properties of Inward Rectification in Vertebrate Vestibular Hair Cells. J.R. Holt* and R.A. Eatock, Box 642, Department of Physiology, University of Rochester Medical Center, Rochester, NY 14642

An inwardly rectifying current (I_{IR}) was noted early on in the study of hair cell currents (Lewis and Hudspeth, Nature, 1983, 304, 538-541), but has received little attention. We are in the process of characterizing this current in acutely dissociated saccular hair cells of the leopard frog using the whole cell configuration of the patch clamp technique.

I_{IR} was isolated using 2 mM Cd^{2+} externally to block inward Ca^+ current directly and thus, $I_{K(Ca)}$ indirectly. A large inward current that increased to steady-state with a monoexponential time course was activated upon hyperpolarization and deactivated upon depolarization. A small steady-state outward current was apparent up to 40 mV more positive than the K^+ equilibrium potential. The sensitivity of I_{IR} to external Cs^+ and its reversal potential indicated that it is K^+-selective. Externally applied Cs^+ completely blocked inward I_{IR}. A 10 fold change in external K^+ shifted the reversal potential approximately 58 mV, the amount predicted by the Nernst potential for K^+.

Steady-state chord conductance (G_{Chord}), at several K^+ concentrations, was plotted as a function of voltage and fit by a Boltzmann curve. Increase in external K^+ concentration was found to increase the maximal G_{Chord}, as well as shift the voltage at which G_{Chord} was half maximally activated. The slope of the curve was such that an e-fold change in G was elicited by a 10 mV change in voltage (equivalent to 2 to 3 gating charges). In these respects hair cell I_{IR} resembles other K^+-selective I_{IR}'s.

Vandenberg (Proc. Natl. Acad. Sci., 1987, 84, 2560-64) showed in cardiac cells that inward rectification is due to a voltage-dependent Mg^{2+} block. To test the Mg^{2+} dependence in hair cells we added 10 mM EDTA internally to chelate Mg^{2+} to less than 100 nM. The presence of EDTA in the cell was verified by an 80% reduction in $I_{K(Ca)}$, yet low Mg^{2+} had no effect on the steady-state rectification of I_{IR}.

588.6

OUTER HAIR CELL MOTILITY UPON FREE-FIELD ELECTRICAL STIMULATION. M.L. Whitehead*[1] and W.E. Brownell[2]. [1]University of Miami Ear Institute (M805), P.O. Box 016960, Miami, FL 33101. [2]Department of Otolaryngology-Head & Neck Surgery, Johns Hopkins University, 522 Traylor, 720 Rutland Ave, Baltimore, MD 21205.

Isolated outer hair cells (OHCs) undergo fast length changes upon a.c. voltage stimulation, which have been proposed to result from shifts of *position* of elements (electrophoresis) or fluid (electro-osmosis) in response to axial voltage drop, or from summed *length* changes of "molecular motors" in response to transmembrane voltage. To distinguish between these models, guinea-pig OHCs were placed in phosphate-buffered saline between Ag/AgCl electrodes, and exposed to transcellular electric fields of ± 0.75 V/mm. When held at one end, in electric fields parallel to the long axis, ~80% of OHCs shortened and ~20% elongated when the apex was near the cathode. For a fixed cell orientation, the polarity of the length change was independent of which end of the OHC was held, inconsistent with electrophoretic and electro-osmotic models. Fast length changes continued for some minutes after membrane ruptures sufficient to prevent fluid pressure gradients within the cell, arguing against electro-osmotic models, which require such pressure gradients. The middle of all intact OHCs bowed towards the anode in electric fields orthogonal to the long axis, consistent with shortening of the depolarized side, and elongation of the hyperpolarized side, as implied by molecular-motor models. Free-floating OHCs demonstrated slow translocations towards the anode of $\leq 5 \times 10^{-9}$ m^2/Vs. Calculations based on this electrophoretic mobility indicate that electrophoresis contributes insignificantly to fast OHC-length changes.

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588.7

ACETYLCHOLINE MEDIATED CURRENTS IN SOLITARY TURTLE COCHLEAR HAIR CELLS. M. B. Goodman* and J. J. Art. Committee on Neurobiology and Dept of Pharmacology & Physiology. The University of Chicago, Chicago, IL. 60637.

The efferent post-synaptic potential in turtle hair cells consists of a fast depolarization followed by a longer-lived hyperpolarization. Acetylcholine (ACh) is the probable neurotransmitter at the efferent fiber-hair cell synapse. In the turtle, the entire post-synaptic potential is blocked by atropine and *d*-tubocurare. In addition, superfusion with ACh hyperpolarizes the hair cell and eliminates its response to subsequent efferent stimulation (Art, Fettiplace & Fuchs, 1984, *J. Physiol.* **356**: 525-550). The underlying ionic conductances have been studied in solitary hair cells exposed to ACh. Rapid superfusion of ACh produces a net outward current (20-100 pA) in most cells voltage-clamped at their resting potential (22 of 45). The I-V relation of these cells is most simply interpreted as an increase in the amount of $I_{K(Ca)}$ active at all potentials depolarized to rest. This current may reflect a global increase in intracellular Ca^{2+}, which declines in the continued presence of ACh. A few cells (9% of total) generated a fast inward current followed by an outward current when exposed to ACh and voltage-clamped at rest. The time course of the response in these cells is similar to that of the post-synaptic potential observed in the turtle half-head. The I-V curve for the late component reverses near the potassium equilibrium potential, consistent with the notion that an additional K^+ conductance hyperpolarizes the cell during the late, inhibitory post-synaptic potential. Work supported by: DC00454-04 (NIH) to JJA and a Howard Hughes Predoctoral Fellowship to MBG.

588.8

EXCITATORY AMINO ACID ANTAGONISTS BLOCK THE BASAL AND THE EVOKED ACTIVITY IN THE INNER EAR AFFERENT FIBERS. Enrique Soto*, Amira Flores y Rosario Vega. *Ciencias Fisiológicas - ICUAP, Univ. Autónoma de Puebla, México.*

To determine the role of excitatory amino acid (EAA) receptors in the inner ear hair cell- primary afferent synapse. We studied the effect of EAA antagonists on the mechanical response (sinusoidal accelerations), and on the basal activity of semicircular canal afferent fibers of the axolotl (*Ambystoma tigrinum*) inner ear.

The extracellular electrical discharge of the afferent fibers was recorded by means of a suction electrode. Drugs 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX) 0.01 μM - 0.1 mM; 2-amino-5-phosphono pentanoic acid (AP5) 1 μM - 3 mM; 7-Chloro kynurenic acid (7ClKyn) 1 μM - 1 mM and kynurenic acid (Kyn) 1 μM - 1 mM, were applied by pressure ejection. CNQX and 7ClKyn exerted a specific dose dependent inhibitory action on both the evoked and spontaneous activity, while AP5 and Kyn did not significantly modify the mechanical response, except when used at high concentrations. CNQX shows a thousand fold greater potency than AP5. The rank order of potency was CNQX > 7ClKyn > Kyn > AP5. These results further support the idea of a non-NMDA EAA receptor mediating both the spontaneous and the mechanically evoked afferent transmission in the vestibular system.

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588.9

SYNAPTIC PLASTICITY IN VESTIBULAR HAIR CELLS OF ANIMALS EXPOSED TO MICROGRAVITY. M.D. Ross and T.C. Chimento* Biocomputation Center, NASA, Ames, MS 239-11 Moffett Field, CA 94035-1000

Do synapses in maculas change in number, size or type as a result of exposure to altered gravity during space flights of short duration? This report focuses on results obtained from three maculas of rats flown for nine days on the Spacelab Life Sciences 1 shuttle, and from three matched ground controls. Tissues were obtained within six hours of landing; fixed, post-osmicated, dehydrated, microdissected, and prepared for transmission electron microscopy according to usual methods. Serial sections (0.2 μm) were cut from the medial border of the macula. More than 600 hair cells, containing more than 3000 ribbon synapses, were studied. Synapses in type I hair cells of flight animals increased by 41% compared to type I hair cells of ground controls, and synapses in type II hair cells increased by 57%. This increase was greater in spherule than in rod synapses. There was a significant rise in spherules in both type I (53%; $p \leq .005$, N=301) and type II hair cells (74%; $p \leq .005$, N=320). Most of the additional spherule synapses were present in multiples. In type I hair cells the number of synapse pairs increased from 9% in controls to 18% in flight animals, and in type II hair cells the number of synapse pairs increased from 19% to 52%. Groups of 3-6 spherule synapses in type II cells increased from 2% to 21%. The more numerous multiple synapses in type II cells contacted collaterals rather than calyces. The size of synapses was not significantly different between flight animals and ground controls. The results indicate that macular hair cells exhibit plasticity and that the macular neural network can adapt to an altered gravitational environment.

This work supported by National Aeronautics and Space Administration.

588.10

POSSIBLE MORPHOLOGICAL FEATURES TO DISTINGUISH CALYX VS DIMORPHIC TYPE I VESTIBULAR HAIR CELLS IN THE CHINCHILLA CRISTAE. A. Lysakowski* Dept. of Pharmacol. and Physiol. Sciences, The University of Chicago, Chicago, IL 60637.

A type I vestibular hair cell is enclosed within an ending termed a calyx. A calyx, however, can belong to either a dimorphic unit or to a pure calyx unit (Fernàndez et al., *J. Neurophysiol.* 60:167-181, 1988). Previously, it has been difficult to distinguish type I hair cells belonging to pure calyx units from those belonging to dimorphic units without some form of dye labeling of the afferent fiber. Recently, however, we have observed some morphological features which may serve to distinguish the two types in unlabelled material. Based upon serial section analysis of ten samples from cristae in four animals, there appear to be at least two classes of type I hair cells. The first class has large mitochondria (0.52 μm ± 0.23 in diameter) subjacent to the cuticular plate and thicker stereocilia. These mitochondria were twice as large as those found elsewhere in the same cell. Cells containing these larger mitochondria were found mainly in the central zone at the apex of the crista and occasionally in the intermediate zone. So far they have not been observed in the peripheral zone. The second class of type I hair cells has smaller subcuticular mitochondria (0.24 ± 0.06 μm diameter) and thinner stereocilia. This second class was found in units identified as dimorphic units by the presence of calyceal collaterals. Type II hair cells had subcuticular mitochondria similar in size to the second class. We have confirmed this difference in several HRP-labelled calyx and dimorphic units. Further confirmation will be obtained using an antibody to calretinin, a calcium binding protein apparently found only in calyx units. (Supported by RO3-DC01474.)

588.11

IMMUNOHISTOCHEMICAL LOCALIZATION OF S-100 PROTEINS IN AUDITORY AND VESTIBULAR END ORGANS OF THE MOUSE. J.D. Foster[1,2], M.J. Drescher[1,2], S.F. Myers[2]*, and D.G. Drescher[1,2,3]. [1]Lab. of Biotology, Depts. of [2]Otolaryngology and [3]Biochemistry, Wayne State University, Detroit, MI 48201.

Calcium-binding proteins are thought to be involved in the transduction of intracellular calcium signals which mediate cellular responses to extracellular events. In auditory and vestibular end organs, calcium-binding proteins may modulate certain calcium-dependent processes. Utilizing immunohistochemical procedures, we have localized in the mouse labyrinth S-100-like immunoreactivity, the S-100 proteins representing one subfamily of the EF-hand family of calcium-modulated proteins.

Cochlear inner hair cells and neural elements of the spiral ganglion were weakly immunoreactive. The cytoplasm of Deiters' cells and the basal regions of outer hair cells were strongly positive for S-100-like immunoreactivity, while the supranuclear regions of outer hair cells were unreactive. Fibers in the spiral ligament were strongly immunoreactive.

In the vestibular end organs, including the saccule, utricle, and semicircular canals, nerve fibers underlying the sensory epithelium and nerve calyces surrounding type I hair cells were immunoreactive. Types I and II vestibular hair cells appeared weakly immunoreactive, while epithelial supporting cells showed no immunoreactivity.

The localization of S-100-like immunoreactivity to the basal regions of cochlear outer hair cells and to presumed afferent nerve calyces enveloping vestibular type I hair cells is consistent with a functional role for S-100 proteins at these sites in the regulation of calcium-dependent events.

(Supported by NIH Grants DC 00026 and DC 00156.)

588.12

RECEPTION OF LOW INTENSITY MILLIMETER-WAVE RADIATION BY SENSORY ORGAN. G. N. Akoev, V. D. Avelev, P. G. Semenjkov and R. N. Stiles*. I. P. Pavlov Institute of Physiology, Russian Academy of Sciences, St. Peterburg, Russia, Kara-Dag Biological Station, Krimea, Ukraine, and Department of Physiology & Biophysics, University of Tennessee, Memphis.

The effect of low intensity millimeter-wave electromagnetic radiation at 33-55 GHz on electroreceptors (ampullae of Lorenzini) was studied in skates (*Raja clavata*). The onset of millimeter-wave irradiation at a power intensity of 1-5 mW cm^{-2} to the duct opening at 1-10 mm distance caused transient increases in the firing rates of single afferent units, followed by adaptation during 2-5 min to the initial level. The reverse effect was observed after offset of irradiation. When the power intensity was increased, inhibitory responses were also observed. The inhibitory responses were more pronounced in receptors with higher electrical thresholds; these did not show any excitatory response. On the contrary, on irradiation from a distance of 15-20 mm from the duct opening, the receptors responded with a prolonged excitatory activity lasting up to 20 min. Direct irradiation of the sensory cells irrespective of their electrical threshold produced only an inhibition. The maximal effect of millimeter-wave radiation was shown to be at 55 GHz. The mechanism of the effect of low intensity millimeter-wave radiation on the ampullae of Lorenzini will be discussed.

588.13

REGENERATIVE CELL PROLIFERATION IN THE MAMMALIAN UTRICLE IN VITRO. M.E. Warchol*, B.J. Goldstein, and J.T. Corwin. Dept. of Otolaryngology-HNS and Dept. of Neuroscience, University of Virginia, Charlottesville, VA 22908

Postembryonic proliferation of supporting cells, which leads to the addition of new hair cells, has been demonstrated in the inner ear sensory epithelia of fish, amphibians, and birds. The vestibular sensory epithelia in mammals share most morphological traits with their counterparts in those groups, but cell proliferation in mammalian vestibular epithelia has been thought to occur only during embryonic development. We have tested this assumption by explanting utricles into organ culture and have observed that regenerative proliferation of mammalian supporting cells can occur.

Utricles were removed from juvenile and sexually mature albino guinea pigs (wt: 400-700 gm), and cultured in Rose chambers at 37°C. Hair cells were lesioned by incubating the cultures in media that contained ototoxic antibiotics (either 0.5-1.0 mM neomycin or 1.0 mM gentamicin). Then the cultures were incubated for 2-6 days in aminoglycoside-free media that contained either ^{3}H-methyl-thymidine (0.8 μCi/ml) or bromo-deoxyuridine, so that DNA would be labeled in any cells that had proliferated during the culture period.

At least four labeled cell nuclei were present in the sensory epithelium in each case. The occurrence of the labeled cells demonstrates that mammalian hair cell epithelia can respond to trauma by renewed proliferation. In addition, these results and others (Forge et al.,submitted) suggest that mammalian vestibular organs are capable of a heretofore unexpected degree of self-repair after hair cell loss.

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588.14

THE ORGAN OF CORTI RESPONDS TO RETINOIC ACID (RA) AND CONTAINS RA, A NUCLEAR RA-RECEPTOR, AND CRABP DURING THE PERIOD OF HAIR CELL DEVELOPMENT. M.W.Kelley[1]*, X.-M.Xu[1], M.A.Wagner[2], and J.T.Corwin[1]. [1]Dept. of Otolaryngology-HNS and Dept. of Neuroscience, University of Virginia, Charlottesville, Virginia, 22908, [2]Howard Hughes Medical Inst., Columbia University, New York, New York, 10023.

We have demonstrated that the addition of 10 nM retinoic acid to cultured cochleas from mouse embryos causes a significant number of cells to alter their normal developmental fates and to differentiate as supernumerary hair cells. This suggested that retinoic acid might participate in the control of differentiation during the normal development of the organ of Corti.

If retinoic acid does function in the normal development of this organ, then retinoic acid and at least one of its nuclear receptors (RAR's) should be present during development. To test for the presence of endogenous retinoic acid, we have cultured embryonic (E14-P0) organs of Corti on monolayers of F9 teratocarcinoma cells that were engineered to express a ß-galactosidase gene when exposed to retinoic acid (Wagner and Jessell, in press). Intense blue staining in the X-gal reaction demonstrated that retinoic acid is produced by the organ of Corti, but not by negative control tissue, such as skin from the head. Western blots were used to test for the presence of RARß and CRABP. Organs of Corti were dissected from mouse cochleas at three developmental time points (E14, E16, P0), and separately homogenized, run through PAGE, transblotted to nitrocellulose, and incubated with monoclonal antibodies specific for those markers. At each time point, a single band at approximately 50 kD was labeled in blots exposed to anti-RARß. A single densely stained band at 16 kD was observed in blots exposed to anti-CRABP.

The results are consistent with a potential role for retinoic acid in the control of hair cell differentiation during the normal development of the organ of Corti.

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588.15

EXPRESSION OF A PROTEIN WITH EGF-LIKE IMMUNOREACTIVITY IS TRIGGERED BY TREATMENTS THAT EVOKE HAIR CELL REGENERATION.

X.-M. Xu, and J.T. Corwin*. Dept. of Otolaryngology-HNS and Dept. of Neuroscience, University of Virginia, Charlottesville, VA 22908.

We have used the trauma-evoked response of the avian cochlea to investigate biochemical changes that lead to regeneration of hair cells. Multiple experimental groups of 7 to 9-day-old white leghorn chicks were each given an injection of gentamicin at 100 mg/kg body weight and then exposed to tonal sound stimulation at 1.5 kHz and 120 dB SPL for 24 hr, so as to damage hair cells and trigger regeneration in the cochlea. Immediately after the treatment, the chicks were euthanized and their cochleas were removed. The sensory epithelium was dissected from each and homogenized in a detergent buffer containing protease inhibitors. Supernatants were run through SDS-PAGE under reducing conditions along with samples from age-matched controls. Nitrocellulose blots were reacted with a polyclonal antibody raised against epidermal growth factor from the mouse submaxillary gland.

Our preliminary findings include significant anti-EGF labeling of a protein that runs at approximately 14 kD in all samples from the experimental groups. The strong labeling of this protein in samples from treated cochleas contrasted with an absence of labeling or barely detectable labeling at that position in control lanes. Bands at higher molecular weights were comparably labeled in both controls and experimentals, providing an internal control for the assay. The increased expression of this protein is detectable immediately after a treatment which causes extensive hair cell loss and which evokes hair cell regeneration. This suggests that a small protein with an EGF-like domain may participate in the cascade of events that initiates the replacement of hair cells.

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588.16

HAIR CELL AND SYNAPTIC RECONSTRUCTIONS IN THE AMPHIBIAN PAPILLA IN LEOPARD FROG, *RANA PIPIENS PIPIENS*.

C. Bertolotto , D.D. Simmons, M. Leong, and P.M. Narins* Dept. of Biology and Brain Research Institute, UCLA, Los Angeles, CA 90024-1606

Amphibians have two auditory organs in each inner ear that are specialized for the reception of airborne sound: the amphibian papilla (AP) and the basilar papilla. In the leopard frog, the AP is innervated by fibers with characteristic frequencies from 100 - 1250 Hz. The AP is believed to be tonotopically organized. To address ultrastructural differences in different parts of the AP, hair cells and their synapses were reconstructed from plastic cross sections. Hair cells from the rostral AP had a mean length of 43.8 μm and a mean area of 519 μm^2. Hair cells from the caudal AP had a mean length of 27.2 μm and a mean area of 292 μm^2. Although the lengths in both caudal and rostral extensions vary systematically, within each region the length decreases along the posterior to anterior axis. In the rostral AP hair cells had 9 to 11 afferent synapses and at least one efferent synapse. In contrast caudal AP hair cells had only 3 to 5 afferent synapses and efferent synapses were not readily observable. Although the present study shows that there are definite morphological correlates of a tonotopic organization, these data additionally suggest that there may be fundamental differences between the rostral and caudal portions of the AP.

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588.17

RAT UTRICULAR MACULA: BLOOD FLOW AND STEREOLOGICAL ASSESSMENT OF CAPILLARY MORPHOLOGY

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Vascular compromise has long been proposed as a cause for inner ear disorders. However, the examination of blood flow and its control mechanisms in the vestibular system has been very limited. Combining stereological techniques with the microsphere injection technique, capillary morphology and regional blood flow were determined for the young adult rat utricular macula. Results are: total utricular blood flow, 0.1581 ±0.0783 μl/min; blood flow to the neuroepithelium (excluding nerve), 0.0995 ± 0.0466 μl/min; blood flow/unit volume, 7.71 ±4.31 $\mu l/min/mm^3$; neuroepithelial volume 0.01322 ±0.0018 mm^3; capillary surface area, 11.75 ±1.78 mm^2/mm^3; mean capillary diameter, 5.84 ±0.56 μm; capillary length/unit volume 627.4 ±78.0 mm/mm^3; volume fraction of capillary lumen, 0.018 ±0.004. Comparisons to data for the posterior canal ampulla (Wanamaker and Lyon, Otolaryngol Head Neck Surg 1990;103:586) indicate that: the mean capillary diameter in the rat utricular macula is smaller; the capillary length/unit volume is greater; the end organs are similar with respect to neuroepithelial volume, capillary surface area/unit volume and blood flow/unit volume. The size of the microsphere used in the present study (9.21 μm), in comparison to the mean capillary diameter (5.84 μm) of the utricular neuroepithelium would indicate that the blood flow data likely represents a minimum value. These findings indirectly indicate that the metabolic rate of the utricular macula is greater than that of the posterior canal ampulla. If there is as much variation in capillary diameter between the neuroepithelium of the other end organs then regional vestibular blood flow studies would have to be performed using microspheres sized appropriately for each end organ

588.18

PRESERVATION OF INNER EAR TISSUES IN THE CHICK FOLLOWING PERFUSION AND MICROWAVE FIXATION.

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The sensory structures of the inner ear are difficult to preserve with conventional fixation methods because such structures are surrounded by bodies of fluid (endo & perilymph). Even intracardiac perfusion can lead to diffusion of molecules from cells and tissues, thus producing poor fixation. This study looks at microwave irradiation as a possible alternative method for fixation of sensitive tissues. The structural preservation of vestibulocochlear tissues is used to compare techniques which utilize a) immersion in primary fixative combined with microwave irradiation; b) intracardiac perfusion combined with microwave irradiation; c) conventional fixation methods of immersion without microwave irradiation; and d) conventional perfusion without microwave irradiation.

Sixteen temporal bones (TB) were harvested from eight chicks and immersed in a 10% formalin solution. Eight of these TB were then fixed by microwave irradiation techniques. Another sixteen temporal bones were harvested from eight chicks perfused intracardially with phosphate buffered saline, followed by 10% formalin. Eight TB from this group were also fixed by microwave irradiation. TB were then embedded in paraffin, sectioned at 6-10μm, stained with hematoxylin & eosin, and inner ear structures were identified. Two blinded investigators then performed a histopathological analysis using light microscopy and graded structural preservation based on a subjective, multi-criterion scale; a value of "1" represented poor preservation,"2" - intermidiate, and "3" - good preservation. The mean values for the four groups are as follows: immersion & microwave, n=2.57; perfusion & microwave, n=2.35; conventional immersion, n=2.33; conventional perfusion, n=2.68.

The general trend of results indicate that tissues perfused intracardially provide the best histological preservation. However, when immersion is used in place of perfusion, microwave irradiation significantly enhances histological preservation and thus may parallel conditions seen in intracardiac perfusion. We are now in the process of determining antigenicity preservation in tissues fixed with the above protocol.

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588.19

EFFECTS OF SOURCE DISTANCE ON THRESHOLD DETECTION AND SOURCE LEVEL DISCRIMINATION BY THE LATERAL LINE SYSTEM OF THE MOTTLED SCULPIN.

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The feeding response of the mottled sculpin was used to measure (1) threshold levels of detection for 50 Hz vibrations of a small (6 mm diameter) spherical source and (2) just-detectable level increments in on-going 50 Hz vibrations of the same source placed near the head or trunk of the fish at varying distances. At distances between 15 and 60 mm, source level at mean threshold detection (4 fish) increased with distance at an average rate of around 14 dB/distance doubling. Mean level discrimination limens (5 fish) for equivalent 10 dB sensation levels did not change with distance and were around 6.5 dB. These results show that (1) incompressible flow fields (falling off at 18 dB/distance doubling) are more likely to govern detection responses than pressure (falling off at 6 dB/distance doubling), but that (2) spatial patterns of flow amplitude, which change dramatically with distance near the source, do not affect the ability of mottled sculpin to discriminate source levels.

588.20

IN SKATE ELECTRORECEPTORS (AMPULLAE OF LORENZINI) INCREMENTAL SENSITIVITY RECOVERS IN THE PRESENCE OF INITIALLY SUPRAMAXIMAL DC SHIFTS, AN ADAPTIVE FEATURE FOR HIGH SENSITIVITY.

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These receptors respond to low frequency μV potentials applied across the receptor epithelium. The afferents are tonically active at c.15/s, and opposite sign stimuli modulate frequency up or down (max. c. 80/s). We recorded from afferent fibers intracranially, stimulated locally close to canal openings to measure sensitivity, and applied DC with a monopolar electrode in the gut. Test responses to weak pulses (e.g. 10μV, 2s) were blocked initially by well suprathreshold DC shifts up to ±1 mV (which is c. 10^3 X threshold), but sensitivity returned to near normal within minutes. As response frequency declined during large excitatory shifts (and after large inhibitory ones) sensitivity often transiently increased above baseline. During large inhibitory shifts (and after large excitatory ones) spontaneous activity returned before recovery of sensitivity. Cellular mechanisms of adaptation appear not to involve widespread changes in $[Ca^{2+}]_i$ (see abstr. by C.J.H. Wong et al.). Adaptation to relatively large DC potentials, such as associated with secretion of Cl^-, should help to maintain receptor function under changing conditions.

588.21

$[Ca^{2+}]_i$-IMAGING OF RECEPTOR CELLS IN AMPULLAE OF LORENZINI OF THORNBACK RAYS. C. J. H. Wong[1], V. Lev-Ram[2], E. L. Winter, M. H. Ellisman[1], R. Y. Tsien[2&3*] and M. V. L. Bennett[4]. San Diego Microscopy and Imaging Resource, [1]Departments of Neurosciences, and [2]Pharmacology and [3]HHMI, Univ. of Cal. San Diego, La Jolla, CA 92093; [4]Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Ampullae of Lorenzini are elasmobranch electroreceptors sensitive to microvolt signals across their sensory epithelia. Neuroepithelial receptor cells transduce these signals and excite primary afferents via glutamatergic synapses. Tonic impulse activity in afferent fibers is increased by lumen-negative stimuli and decreased by lumen-positive ones. Clusin and Bennett (J. Gen. Physiol 73:703, 1979) concluded that apical Ca^{2+}-influx, induced by lumen-negative stimuli, depolarized the basal faces, activating voltage-sensitive Ca^{2+}-channels in them and causing transmitter release. Confocal ratio imaging of indo-1 loaded receptor cells at 0.1-15 frames/sec showed high $[Ca^{2+}]_i$ which did not change during stimulated or spontaneous electrical activity. $[Ca^{2+}]_i$ could be lowered by loading the AM-ester in Ca^{2+}-free EGTA medium or raised by metabolic poisons. Some cells loaded with fluo-3 via its AM-ester showed spontaneous but not externally stimulatable changes in fluorescence, suggesting endogenous fluctuations in $[Ca^{2+}]_i$. Activity-related $[Ca^{2+}]_i$ transients may not have been detected because extensive convergence on afferent fibers means that only a few receptor cells needed to be active. Alternatively, rapid physiologically effective changes in $[Ca^{2+}]_i$ may be restricted to highly localized regions of the receptor cells and not visualizable with these methods.

REFLEX FUNCTION I

589.1

MODULATION OF LARYNGEAL REFLEXES IN HUMANS DURING REPEATED STIMULATION AND VOLITIONAL TASKS. C.L. Ludlow*, G.M. Schulz, T. Yamashita, and J. Koda. Voice and Speech Section, NIDCD-NIH, Bethesda, MD 20892.

In awake humans, electrical stimulation of the superior laryngeal nerve, containing mucosal afferents, elicits two types of responses in the thyroarytenoid muscles, an early ipsilateral R1 response around 17 ms and a late bilateral R2 around 60 ms. Our purpose was to examine inhibitory mechanisms controlling these muscle responses in awake humans during 1) pairs of nerve stimuli presented at rest and 2) when single stimuli are presented at rest and during volitional laryngeal tasks. With double stimulation at different inter-stimulus intervals (ISIs), conditioned R1 amplitudes decreased linearly as ISI durations were reduced from 10 s to 100 ms. Conditioned R2 responses were reduced in amplitude in relation to the time interval between stimuli but became infrequent particularly at the 500 ms ISI. Reflex responses to a single stimulus were studied during quiet respiration and three tasks: forced inspiration, effort closure and phonation. The occurrence and amplitudes of R1 responses were unaffected by task performance. R1 amplitudes were equivalent to the response amplitude in quiet added to the increased baseline activity level for a task. R2 responses, however, became infrequent during task performance in comparison with rest. The R2 response inhibition suggests that these reflexes are normally suppressed during speech. The inhibitory interval affecting the occurrence of R2 responses between 250 and 500 ms following stimulation, may contribute to the cyclic nature of repeated coughs on one exhalation. The results suggest that R1 and R2 are independently modulated.

589.2

MECHANICALLY EVOKED PERIORAL REFLEXES IN INFANTS, CHILDREN, AND ADULTS. S.M. Barlow *, D.S. Finan, P.T. Bradford, and R. Andreatta.
Department of Speech and Hearing Sciences and Program in Neural Science, Indiana University, Bloomington, IN 47405.

Experimental observations of upper limb motor function suggest that the transition of simplex reflex processing to more elaborate forms of sensorimotor actions for voluntary reactions is fundamental to motor skill acquisition (Bawa, 1981: *EEG & Clin Neurophys*, 52: 249). Mechanically evoked activity in the orbicularis oris superior and inferior muscle was studied in young infants (N=7), school-age children (N=10), and adults (N=10) during periods of lip muscle activation. A specially designed multi-point array skin contactor, coupled to a servo controlled linear motor, was used to deliver precise mechanical inputs to the lip vermilion in order to characterize the nature of the perioral reflex as a function of age. The evoked R1 response obtained from the infant was of low amplitude relative to the children and adults, and lacked the degree of muscle specificity characteristic of the adult form. The emergence and maturation of mechanically evoked perioral reflexes is discussed in relation to the acquisition of motor skills involving the lower face. Supported by NIDCD (R01 DC 00365-05).

589.3

EFFECTS OF ANKLE POSITION ON M- AND H-WAVE AMPLITUDES IN HUMAN SOLEUS HOFFMAN REFLEX TESTING. S.C. Allison and L.D. Abraham*, Kinesiology & Health Education and Institute for Neuroscience, University of Texas, Austin, TX 78712 and Healthcare Rehabilitation Center, Austin, TX 78745.

Numerous researchers have reported a somewhat paradoxical finding of soleus H-reflex suppression with passive ankle dorsiflexion. This study investigated the effects of passive placement in three ankle positions in five healthy adult males. Hugon's (1973) methodology was meticulously followed to reduce extraneous influences. The subjects were tested at nine stimulus intensities in three randomly sequenced ankle positions (zero degrees neutral, five degrees dorsiflexion, and 20 degrees plantarflexion) and the entire procedure was repeated on a second day.

Analysis of variance revealed significant differences ($p < .05$, 2 & 18 df) among H-wave amplitudes in 114 (84.4%) of 135 pairwise positional comparisons. There was a consistent trend of H-wave suppression with ankle dorsiflexion and greater H-wave amplitude with ankle plantarflexion. However, M-wave amplitudes were also significantly different ($p < .05$, 2 & 18 df) in 118 (87.4%) of the same 135 positional comparisons. These results suggest that changes in ankle position during H-reflex testing may consistently alter the effective activation of tibial nerve fibers. For this reason assessment of position-dependent changes in H-wave amplitudes requires greater attention to M-wave changes than has been reported previously.

589.4

CONCURRENT BRACHIORADIALIS CHANGES DURING TRAINING OF BICEPS BRACHII SPINAL STRETCH REFLEXES IN SPINAL CORD INJURED PATIENTS. R.L. Segal* and S.L. Wolf. Div. Phys. Ther., Depart. Rehab. Med., Emory Univ. Sch. of Med., Atlanta, GA 30322.

This abstract reports the effects of downtraining the biceps brachii spinal stretch reflex (SSR) on the SSRs of the brachioradialis. Sixteen spinal cord injured subjects with involvement of C-5 and C-6 spinal levels have participated or are participating in the study. Subjects were randomly assigned to control (n=8) and treatment (n=8) groups. For both groups the first 6 sessions (baseline) and last 4 sessions (follow-up) involved no operant conditioning of the biceps brachii. The middle 24 sessions involved operant conditioning for the treatment group while all sessions were the same as baseline and follow-up for the control group. The mean biceps SSR decreased from baseline during training by an average of 25.6% while the mean biceps SSR increased by 1% from baseline for control subjects. The brachioradialis SSR, which was not the target of training, decreased by an average of 32% during training and decreased an average of 15% for control subjects. The results appear to indicate that the non-targeted brachioradialis is downtrained during the downtraining of the biceps brachii in spinal cord injured patients. Supported by the AMERICAN PARALYSIS ASSOCIATION

589.5

VIDEO MOTION ANALYSIS OF KNEE SWING IN SPASTIC SPINAL CORD INJURED PATIENTS BEFORE AND AFTER PERINEAL ELECTROSTIMULATION. P.W. Nance*, L.S. Halstead, S.W.J. Seager, Dept. Medicine, University of Manitoba, Winnipeg, Manitoba, Canada R3A 1M4 and the National Rehabilitation Hospital, Washington, D.C. 20010-2949

Detection of changes in spastic leg tone using the pendulum test can be quantified by computerized motion analysis of the videotaped movement (Nance, et al. Soc Neurosci Abs, #187.20, 1991). Rectal probe electrostimulation (RPES) has been used in the treatment of male infertility for the purpose of evoking ejaculation; however, it has been noted qualitatively that SCI patients experience a reduction of leg tone after RPES (Halstead, et al. Paraplegia, 29:43-47, 1991). The pendulum test of the left leg of five spinal cord injured (SCI) patients with problematic spasticity, 3 men and 2 women, were videotaped before and immediately after perineal stimulation in the form of RPES. All subjects in the present study showed improvement in the pendulum test after RPES by a significant increase in the amplitude of knee angle displacement oscillations after the initial swing and an orderly decrease in knee angular velocity as the angular displacement gradually decreased. A phase plane plot illustrated the closer approximation of normal tone by the appearance of large concentric circles which appear similar to a "whirlpool" (Brown, et al. J Neurol Neurosurg Psychiatry 51:1178-86, 1988). The present study demonstrates a quantified antispasticity effect of RPES in SCI men and women. (Supported by the Health Sciences Centre Foundation, Winnipeg, Manitoba and NIDRR#HI33G00044-91.)

589.6

Sensory input during treadmill training alters rhythmic locomotor EMG output in subjects with complete spinal cord injury. B.H. Dobkin,* V.R.Edgerton, E. Fowler, Depts. of Neurology and Physiological Sciences, UCLA, Los Angeles, CA 90024.

Previously, we demonstrated that the human spinal cord can generate locomotor-like EMG activity in a Frankel A, thoracic (T6) SCI with complete transection. In this study, we compare how EMG activity changed in that subject and another Frankel A (T4) subject who, by virtue of having EMG activity induced by an upper extremity Jendrassik maneuver, presumably had some supraspinal input. Both chronic paraplegic subjects were suspended in a harness by a hydraulic lift so that the amount of loading on the legs could be adjusted as they were manually placed through a stepping motion on a treadmill belt moving at 0.6 to 1mph. Surface EMG was recorded in tibialis anterior, gastrocnemius, soleus, vastus lateralis, rectus femoris and hamstrings.

With training, reciprocal EMG bursts in a stepping pattern developed for each patient. The amplitude, discretness and timing of bursts were modulated by the pattern of weight support (air stepping vs varying the level of weight supported by a leg) and speed of the treadmill belt. The activity of agonists, antagonists and synergists during different sensory conditions was best appreciated by joint probability density plots (de Guzman, Brain Res 555:202, 1991).

Rhythmical oscillation of the lower extremities enables lumbosacral neurons to organize sensory input to generate locomotor-like output when there is little or no clinical evidence of supraspinal influence.

589.7

COORDINATION OF MOTOR POOLS IN SYNERGISTS AND ANTAGONISTS DURING PARTIAL BODY WEIGHT SUPPORT LOCOMOTION IN HUMANS. H. NISHIZONO, E. FOWLER, B. DOBKIN, R. GREGOR*, V. R. EDGERTON. Department of Physiological Science, Brain Research Institute and Department of Neurology, UCLA, Los Angeles, CA 90024.

After complete transection of the spinal cord (T12-13) in the adult cat, full weight-supporting locomotion can be generated (Lovely et al. Exp Neurol 92:421, 1986). Further, it has been shown that proprioceptive modulation related to the loading of the hindlimbs during stepping can affect selected phases of the step cycle of the chronic spinal cat (Edgerton et al. J Neurotrauma 9:s119, 1991). Initial results have shown that humans with a midthoracic "complete" spinal injury also can generate stepping-like EMG patterns on a treadmill when the body is suspended so that the subject's weight can be partially supported and the stepping is assisted manually. To more clearly understand how the level of weight support during stepping in spinal subjects affects EMG patterns, variable levels of weight bearing were studied in 5 control subjects, i.e. free of spinal cord injury, during treadmill locomotion at speeds ranging from 0.6 to 1.8 mph. With increased weight support, EMG amplitude decreased in the medial gastrocnemius (MG) and was unaffected in the soleus (Sol) and tibialis anterior (TA). The MG EMG amplitude increased while Sol EMG amplitude was unchanged as speed increased. The antagonist and agonist EMG temporal patterns observed between the TA and MG and the MG and Sol were unaffected by varying the amount of weight support or treadmill speed. During passive, manually assisted stepping with partial weight support, EMG amplitude decreased markedly in the Sol and TA and slightly in the MG. In addition, the agonist and antagonist EMG amplitude relationships were markedly altered during passive stepping with partial weight support. During voluntary air stepping (no weight bearing) minimal activation was observed in the MG and Sol while TA activity was similar to that observed during weight bearing stepping. These results demonstrate that the activity of motor pools of the lower limb of control subjects are altered significantly during locomotion under reduced loading conditions. (Supported by NIH Grant NS16333)

589.8

POSSIBLE ROLE OF STRETCH REFLEX AND RECURRENT INHIBITION IN SHAPING STATIC ELBOW TORQUE-ANGLE RELATIONSHIPS (INVARIANT CHARACTERISTICS). U.R. Windhorst*. Dept. of Clinical Neurosciences, University of Calgary, Calgary T4N 4N1, Canada

Static torque-angle relationships (invariant characteristics: ICs) as measured by Feldman (1980) at the human elbow joint for constant voluntary effort have a monotonic convex shape determining angle-dependent stiffness. In contrast, for constant activation of elbow flexors, the torque increases, peaks and decreases again with increasing angle because of related moment arm alterations (Hasan and Enoka 1985). Conversion of such constant-excitation torque-angle (CETA) shapes into an IC might result from action of the stretch reflex which adds excitation with increasing joint angle. To test its possible contribution, the following assumptions were made. (i) Muscle fiber length increases nearly linearly with joint angle. (ii) Reflex muscle excitation (EMG) is linearly related to muscle (fiber) length. With these assumptions, IC shape cannot be derived from CETAs because it would be sigmoid at low and straight at large joint angles, whilst real flexor ICs are more convex at large than small angles. A contribution to bending the ICs at large angles may come from recurrent inhibition via Renshaw cells. These show a nonlinear saturating dependence on motor axon input rate and amount of excitation, i.e. number of active axon collateral synapses (Cleveland et al. 1981). These relationships can contribute to shape motoneuron output so as to yield convex ICs. Since recurrent inhibition is increasingly depressed with increasing voluntary muscle activation, its effect on motoneurone output would be consistent with the experimental IC's shape-dependence on joint angle. Together with reflex feedback, recurrent inhibition thus contributes to fine-regulate joint stiffness. (Supported by AHFMR)

589.9

LATENCY MEASURES OF SPASTICITY IN MULTIPLE SCLEROSIS PATIENTS. P.A. Anderson, C.T. Bever and G.V. Smith* Depts. of Physical Therapy and Neurology, School of Medicine, University of Maryland, Baltimore, MD 21201-1587.

Spasticity long has been defined as a "velocity-dependent increase in tonic stretch reflexes". However, recent experiments in our lab challenge this definition. As part of an ongoing clinical study of Multiple Sclerosis,* 19 patients were examined using a Kin-Com^R isokinetic testing device. We examined the latency of movement-induced activity in the quadriceps and hamstring muscles during passive isokinetic flexion/extension movements at the knee of 20°-, 100°- and 180°/sec.. The data were analyzed using an Ariel Motion Analysis System^R. The results suggest that although the latencies to the onset of muscular activity were highly variable, they indicate a trend where the relationship between the velocity of movement and the appearance of muscular activity were reversed. While further study is necessary, these results question the standard definition of spasticity, at least, in the MS population. * This study was supported in part by a MS Society Grant # RG-2127-A to C.T.B.

589.10

ANESTHESIA AND STRETCH REFLEX ACTIVITY IN THE KETAMINE TREATED CAT AS MEASURED WITH EEG AND EMG ANALYSIS TECHNIQUES. J.S.Taylor*, A.Vasanthakumar, C.J.Vierck and J.B.Munson. Dept. of Neuroscience, Univ. of Florida, Gainesville, FL 32610.

In the course of developing a behavioral model of spasticity in the cat, we have investigated the role of ketamine alone or in combination with fentanyl in maintaining an adequate level of anesthesia while preserving triceps surae stretch reflex activity. Anesthetic properties were determined by characterizing the EEG arousal response following application of a controlled noxious or innocuous mechanical stimulus to the tail. Stretch reflex EMG and torque activity were recorded from the triceps surae group in response to ramp and hold dorsiflexion of the feet in both the unanesthetized and ketamine anesthetized cat. We conclude that intravenous infusion of ketamine at 22mg/kg/hr can block arousal in response to noxious stimulation of peripheral somatic structures while maintaining hindlimb stretch reflex activity. We hope to directly correlate spinal injury-induced reflex changes between the conscious and the acute electrophysiological preparation without the confounding problems associated with anesthetic agents. Supported by NS27511, NS15913, NS17474, MH15737.

589.11

EFFECTS OF REPETITIVE CUTANEOUS ELECTRICAL STIMULATION ON MOTONEURONAL EXCITABILITY. C. Goulet, M. Levin, A.B. Arsenault*, D. Bourbonnais, M. Goyette. Physiotherapy Program, University of Ottawa, School of Rehabilitation, University of Montreal, and Research Centre, Montreal Rehabilitation Institute, Montreal, Quebec, Canada.

Cutaneous electrical stimulation in the form of TENS has been shown to decrease spasticity. However, effects of repetitive stimulation in normal subjects are controversial and may originate from the use of different methodologies. The present study was aimed at elucidating the effects of repetitive cutaneous electrical stimulation on motoneuronal excitability. Eleven normal subjects with no sensory deficits in the lower limbs were tested in five experimental sessions. The purpose of each session was to evaluate different parameters of stimulation such as stimulation of a mixed nerve versus a sensory nerve (the common peroneal and sural nerves) at two frequencies of stimulation (50 and 99 Hz). A session where no modality was applied provided baseline values. Motoneuronal excitability was quantified using the soleus H-reflex elicited according to the classic protocol of Hugon (1973). The intensity of stimulation of the posterior tibial nerve was chosen to elicit a peak-to-peak amplitude of $H_{max}/2$ as well as a small M response. Within each session, H-reflexes were recorded every 30 s for 45 min. Control values were taken for 5 min prior to (H_{ctrl}) and for 10 min after a 30 min cutaneous stimulation was administered at twice the sensory threshold (TENS Medtronic-Selectra; 250 μs pulses). Although no statistically significant group effects (ANOVA for repeated measures: $p>.05$) were shown, there was a tendancy towards inhibition (≥20% H_{ctrl}) of the H-reflex in 63% of the subjects after 30 min of repetitive stimulation at 99 Hz of either nerve. Thus, these parameters of stimulation appear to be optimal for decreasing motoneuronal excitability in normal subjects. Further experiments will test for the efficacy of these TENS parameters on spasticity.

Hugon M. (1973) in New Dev in Electromyogr & Clin Neurophysiol, Vol 3: 277-293.

589.12

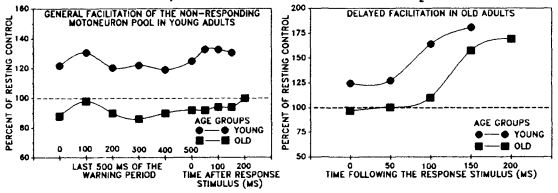
FAILURE OF LOCAL TWITCH RESPONSES IN RABBIT SKELETAL MUSCLES WITH REPETITIVE NEEDLING. **C-Z. Hong***, **Y.Torigoe** , and **D.G. Simons**, Dept. of Phys. Med. & Rehab., University of California Irvine, Irvine, CA 92717.

In rabbit skeletal muscle fibers, responses similar to **local twitch responses (LTRs)** of myofascial trigger point in man could be elicited by mechanical stimulation on certain "responsive bands", and could be confirmed with electromyographic (EMG) recordings. This study investigated the failure of LTRs by needling the "responsive band". A 27-G needle was inserted into the most responsive site of the band repetitively at a rate of one per second. A bipolar EMG recording needle was inserted into the other relatively "inert" end of the band. The visible LTRs and the EMG activity failed to appear after 3-15 times of repetitive needling. If the lidocaine (0.5%, 0.1-0.2 ml) was injected simultaneously during needle insertion, the visible LTR and EMG activity disappeared with only 1-3 needle insertions. The above findings are consistent with clinical experience that trigger point injections eliminate LTRs at that site.

589.13

AGE-RELATED CHANGES IN THE EXCITABILITY OF THE RESPONDING AND THE NON-RESPONDING MOTONEURON POOLS PRIOR TO MOVEMENT ONSET. J.R. Burke*[1] and G. Kamen[2]. (Motor Control Laboratory, Indiana University, Bloomington, IN). [1]Department of Exercise Science, University of South Carolina, Columbia, SC and [2]Department of Physical Therapy, Boston University, Boston, MA.

Young (n = 20) and old (n = 20) adults were required to perform a rapid plantar flexion in response to a visual stimulus. The changes in the excitability of the responding (right soleus) and non-responding (left soleus) motoneuron pools were assessed by eliciting simultaneous right and left leg tibial nerve H-reflexes (50% of the maximum H-reflex) during the last 500 ms of a 1 second warning period at increments of 100 ms and at increments of 50 ms between the presentation of the response stimulus and the onset of movement. On each trial, simultaneous bilateral H-reflexes were elicited at one of these test intervals. The results supported a general facilitation of the non-responding motoneuron pool prior to movement onset in the young adults only and a delayed facilitation of the responding motoneuron pool in the old adults, as shown for H-reflex amplitude.



589.14

EVIDENCE FOR CNS INGROWTH CAUDAL TO CERVICAL SPINAL CORD INJURY IN MAN. Blair Calancie* and James G. Broton, The Miami Project to Cure Paralysis, The University of Miami, Miami FL.

In a recent study we reported the existence of short-latency reflexes evoked in upper extremity (UE) distal musculature by lower extremity (LE) nerve stimulation in subjects with C6 or higher chronic and motor-complete spinal cord injuries (Group A). Conversely, those subjects whose neurological deficits at the acute stage were clinically indistinguishable from those in Group A, but who subsequently recovered even marginal strength in muscles innervated by levels at or caudal to C7, do *not* show such interlimb reflexes, nor do control subjects.

Here, we further characterize properties of these reflexes: 1) LE electrical stimulation of both muscle *and cutaneous* afferents routinely leads to 1:1 following by UE single motor units (SMU) at rates of 15 - 20 Hz, and which may go as high as 85 Hz; 2) intense UE SMU discharge (as high as 220 Hz over a 200 ms period) has been observed in response to application of ice to discrete LE receptive fields; 3) UE SMU 'jitter' can be < 2 ms for LE stimulation; 4) reflexes were *not* observed within 4 months of a functionally-complete cervical transection above C6 (N = 3 subjects), but became evident 9 months post-injury (N = 1 subject). These data show that both cutaneous and muscle afferents are forming strong (probably monosynaptic) connections with upper extremity motoneurons in these individuals.

We suggest that LE primary afferents which are known to pass within the fasciculus gracilis send out collaterals caudal to the region of SCI which excite UE motoneurons. The distribution of muscles which show this reflex activity (hand intrinsics = wrist extensors > wrist flexors) parallels the dependence of these muscle groups on corticospinal input. Thus loss of corticospinal-derived synaptic input to these UE motoneuron pools may make them more susceptible to ingrowth from alternate neural populations. These findings lend encouragement to attempts to promote CNS regeneration for the restoration of function.

589.15

NEUROPHYSIOLOGICAL BASIS OF FUNCTIONAL RECOVERY IN NEONATAL SPINALIZED RAT. **J.W. Commissiong***, LMCN-NINDS-NIH†, Bldg. 10/5N214, Bethesda, MD. 20892.

We have recently shown that in the neonatal rat that is spinalized at 7 days after birth (PN7), spontaneous recovery of coordinated stepping in the hindlimbs occurs, and is associated with a robust connectivity of Gp Ia/α-MN in the hindlimb muscles. There is also a widespread bilateral convergence of cutaneous afferents from the hindquarters to the α-MNs of the lateral gastrocnemius (LG) muscle (Exp. Brain Res. 78: 597-603, 1989; Brain Res. Bull. 27: 1-4, 1991; Soc. Neurosci. Abs. 1-7: 197, 1991). Rats that were spinalized at PN14 remained paralyzed, and exhibited severely depressed H-reflexes. Using the same non-anesthetized, intecollicular, decerebrate preparation, we have recorded H-reflexes ipsilaterally from the right tenuissimus anterior (TA) and LG muscles, in response to electrical stimulation of the sciatic nerve. In the PN7 preparation, there was a 2.5 msec delay between the H-reflexes elicited from TA and LG. Since the conduction velocities of the Gp Ia fibres from TA and LG were not different, the results suggest that a temporal delay between the Gp Ia inputs from antagonist hindlimb muscle spindles to α-MNs occurs within the spinal cord, and raises the possibility that Gp Ia/α-MN transmission in this preparation might not be always monosynaptic. We have also transplanted E14 dopaminergic cell suspensions into the middle lumbar spinal cord of spinalized PN14 and young adult rats. In both cases, a rich dopaminergic innervation of the transected lumbar cord was present by two weeks. However, only the PN14 rat exhibited the indices of functional recovery that are characteristic of the untreated, PN7 preparation. These results indicate that there is considerable plasticity of the lumbosacral CPG in the lumbosacral spinal cord of the rat. The results also indicate that morphological reinnervation and functional recovery do not always correlate.

589.16

THE INFLUENCE OF GLYCINE ON SPINAL CORD INJURY INDUCED SPASTICITY. R.K.Simpson*, A.A. Leis, M.M. Gondo, C.S.Robertson, and J.C.Goodman, Neurosurgery Department,Baylor College of Medicine,Houston,TX,77030

Spasticity is a frequent and complex sequel to central nervous system injury. The neurochemical basis for the origin of spasticity is largely unknown. Various neurotransmitters have been implicated such as γ-amino butyric acid (GABA). Drugs designed to mimic GABA activity can influence the severity of spasticity but are limited by side effects. Glycine is among the most abundant neurotransmitters in the spinal cord. However, its role in spasticity has received little attention. We have hypothesized that glycine may modify abnormal motor behavior expressed as spasticity.

Rabbits subject to percutaneous, mid-thoracic spinal cord transections were followed and studied after stabilization of spasticity, as determined clinically by the Ashworth score. A catheter was inserted into the cisterna magna and the spinal cord was bathed with either 100 mmol/l glycine,1 mmol/l strychnine, or artificial CSF for 4 hours. H-reflexes were periodically monitored before and during infusion by stimulating the posterior tibial nerve and recording from the plantar surface of the foot. Amplitudes of the H and M waves at various stimulus intensities were measured and H/M curves were constructed.

Glycine was found to depress the exaggerated H response in spastic animals after approximately 90 minutes of infusion. In addition, the stimulus intensity required to produce the H wave was higher than for controls. Strychnine, in contrast resulted in heightened H waves with prolonged durations and aberrant configurations. The stimulus intensity required to produced these H waves was less than for controls.

Our results indicate that glycine may suppress the degree of spasticity and that a potent glycine antagonist, strychnine, can exacerbate it. Glycine activity may play a role in the development of spasticity. Glycine and glycinergic compounds may, therefore, modify the spasticity observed in patients following neurotrauma and require further investigation.

590.1

INFLUENCE OF AUDITORY PRECUEING ON AUTOMATIC POSTURAL RESPONSE. J.W. McChesney, H. Sveistrup, and M.H. Woollacott*. Exercise and Movement Science, University of Oregon, Eugene, OR 97403.

An experiment was conducted to evaluate the influence of precueing on posture control. Two series of trials were run on each subject to assess the influence of a warning signal on automatic postural response muscle onset latency times in the gastrocnemius (G) and tibialis anterior (TA) respectively.

While standing on a moveable platform, each of four subjects was exposed to an audible tone of 50ms duration that preceded a balance disturbance by 500ms. This tone was used as a precue to warn of the forthcoming balance perturbation. The perturbations were anterior and posterior translations (3cm) at 30 cm/sec. Unilateral electromyograghic activity was recorded from G and TA. In the first half of the experiment (series A), a white noise tone precue as well as no precue and catch trials were utilized in 54 random trials. In the second half of the experiment (series B), 60 random trials utilizing a high/low, directionally specific tone precue, no precue, and catch trials were run.

In series A, a decrease in mean muscle onset latency time was observed in TA (4%: 94 ±8ms to 91 ±11ms) and G (10%: 98 ±11ms to 88 ±8ms) following forward and backward platform perturbations respectively. During series B, the TA and G latencies were decreased by 12% (96 ±10ms to 84 ±11ms) and 13% (100 ±9ms to 86 ±10ms) respectively. In both series, G onset was more sensitive to decrease than TA. The directionally specific tone proved to be more effective as a precue. We thus conclude that prior knowledge of a forthcoming balance perturbation can reduce postural muscle onset latency times.

590.2

MECHANICS AND CONTROL OF HUMAN STANDING: ANALYSIS OF THE HIP AND THE ANKLE STRATEGIES. A. D. Kuo and F. E. Zajac*. Mech. Eng. Dept., Stanford University, Stanford, CA 94305, and Rehabilitation R & D Center, VA Medical Center, Palo Alto, CA 94304.

Humans prefer to use the "ankle strategy" over the "hip strategy" in response to small perturbations of the support surface (Horak et al., Exp Brain Res 82:167-177, 1990). The reasons for this preference remain largely speculative. We used a musculoskeletal model of the human body to study the effectiveness of these control strategies in relation to stability and control constraints.

Musculotendon actuator models for 14 muscle groups were combined with a model of the mechanics of the body to produce the dynamical equations of motion, which were used to map the set of all possible muscle forces into the set of all feasible accelerations. When visualized in a coordinate system with axes corresponding to angular accelerations about the ankle, knee, and hip joints, the feasible acceleration set appears as a 3-D polyhedron. A constraint to keep the knees straight, approximating behavior seen experimentally, reduces the set to a polygon. Constraints corresponding to the desire to maintain the feet flat on the ground were also displayed in relation to the polygon.

We found that these flat-feet constraints greatly restrict the feasible accelerations of the body when the ankle strategy is used (i.e., only acceleration about the ankle is permitted) but not when the hip strategy is used (i.e., when ankle flexion is combined with hip extension, or vice versa). This corroborates with data showing that the ankle strategy is used in response to small disturbances, but beyond a certain size of disturbance, the hip strategy is employed.

Analysis of muscular effort indicates that the hip strategy is more efficient and faster than the ankle strategy. This leads to the question of why the ankle strategy is preferred, even for small disturbances. Results from optimal control analysis suggest that neither speed, energy, stabilization of the center of mass, nor maintenance of a stable platform for the head are considerations which explain the preference for the ankle strategy. (Supported by NIH grant NS 17662 and the Dept. of Veterans Affairs.)

590.3

POSTURAL CONTROL DURING VOLUNTARY ARM MOVEMENTS: CENTRE OF MASS REGULATION VERSUS COUNTERACTION OF THE REACTIVE FORCES

J.J. Eng*, D.A. Winter, A.E. Patla and C.D. MacKinnon. Dept. of Kinesiology, University of Waterloo, Ontario, Canada N2L 3G1.

Voluntary arm movements result in the application of reactive forces to the body and the alteration of the whole body centre of mass (COM) position. In this study, the effects and interactions of these mechanisms on the postural response during the upright stance position were assessed. Subjects were instructed to rapidly move their arms during four conditions which combined the polarity of the reactive forces applied to the body by the arms and the polarity of the displacement of the mass of the arms. Kinematic, kinetic and EMG analyses of the task were performed. A simulation model was developed to further examine the effects of the reactive forces. Two model conditions were considered a) no counteraction, and b) complete, instantaneous counteraction of the reactive forces. Experimentally, the postural moments (torques) of the hip, knee and ankle peaked simultaneously and were opposite in polarity to the focal moment. The hip/knee peak moment ratio was invariant while the knee/ankle peak moment ratio was highly variable suggesting that the final fine-tuning control of the postural response was the responsibility of the ankle muscles. Furthermore, the experimental and simulation results suggest that the effects of the COM regulation and the counteractive postural moments are inextricably linked to each other. (Supported by NSERC and MRC)

590.4

AN ELECTROMYOGRAPHIC STUDY OF THE STARTLE RESPONSE DURING STEADY SPEED SWIMMING OF FISH. B. C. Jayne* and G. V. Lauder. Dept. Biol. Sci. (ML 6), Univ. Cincinnati, OH 45221; Dept. Ecol. Evol. Biol., Univ. California, Irvine, CA 92717

We recorded muscle activity (EMGs) from both sides in each of five bluegill sunfish, *Lepomis macrochirus*, from fine wire electrodes implanted in superficial myomeric muscle at four standard locations along the length of each fish and from deep myomeric muscle at two positions. All trials were videotaped at 400 images/s, and we placed the fish in a flow tank to obtain steady swimming speeds of about 1.6 lengths/s when needed. At 30 min intervals, we dropped an object into the water to elicit startle responses (C-starts) both from fish during swimming and at a standstill. We obtained a total of 40 C-starts during swimming and 20 from a standstill with equal numbers of observations for each individual. The general features of the EMGs from both types of C-starts were similar, with synchronous onset of high amplitude activity from both superficial and deep muscle along the entire side of the fish that forms the concave side of the C. During steady swimming, the EMGs from the superficial muscles were unilateral and propagated posteriorly, whereas the deep muscles were inactive. For C-starts during steady swimming, the onset of large amplitude EMGs involved in C-formation occurred at any time with respect to the rhythmic pattern of low amplitude EMGs involved in steady swimming. These data suggest that the circuitry involved in the startle response can override the motor pattern generated for continuous swimming.

590.5

DIFFERENT CONTROL STRATEGIES FOR MOVEMENT AND POSTURE. M. Dornay*, Y. Uno, M. Kawato and R. Suzuki. ATR Human Information Processing Research Laboratories, Soraku-gun, Kyoto 619-02, and Tokyo Univ. Japan.

An appealing motor strategy for the control of arm posture and movement, suggested by E. Bizzi, N. Hogan, T. Flash and others, suggested that the brain could use a unified treatment for posture and movement: Control of posture results from setting the co-activation levels of agonist and antagonist muscles so that a single attractive equilibrium position of the hand is coded. According to the *virtual trajectory hypothesis*, the ability to maintain stable postures is a building block for arm movements. The nervous system continuously vary the activities of the muscles and generates a time-sequence of attractive equilibrium positions called *a virtual trajectory*. The strategy is attractive if an easy to compute virtual trajectory can reproduce realistic movements (Flash, 1987). The size of the hand stiffness predicted by Flash, were much larger than values measured in humans during posture control.

We proposed an alternative control for hand movement, the *minimum-muscle-tension-change model*. It assumes the nervous system must solve inverse dynamics for generating the muscle tensions during movements. We simulated arm movements using a 17-muscle model of the monkey's arm, and a new algorithm which allows non-zero muscle tensions at the beginning and end points. The simulated horizontal arm movements are quite similar to experimental findings in humans. Furthermore, our simulations predict antagonistic co-activation at the initial and final (equilibrium) points. During the movement, however, the model predicts reduced co-activation of the antagonists in a coordinated manner. Therefore, the hand stiffness was reduced during movement relative to the level during posture control. This is contradictive to the requirements of the virtual trajectory hypothesis (Flash 1987). Our predictions for reduced stiffness during movement are supported by experimental data (Bennet et al, 1992). We conclude that the unified approach for the control of posture and movement, based on a simple virtual trajectory, is not supported by our findings.

590.6

IMPAIRED PROGRAMMING OF ANTICIPATORY POSTURAL ADJUSTMENTS IN PARKINSON'S DISEASE. F. Viallet*, R.G. Lee, J. Tonolli, R. Aurenty and J. Massion. Laboratoire de Neurobiologie et Mouvements, CNRS, Marseille, France.

Anticipatory postural adjustments were investigated in 5 patients with Parkinson's disease (PD) and in 5 age matched control subjects. While standing on a force platform , subjects were required to rapidly raise one leg in a lateral direction to an angle of about 45°. Kinematics were monitored using an ELITE system with reflective markers placed at multiple sites on the trunk and legs. Surface EMG recordings were obtained from 4 muscles in each leg. To maintain equilibrium during this task requires a number of postural adjustments which have been well characterized in young normal subjects (Mouchnino et al., J. Neurophysiol. in press). Prior to the onset of lateral displacement of the moving leg (time T2) there is a shift in the center of pressure on the force platform (onset at the time T1), initially toward the side of the moving leg and then a transfer of weight to the support leg. The mean value of T1-T2 interval was 570 ms. These force changes are accompanied by displacements of the trunk markers toward the support side. In the more severely affected patients with clinical evidence of postural instability the following abnormalities were observed: 1. the amplitude of the initial change in the center of foot pressure was diminished. 2. the T1-T2 interval was markedly prolonged (mean value: 1331 ms). 3. the amplitude of displacement of the trunk markers toward the support side was reduced. 4. EMG recordings revealed that most muscles were tonically active and did not show bursts of activity or periods of inhibition around the T1-T2 times which were a consistent feature in the normal subjects. These results suggest that mechanisms for programming anticipatory postural adjustments are impaired in some patients with PD.

590.7

THE ROLE OF PERCEIVED VISUAL MOTION AND EXTRARETINAL INPUTS IN POSTURAL SWAY CONTROL M. K. Holden*, P. DiZio, and J. R. Lackner. Ashton Graybiel Spatial Orientation Laboratory, Brandeis Univ., Waltham, MA. 02254.
Extraretinal information influences motion perception, and motion perception influences postural control. Thus, extraretinal inputs might play a significant role in posture control, particularly if retinal inputs are minimal. We tested this idea by evaluating postural sway in sharpened Romberg stance in 32 young subjects for 4 fixation conditions: World-fixed visual (V) or remembered (R) target, Head-fixed visual target (H) and No target (N), in a dark room. Subjects let their eyes wander in the N condition. Sway measures were derived from force platform center-of-pressure outputs; eye movements were measured via AC-coupled electroculograms (EOG). Sway was reduced for conditions V and H, but not R, vs N ($p<0.05$). Subjects maintained good fixation stability in V, H, and R conditions, and made larger and more frequent saccades in the N condition ($p<0.05$). Better fixation was associated with improved performace on posture scores in selected conditions. Our results indicate that extraretinal inputs associated with fixation supression of the vestibular-ocular reflex contribute to posture control, especially when retinal input is limited, and have functional implications for falls among the elderly in reduced light conditions. Supported by NASA Grant NAG 9-295.

590.8

STABILIZATION OF POSTURAL SWAY WITH HAPTIC INFORMATION. J.J. Jeka* and J.R. Lackner. Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, MA 02254.

Different forms of sensory information such as vision and touch influence the ability to maintain an upright posture. We studied the role of haptic information on the stabilization of postural sway while subjects (N=5) maintained a tandem Romberg stance on a Kistler force platform. A metal bar placed laterally aside the force platform was used to measure the amount of force applied with the right index finger during 24 s trials. Subjects were tested in three touch conditions: no support, light touch (< 100 g of pressure) or pressure support (as much force as desired) both with eyes open and eyes closed.

The results showed a significant touch x vision interaction effect ($p<.001$) on mean sway amplitude (MSA). MSA with no support-eyes closed was more than twice that of any other condition. Morever, MSA with light touch-eyes closed was significantly less than with no support-eyes open ($p<.01$). Thus, touch information is more effective than vision in decreasing MSA in the tandem Romberg stance. Average applied force with pressure support (370 g) was almost ten times that of touch support (38g). The latter force has been shown to be less than that necessary for mechanical support (Holden, Ventura & Lackner, (1987). Soc. Neurosci. Abs., 13 (1), p. 348). Supported by NIH Grant F32-NS09025-01 and NASA Grant NAG9-515.

590.9

SEPARATE SYSTEMS FOR TONIC, TRIGGERED, AND CENTRALLY INITIATED POSTURAL CONTROL: EFFECTS OF LEVODOPA F.Horak*, J.Frank, C.Shupert, M.Stephens, J.Nutt, and A.Burleigh, R.S.Dow Neurological Sciences Inst. of Good Samaritan Hospital, Portland, OR 97209

Levodopa had an opposite effect on triggered postural responses and on centrally initiated postural movements in 15 parkinsonian subjects. Levodopa increased phasic muscle bursts associated with centrally initiated toe rise movements but decreased the size of phasic bursts triggered in response to surface displacements. Levodopa also reduced baseline tonic muscle activity which reduced resistance to external displacements but increased the ability to generate centrally initiated toe rise movements.

These results suggest that there are three separate postural systems: 1) background tonic, 2) centrally initiated, and 3) peripherally triggered. Parkinsonism affects all three postural systems. However, dopamine replacement directly improves only the tonic and centrally initiated postural systems. Supported by grants from NIA and NSERC.

590.10

Modification of Motor Evoked Potentials (MEPs) in Lower Limb Muscles by Motor Task

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Magnetic transcranial motor cortex stimulation elicits both early (MEP1) and later (MEP2 and MEP3) motor evoked potentials appearing with different latencies in muscles of the limbs. In a group of 7 healthy subjects, age 36.9 ± 13.2 yrs., in the supine position, MEP1 was recorded in relaxation with a latency of 24.2 ± 2.8 ms. from the quadriceps, 27.1 ± 2.9 ms. from the medial hamstring, 26.7 ± 1.6 ms from the lateral hamstring, 31.2 ± 7.9 ms from the *tibialis anterior and 35.0 ± 2.6 ms from the soleus muscle during relaxation*, passive stretch and active ankle dorsi- and plantar flexion. Stimulation was applied over the C_z (10-20 EEG placement system) using a 9 cm coil. MEP1 in all muscles was increased during the active motor task while MEP1 in the agonist muscle showed a much larger increase than in the antagonist. Moreover, the later MEP in the soleus muscle occurring in relaxation at 94.9 ± 53.9 ms latency and 405.6 ± 173.2 μv amplitude decreased in latency to 86.8 ± 3.2 ms and increased in amplitude to 711.5 ± 357.7 μv during voluntary dorsiflexion. Passive movement did not bring such changes when compared to relaxed values. Volitional activity and response to motor cortex stimulation are convergent giving selective modification of MEPs based on the motor task.

590.11

INSIGHTS INTO LENGTH-GAUGING MECHANISMS OF MUSCLE SPINDLES IN THE CAPSULARIS MUSCLE OF THE CAT. E. Eldred*, L. Yung, P. Tam and R.R. Roy. Brain Research Institute and Department of Anatomy and Cell Biology, UCLA, L.A., CA 90024.

To gain a better understanding of how muscle spindles function as gauges of muscle length, measurements were taken on 18 spindles identified in serial sections of the capsularis, a small, predominantly slow flexor acting at the hip joint. The muscle was fixed in situ in formalin with the hip extended. The muscle then was embedded in paraffin, sectioned transversely at 10 mm thickness throughout its length and the sections stained with H&E. Individual spindles were traced throughout their lengths using a light microscope and a Eutectics Graphics System. Mean spindle length was 5.1 mm, with the capsule comprising approximately 43% of the length. Fifty percent of the ends of the 108 intrafusal fibers measured did not emerge from the capsule. Thus, for these intracapsular fibers the effects on their sensory wrappings would be secondary to deformation of the capsule. In contrast, two fibers at each pole of the spindle continued, generally in isolation, for distances that together comprised approximately 50% of the spindle length. These presumably nuclear bag fibers, therefore, would be expected to be affected by length changes, passive or active, induced over an extent of muscle septum matching the entire spindle length. If sarcomere lengths shortened by 20% when activated, the nuclear bag regions would be expected to lengthen by 1 mm. This outcome is highly unlikely, however, since the capsular space was only 1.05 mm long. Perhaps mere contractile stiffening of these long fibers would permit length changes to appear at the sensory wrappings primarily as inflections of force. In contrast, short fibers with capsular insertions would be subject to in series tension pulling on the capsule and to tension from the tensing of muscle fibers surrounding the bulging spindle equator. These data suggest that the longer spindle fibers may better suited to monitor length and the shorter fibers stiffness changes in the muscle. (Supported in part by NIH Grant NS16333)

590.12

MOTOR UNIT SYNCHRONIZATION ASSESSED FROM SURFACE EMG INCREASES WITH DISCHARGE RATE OF REFERENCE MOTOR UNIT. G. Yue*, A.J. Fuglevand, M.A. Nordstrom and R.M. Enoka. Depts Exercise & Sport Sciences and Physiology, Univ Arizona, Tucson, AZ 85721

One method used to assess the degree of motor unit synchronization involves obtaining averages of unrectified and rectified surface EMGs that are triggered from the discharge of a reference motor unit (Milner-Brown et al. *J Physiol*, 228: 285-306, 1973). The influence of motor unit discharge rate on this estimate of synchronization has not, to our knowledge, been delineated. The purpose of the study was to determine the effect of motor unit discharge rate on surface-EMG estimates of synchronization and to compare these estimates to those obtained from cross-correlograms of motor unit pairs. Motor unit (N=28) synchronization ratio, estimated from the surface EMG of human first dorsal interosseus muscle, was found to increase significantly ($p<0.001$) when the discharge rate of the reference motor unit increased (mean±SD, range = 3.33±1.66, 0.36-6.36 Hz). In contrast, synchronization indices based on the cross-correlogram decreased with increased motor unit discharge rate (Nordstrom et al. *J Physiol*, in press). Simple regression analysis indicated a significant, positive relationship ($p<0.001$) between an upward shift of the rectified surface EMG (due to an increase in both discharge rate and recruitment) and the surface-EMG estimate of synchronization. These findings suggest that the surface-EMG method of assessing motor unit synchronization needs to be modified to account for changes in the level of EMG activity.

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590.13

EQUILIBRIUM POINT HYPOTHESIS: EXPERIMENTAL VERIFICATION OF THE CONCEPT OF MUSCLE ACTIVATION AREA. H.Qi, T.E. Milner, M.F.Levin and A.G.Feldman*. Ctr Recherche, Inst. de réadaptation de Montréal, Inst. de génie biomédical, Univ. de Montréal, Montreal, Canada H3S 2J4.

Motoneuronal (MN) recruitment is a function of kinematic variables (position and velocity), independent control variables, as well as variables characterising the interaction between MN pools mediated by interneurons via reflex and descending pathways. These properties have been integrated in the concept of muscle activation area (MAA) which has been used to explain EMG patterns associated with single and multi-joint movements (Feldman et al. 1990). To test this concept, we recorded EMG signals from wrist flexor and extensor muscles as well as position and velocity during unloading of the wrist to different torque levels. Initial wrist position was neutral (0°) and torque was fixed at 60% of flexor MVC. Subjects were instructed not to correct the deflections of the wrist arising from unloading. This instruction is assumed to be associated with an invariant control signal. Sudden unloading produced a silent period in flexor muscles and a stretch reflex in extensor muscles, and the wrist stabilized at a new final position dependent on the final torque. The combinations of position and velocity associated with the onsets and offsets of the silent periods were represented by points on the phase plane. The set of points associated with different levels of unloading produced a straight line on the phase plane diagram corresponding to the border of the MAA for given initial conditions. When experiments were repeated from an initial wrist angle of 30° flexion, the border of the MAA shifted correspondingly. The change in the initial position (λ) is assumed to be associated with a change in the control signals and our results suggest that this change can be visualized as a shift in the position of the border of the MAA. The EP hypothesis suggests that the position of the border may be an indirect measure of descending influence to α and static γ MNs, whereas its slope may be a measure of the level of γ dynamic activity of the system (damping coefficient).

590.14

RESPONSES OF CAT SOLEUS GOLGI TENDON ORGANS TO DISTRIBUTED VENTRAL ROOT STIMULATION AT TIME-VARYING RATES. M. Hulliger*, J.L.F. Weytjens and U. Windhorst. Dept. of Clinical Neurosciences, University of Calgary, Calgary, Alberta T2N 4N1, CANADA.

Whilst it has been well established that Golgi tendon organs (GTOs) are very sensitive to contractions of the muscle fibers inserting into the collagen fibers they encapsulate, it remains a matter of debate whether or not the afferent signal sent to the central nervous system accurately represents muscle force. The question is often addressed by comparing GTO afferent firing with forces produced by reflex contraction or by stimulation of single or small numbers of motor units (MUs). We have reinvestigated the problem by stimulating most of the soleus MUs in a distributed and rate-modulated way, to assess whether (1) single GTOs and/or (2) populations of GTOs, activated by populations of MUs, carry signals which reflect whole muscle force.

In pentobarbitone-anesthetized cats, GTO afferents from soleus were recorded from dorsal root filaments. Eight ventral root filaments, prepared so as to yield approximately one eighth of the total force on tetanic stimulation, were stimulated in a staggered way such that the times of occurrence of the stimuli were equally distributed over the interstimulus intervals of the leading train. Four different stimulus patterns were used: (1) sine waves; (2) staircase functions, incrementing and decrementing in steps of 5 stimuli/s up to 35/s; (3) ramp functions starting at 5/s, with the stimulus patterns to different filaments starting at different times, so as to mimic recruitment; and (4) staircase functions temporally organized as in pattern (3).

GTOs reacted with vigorous bursts to unfused contractions of the MUs exciting them. At intermediate force levels, instantaneous frequency followed force closely. It plateaued at forces below maximum, and generally also showed adaptation. Beyond those general response characteristics, individual GTOs displayed great variability in their responses to similar stimulus patterns, in particular when staircase functions were used; e.g., a GTO might fire during the entire period of activation, or only during individual steps. Faithful whole muscle force monitoring can therefore at best be provided by populations of GTOs. (Supported by AHFMR and MRC, Canada)

590.15

FORCE FIELDS AND MUSCLE USE STRATEGIES THAT UNDERLIE REFLEX BEHAVIORS IN THE SPINAL FROG. Simon Giszter*, Department of Brain and Cognitive Science, M.I.T., Cambridge, MA 02139.

Spinal frogs perform motor tasks requiring complex multi-joint limb motion. We have suggested this ability rests on a few movement primitives residing in the spinal cord. To test this hypothesis the three-dimensional forces that underlie different reflex behaviors were measured in spinal frogs.

The frog's limb was attached to a six-axes force transducer. The muscle activity and force responses were measured in the leg when the reflex behaviors were activated by skin stimulation. The limb was moved to a set of locations forming a 3-D grid. The force response patterns through space were analyzed as three dimensional force fields. These force fields and the electromyogram activity were compared with activity and restraining forces measured during and following free limb responses to the same stimulus.

The analyses showed that: (1) the structures of force field patterns for a particular behavior were almost constant between trials and frogs. (2) Two qualitatively different types of force field patterns were found: position control and force control. (3) The force field patterns showed very little adjustment to alterations of body orientation to gravity. (4) While the active force patterns had fixed structures, the kinematics generated showed variability due to amplitude variations of the elicited force fields. (5) A small set of fixed muscle synergies underlay these force fields. (6) Similarly structured force fields could also be measured when the limb was halted after free motion.

A distributed control model is proposed to account for the organization and interaction of primitives in generating spinal reflexes and behaviors, based on Maes' behavior networks (Maes, 1989, AI Memo 1180, M.I.T.). The results here support the idea that fixed muscle synergies and associated force-fields (movement primitives) underlie several behaviors generated in the spinal cord. These primitives include fields implementing force control as well as equilibrium point strategies. These primitives may also form a basis for more complex behaviors.

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590.16

SIMULATION OF RENSHAW CELL-MEDIATED EFFECTS ON MOTONEURON SYNCHRONY. M. Maltenfort* and W. Z. Rymer. Sensory Motor Performance Program, Rehabilitation Institute of Chicago, and Departments of Rehabilitation Medicine & Biomedical Engineering, Northwestern University, Chicago, IL 60611.

To study the effects of increasing Renshaw cell (RC) synaptic strength on motoneuron firing synchrony, an RC-motoneuron pool was simulated using the MacGregor point neuron model. Parameters for RCs and motoneurons were generated by matching published data for input resistance, time constants, and current-rate plots. Synaptic strengths of motoneurones on RCs were set to be sufficient to cause burst firing of RCs. The neurons were arranged on a rectangular grid, and the effects of synapses were spatially limited to ± one row or column.

The synaptic current on the motoneuron from each RC terminal was 0.01 nA. The average number of terminals from each RC to each motoneuron was increased, to enlarge both the magnitude and the statistical variation of IPSPs on the motoneurons. This allowed us to look at the effects of increasing RC strength without introducing extra synchrony.

The motoneuron pool was stimulated with constant current levels. Firing synchrony was measured as the coefficient of variation (c.v.) of the total activity of the motoneuron pool; it was assumed that RC-mediated desynchronization would reduce variability in the steady-state firing rate. It was found that the c.v. fell off steeply with the number of terminals until it reached a plateau, then began a shallow rise. This implies that the small magnitude of RC effects is actually optimal for desynchronization of motor pools.

Work is in progress to expand the model to look at effects of RCs on force output, to use simulated afferent activation of the motoneuron pool, and to study the effects of varying synaptic strengths of motoneurones on RCs.

This work was supported by NIH grants NS28076-02 and NS30295-01.

590.17

MECHANICAL PROPERTIES OF CAT SOLEUS MUSCLE ELICITED BY SEQUENTIAL RAMP STRETCHES. D.C. Lin* and W.Z. Rymer. Departments of Biomedical Eng. and Physiology, Northwestern University, Chicago, IL 60611.

The objective of this study was to examine the intrinsic mechanical properties of muscle during constant velocity stretches of sufficient amplitude to induce muscle yield, with the goal of establishing the relative magnitude and time-dependence of viscous and elastic components of the mechanical response. The soleus muscle in a deafferented decerebrate cat preparation was activated by the crossed-extension reflex. Two experimental paradigms were employed on a total of five cats.

The first consisted of applying two ramps (each 2 mm amplitude), separated by a variable time interval. The velocities of the two ramps were usually the same and ranged from 10 to 100 mm/s. The primary result was that during the ramp, the force response after yield remained constant until the end of the ramp, and the force level depended solely on stretch velocity, not on previous mechanical events, similar to a viscous damper. However, the force transient recorded prior to the steady-state response was history dependent. Specifically, stiffness measurements taken shortly after the onset of the second ramp depended upon the time elapsed between ramps (when the muscle was isometric). The recovery of the initial stiffness was very fast, such that the measures returned to control values within 30-40 ms.

The second experimental paradigm consisted of superimposing a single, very fast, length pulse onto a ramp at various times before, during, and after the ramp. The force response to the pulse, which is an indication of the mechanical impedance, was observed to decline after ramp onset, and remained constant until the end of the ramp. We concluded that the muscle mechanical characteristics were relatively constant and simple throughout the ramp after the initial yield.

The characteristics of post-yield muscle have not been adequately described to determine whether it can form a stable interface with mechanical loads. The results in this study show that steady-state properties of post-yield muscle resemble a viscous damper, providing damping in many situations. By comparing deafferented with intact muscle responses, we conclude that the stretch reflex acts to transform the muscle's impedance from primarily viscous-like behavior (in post-yield muscle) to primarily elastic behavior.

This work was supported by NIH grants NS28076-02 and NS30295-01.

590.18

EFFECTS OF MOTONEURON AND MUSCLE FIBER ABNORMALITIES ON THE FORCE-EMG RELATIONSHIP IN A MODEL OF CAT MEDIAL GASTROCNEMIUS MUSCLE. J.J. Gemperline*, C.J. Heckman, and W.Z. Rymer. Departments of Biomedical Engineering and Physiology, Northwestern University, and Rehabilitation Institute of Chicago, Chicago, Illinois 60611.

When the paretic limb of a hemiparetic human subject is compared to the unimpaired limb, it often shows increased EMG and reduced motor unit rates for a given force. To assess the mechanisms of these abnormalities we constructed a computer model of the motoneuron pool and its muscle. This model is based on the available physiologic data regarding size-dependent properties of motoneurons and muscle fibers, primarily from studies of the cat medial gastrocnemius muscle.

We have added an EMG component to an earlier version of this model, presented previously. Each motoneuron is made to fire with interpulse intervals randomly distributed around its central interval, and the neurons of the pool fire asynchronously. The EMG contribution of each unit is modeled as a biphasic triangular waveform whose duration and amplitude depend on unit size.

Using this model, we studied the effects of two proposed mechanisms of muscle weakness. First, motoneuron rates were lowered while muscle fiber properties were left unchanged.We observed compression of recruitment force thresholds, a shift in the force-EMG relationship to produce more EMG for a given force, and a higher mean power frequency for the EMG.

To simulate atrophy we reduced the maximum force of the larger units and slowed their action potentials. We observed a decrease in the EMG mean power frequency, but depending on the assumptions made about changes in contractile speed, EMG could either increase or decrease for a given force.

Data consistent with both mechanisms were obtained from our population of hemiparetic subjects. The effects of reduced motor unit rates and atrophy may counteract each other if both mechanisms were active in a given subject.

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590.19

MUSCULOSKELETAL KINEMATICS DURING CONTROLLED HEAD MOVEMENTS IN CATS. E.A. Keshner*, S.D. Garimella, J. Hanson, and B. W. Peterson. Dept. of Physical Therapy, Univ. of IL at Chicago and Dept. of Physiology, Northwestern Univ. Med. School, Chicago, IL 60611. *Supported by grants BNS9109705 and NS22490.*

Patterns of muscle activation during voluntary head movements in the cat were consistent within each animal, but varied between animals producing the same head movement (Keshner et al., Exp Br Res, 1992). One source of this variability could be the relative arrangement of the cervical vertebrae during the head movement. In this study we recorded simultaneous video-flouroscopic and neck muscle (biventer (BIV), complexus (CPX), occipitoscapularis (OCC), and splenius (SPL)) EMG data from a cat performing ±15° sinusoidal (0.25 Hz) head tracking movements in the sagittal plane in order to identify the relations between intervertebral actions and muscle activation. Video-opaque markers were inserted into the anterior/posterior and lateral aspects of C_1-C_7 to measure vertebral displacement every 300 ms during 20 sec of sinusoidal head movement. Vertebral movement phase led stimulus position by as much as 45°, and the extent of motion between vertebrae was unequal. C_1 exhibited 1°-1.5° more motion than did C_2, which moved only about 0.5° more than C_3. The differential motion between C_3 and C_7 was about 1°. Unlike the vertebrae, the head moved *in phase* with stimulus position, and exhibited the greatest displacement, moving as much as 5° more than C_1. BIV phase led head extension. CPX and OCC either phase led peak extension of the head, or peaked in phase with peak downward flexion of the head. The latter behavior was accompanied by small shifts in C_2 and C_3 toward a greater phase lead re peak head extension. SPL exhibited 2 response peaks, leading both peak extension and peak flexion of the head. The overall picture suggests a whiplike motion of the head and neck where the movement is initiated in the vertebrae, and the inertial properties of the head are used to produce a position matched response.

590.20

CIRCLING BEHAVIOR IN MICE WITH MISSING OR VARIABLY SIZED CORPUS CALLOSUM. L. Ricceri, D.P. Wolfer and H.-P. Lipp*. Institute of Anatomy, Univ. of Zürich, CH-8057 Zürich, Switzerland.

We studied the consequences of a missing or variably developed corpus callosum (CC) on locomotion of mice (n=107) in an open-field arena by means of computerized analysis of paths during two exposures of 10 min per day. An initial comparison between a strain with a normal CC (C57BL6, n=24) and a strain with congenital agenesis of the CC (I/LnJ, n=39) showed significant strain differences in path length on day 1 and 2 ($p < 0.01$, I/LnJ moving more); in 24h habituation ($p < 0.025$, many I/LnJ showing none) but not in within-session habituation. During the first exposure, I/LnJ mice moved more in the center of the arena ($p < 0.01$) and showed a high incidence of circling either to the left or to the right ($p < 0.001$).

In order to test whether the size of the CC was responsible for the observed strain differences, mice with missing or variably developed CC were bred by means of backcrossing C57 x I/LnJ hybrids to I/LnJ fathers (BX mice). The cross-sectional area of the CC of these animals (12 females, 32 males) was measured after the open-field test and correlated with the behavioral scores. This revealed again a significant negative correlation between callosal size and locomotor activity ($p < 0.05$), closeness to the walls ($p < 0.01$) and circling ($p < 0.01$). For the other behavioral measures, variability increased with decreasing size of the CC.

We conclude that a missing or weakly developed CC in mice is not responsible for hyperactivity itself but facilitates the development of uncontrollable circling during bouts of locomotor activity, while the large behavioral variability of acallosal mice might reflect a more general interhemispheric dyscoordination syndrome. Supp. by SNF 31-9470.

CONTROL OF POSTURE AND MOVEMENT IV

591.1

PROCAINE PARCELS NUCLEUS ACCUMBENS AND LOCUS COERULEUS-MEDIATED EXPLORATORY MOVEMENT OF RATS.
R.M. Chesire*, B.E. Morton+, W.E. Winn+, B.E. Digman, & M.K. Peterman, Departments of Psychology and +Biochemistry, University of Hawaii, Honolulu, HAWAII 96822.

Selective damage of N. accumbens (NA) or locus coeruleus (LC) can increase or decrease locomotion and movement. To identify specific arousal functions of these areas, we first examined their roles in exploration. Six male and female Long-Evans hooded rats survived bilateral implantation of chronic, indwelling cannulae in the LC and NA in a single procedure. After recovery, 0.5 or 1.0 ul of 0.733M, pH 7.4, procaine HCl in normal saline was applied focally to NA, LC, or both structures in counterbalanced order (n=4). Pre- and postdrug movement measures included amount and location of locomotion in an open field, air and surface righting, tonic grasp, position tolerance, tactile and tail-pinch-induced orientation, and movement on an inclined plane. Vocalization, piloerection and form of locomotion were also noted. NA procaine increased amount of forward locomotion, thigmotaxis, barrier-bound loss of postural support, grooming, piloerection and vocalization. LC procaine increased amount of locomotion and non-thigmotactic locomotion and movement. NA + LC procaine abolished forward locomotion and postural support, but left some head scanning intact. Thus, behaviors traditionally associated with "fear" (thigmotaxis, grooming, freezing) are preserved in the presence of accumbens inhibition; behaviors associated with increased exploration (less "fear") exist in the presence of coeruleus inhibition; and loss of most movement occurs when both systems are inhibited. The results suggest that (1) LC and NA subserve different exploratory subsystems (2) the LC and NA subsystems are sufficient to account for exploratory movement in the absence of social stimulation and (3) perhaps these subsystems mediate antagonistic exploratory functions.

591.2

IMPAIRED "NATURAL" RECIPROCAL INHIBITION IN PATIENTS WITH SPINAL SPASTICITY. G.I. Boorman, W.J. Becker*, and R.G.Lee, Dept of Clinical Neurosciences, U of Calgary, Calgary, AB.

Voluntary activation of the tibialis anterior (TA) muscle causes a decrease in the size of the soleus H-reflex (Natural Reciprocal Inhibition, NRI) in normal humans. This occurs during isometric TA contractions producing either constant (static) force or dynamic force changes. We measured NRI in patients with partial spinal cord injury (SCI), and compared these measurements with those from normal subjects.

Subjects were seated with their foot strapped to a metal plate which measured force during isometric TA contractions. At force levels which were comparable during the ramp and during static contractions, the tibial nerve was stimulated. H-reflex recruitment curves were obtained during both tasks. Forces were compared by absolute torque levels and percentages of maximal voluntary contraction.

In normals, TA contraction produced NRI during both static and dynamic contractions. NRI was demonstrated at all test reflex amplitudes, even in normal subjects with large resting H-max/M-max ratios. In contrast, partial SCI patients demonstrated a reduction or loss of NRI. Impaired NRI was especially evident when dynamic contractions were compared. Interference with mechanisms producing NRI may contribute to the clinically observed motor deficits in these patients.

591.3

INFLUENCE OF INTERLIMB DISTANCE ON THE CONTROL OF QUIET STANCE IN THE CAT. J. Fung*, P. Rougier, C.A. Pratt and J.M. Macpherson. R.S. Dow Neurol. Sci. Inst., Portland, OR 97209

This study examined the biomechanics of stance in the cat at various fore-hind interlimb distances. As stance distance narrows, the freely standing cat can either: 1. maintain a horizontal trunk and change the limb inclination or 2. constrain limb inclination and arch the trunk. Cats stood quietly on 4 force plates mounted at fore-hind distances of 33, 30, 25 and 20 cm. The 3D ground reaction forces and kinematics were recorded. Joint moments were computed for the left hind limb.

At 33 cm, the reaction force vector and limb axis (line from toe to hip) were both inclined forwards and inwards. As stance distance decreased, the force vector inclination gradually decreased to become vertical at 20 cm. The limb axis inclination decreased more rapidly, becoming vertical at 30 cm and backwardly directed at 20 cm. Concurrently, the sagittal knee moment decreased while hip and ankle moments increased. However, the sum of absolute 3D moments at the hip, knee, ankle and metatarso-phalangeal joints remained similar. The line joining the shoulder and the hip remained constant in length and horizontally oriented. Thus, in varying stance distance, cats use the first strategy of constraining the trunk and changing limb inclination. These results complement the study of stance on a tilted surface (Lacquaniti et al., J Physiol 1990; 426:177-192), in that the geometry of the trunk and/or limbs may be controlled, depending on the task. A possible outcome may be conservation of the sum of moments across the hind limb joints.
Supported by NIH (NS29025); JF is a Rick Hansen Man in Motion Fellow

591.4

ORGANIZING PRINCIPLES AND SENSORIMOTOR MECHANISMS FOR SKILLED MOTOR BEHAVIOR : EVIDENCE FROM SPEECH MOTOR CONTROL V. L. Gracco*. Haskins Laboratories, 270 Crown St. New Haven, CT 06511.

In attempting to understand human motor behavior and its neural control one approach is to employ novel and constrained tasks to obtain insight into relevant control variables. Such an approach has yielded significant data on the properties of single and multijoint motion with limited application to functional goal-directed behaviors. It is plausible that isolating movement from function provides an incomplete picture of underlying control principles and a limited representation of the underlying neural mechanisms. An alternate approach is to study more complex behaviors attempting to identify organizational principles which provide a framework to interpret characteristics observed at other levels of analysis.

Data from a number of experiments will be presented that provide an empirical foundation for a hierarchical conceptual model of speech production. Components of the model include functional (sound producing) units of action, a global activation and tuning process, a rhythm generating mechanism, provisions for somatosensory modulation of action units, and a cognitive component that drives the motor control system. Inherent in the neuromotor specification for each functional unit is the coordination among the various articulators involved in the production of specific sounds. These units are then sequenced by an underlying oscillatory process providing the serial timing inherent speech rhythmicity. Speed and accuracy are byproducts of overall changes in oscillatory frequency. An assumption underlying the model is that details of most speech motor actions are not explicity controlled. Rather, characteristic control processes exploit the complex geometry and biomechanical diversity of the speech production mechanism. The result is a dynamic modulation of the vocal tract producing complex kinematic patterns from relatively simple control processes. Supported by NIH

591.5

SOURCES OF VARIABILITY OF SIMPLE PRE-PLANNED MOVEMENTS. S.R.Gutman*, G.L.Gottlieb. Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612.

Simple pre-planned movements can be defined as realizations of an original central pattern transformed by a parametric transform. The values of parameters are established by the motor control system prior to the movement. As a first approximation of the transform, we take scaling in space and time. Assume that the original pattern is deterministic and does not contribute to movement variability, and the parameters values are random. Then, variance time profiles of trajectory (Var(x(t))), velocity (Var(v(t))), and acceleration (Var(a(t))) can be expressed as weighted sums of two basic functions by formulae:

$$\mathrm{Var}(x(t))=\frac{\mathrm{Var}_A}{A^2}x^2(t)+\frac{\mathrm{Var}_\tau}{\tau^2}t^2v^2(t),\quad \mathrm{Var}(v(t))=\frac{\mathrm{Var}_A}{A^2}v^2(t)+\frac{\mathrm{Var}_\tau}{\tau^2}(v(t)+t\cdot a(t))^2,$$

$$\mathrm{Var}(a(t))=\frac{\mathrm{Var}_A}{A^2}a^2(t)+\frac{\mathrm{Var}_\tau}{\tau^2}(2a(t)+t\cdot jerk(t))^2,$$

where Var_A and Var_τ are variances of established space scale A and time constant τ, $jerk(t) = d^3x/dt^3$. Note that in the expressions, the first and the second terms are, correspondingly, the spatial and the temporal components of the variances.

Particularly, for a reaching movement, the model predicts that variances time profiles should be uni-modal for trajectories, bi-modal for velocities, and tri-modal for acceleration. The model time profiles of correlations between trajectory, velocity, and acceleration were also calculated. They predict that correspondent curves have to be bi-phasic, tri-phasic, and quadri-phasic. The experimentally measured variance and correlation time profiles demonstrate the predicted properties for different experimental conditions. Their consistency allows one to consider them invariants of reaching movement, analogous to the bell-shape velocity time profile. For a more complex movement, time profiles of variability were also measured and explained by the model.

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591.6

Apraxia Results From a Fractionation of Movement Representations. M. Clark,* A. Merians, H. Poizner, L. Rothi, and K.M. Heilman. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102, VA Medical Center and University of Florida, Gainesville.

Geschwind (1975) proposed a disconnection model in which an apraxic subject is unable to carry out movements to command because the left hemisphere that comprehended the verbal command is disconnected from the right premotor and motor areas which controls the left hand. An alternate model, however, proposes that apraxia results from destruction of spatiotemporal representations of learned movement stored in the left hemisphere (Heilman, 1979). The disconnection hypothesis would predict that apraxic subjects should be able to correctly imitate gestures and correctly use actual objects since these tasks do not require language. The movement representation model predicts that imitation and actual tool use would also be impaired. Motion analyses were performed on selected gestures made in a series of conditions in which contextual cues were introduced in a graded fashion in two left-lesioned apraxic subjects and two matched neurologically intact subjects. Four cue conditions were presented: no cues (verbal command), object present, tool present and both object and tool present. Movements of the hand, wrist, elbow, and shoulder were digitized from neighboring views, reconstructed in three dimensions, and analyzed graphically and numerically. The apraxic subjects showed movement disturbances across the cue conditions. For example, the calibration that normal subjects showed for movement amplitude with movement time and velocity was disrupted. Adding contextural cues did not restablish the normal relationship among these movement parameters. These data support the hypothesis that the left hemisphere is specialized for aspects of higher order motor control, and that apraxia results from the destruction of spatiotemporal representation of learned movement rather than from a disconnection between the receptive language areas of the left hemisphere and the contralateral motor cortices.

591.7

EFFECTS OF PRACTICE AND COMPLEXITY ON HUMAN MOTOR LEARNING IN A CONTINUOUS TASK. Hanneke van Mier* (1,2), Wouter Hulstijn (2), Steven E. Petersen (1). (1)= Department of Neuropsychology, Sch. of Med., Washington Univ., St. Louis, MO 63110, (2)= Nijmegen Inst. for Cognition and Information (NICI), Univ. of Nijmegen, The Netherlands.

Large differences are found between the planning of overlearned movement patterns, like letters, and that of novel, unpracticed movement patterns. This difference stimulated a study in which changes in the planning of a movement were studied as a function of extensive practice. The learning process, in which a novel movement pattern transforms into a well-learned movement pattern, was studied in a maze drawing task. Twelve subjects learned to move a pen through cut-out maze patterns with their eyes closed. Maze patterns consisted of six, eight, ten, or twelve segments that were connected by intersections. Total path length of each maze was 24 cm. Although the mazes could be traced continuously in a clockwise direction, selecting a wrong turn at an intersection led to to a dead end. Performance at intersections was analyzed by determining the number of correct (and incorrect) turns following mechanically forced stops and the number of correctly planned and executed turns without any halt. In addition, mean duration and movement velocity was measured. An increase in the number of correct turns as a function of practice indicated that subjects gradually learned to produce segment chunks of increasing size. A practice-complexity interaction with respect to the number of correct turns showed that chunk size did not increase linearly with the number of maze segments. A non-linear increase in performance speed was found across the six practice sessions.

The results show a gradual change in movement strategy from a sequential, trial-and-error mode in which the planning and execution of movements occurred segment by segment, to a concurrent planning mode in which the preparation of subsequent movements occurred - to a greater or lesser extent - simultaneously with the execution of earlier segments. Finally, the results suggest that this learning process proceeded through qualitatively different learning phases.

591.8

L-DOPA TREATMENT DESENSITIZES BOTH D1 AND D2 MEDIATED ROTATION RESPONSES IN 6-OHDA-LESIONED RATS. D.R. Britton* and P. Curzon. Neuroscience Research, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064.

Rats with unilateral 6-OHDA lesions of the nigrostriatal tract show contralateral rotation in response to l-dopa with no apparent loss of efficacy of the drug over 4 successive days of testing. The response to l-dopa can be blocked with the D1 antagonist, SCH23390 plus the D2 antagonist, haloperidol when they are administered together but not separately. L-dopa treatment causes a pronounced decrease in the subsequent sensitivity of the rotation response to the D1 agonists SKF-38393 and A68930. The present data demonstrated that there is likewise a decreased response to the selective D2 agonists, bromocriptine (0.8 - 3.3 umol/kg, sc) and LY-171555 (0.032 to 1.0 umol/kg, sc). If the maintained sensitivity to l-dopa in the presence of decreased responsiveness to both selective D1 and D2 agonists were due to the ability of l-dopa derived dopamine to simultaneously stimulate the D1 and the D2 receptors together, animals might be expected to be responsive to other approaches to activation of both receptor subtypes. However, rats pre-treated with l-dopa prior to being tested with either the mixed D1/D2 agonist, apomorphine or with a combination of A68930 plus LY171555 showed a significantly diminished response to these drugs as well. These data suggest the possibility that l-dopa may be acting at non-D1, non-D2 receptors in order to maintain its efficacy in the face of diminished responsiveness to selective D1 and D2 agonists.

591.9

DIMINISHED CONCENTRATION OF GLYCINE IN THE CEREBRO SPINAL FLUID OF SPASTIC CHILDREN. **J.A. Lazareff * A. Velázquez J.C. López Flores. Depto. Neurosurgery. Hospital Infantil de México, Dr. Márquez 162 C.P. 06720 México, D. F.**

Glycine is recognized as a mediator in postsynaptic inhibition in the spinal cord. Its depletion has been linked to rigidity after ischaemic damage in experimental animals. The concentration of Glycine in CSF of 10 spastic and 10 non spastic age matched children was determined. The spastic children had antecedents of perinatal asphyxia The values of glycine were measured by HPLC, using a Waters 510 equipment with fluorimetric detector. The aminoacids were separated with a 15 cm. long. Nova Pack C 18 column. A gradient with two solvents was used, solvent A was a phospahte buffer (pH 7), solvent B was 40% of the former buffer and 60% of tetrahydrofurane. The fluoresent intensity was measured at an excitation wavelength of 425 nm. The peak of glycine was identified by comparison with the retention time of the authentic compound. Glycine was quantified by the mesasurement of the peak area using an on-line computer integrator Waters 740 The values detected (mean 2.3 umol S.D. 0.83), in the spastic group were significantly lower ($P<0.001$) than those oserved in the non spastic children (mean 6.6 umol S.D. 1.14). It could be suggested that low CSF glycine reflects loss of inhibitory Renshaw cells. This imbalance could be in part responsible for spasticity

591.10

FUNCTIONAL INTERPRETATION OF NUCLEUS AMBIGUUS NEURONS BY COMBINED PETH AND STA ANALYSES. C.R. Larson* and Y. Yajima. Dept. of Communication Sciences and Disorders, Northwestern University, Evanston, IL 60208, and Dept. of Physiology, Hyogo College of Medicine, Nishinomiya, Hyogo, 663 Japan.

A variety of neurons are located within the nucleus ambiguus (NA), such as laryngeal, pharyngeal, palatal and esophageal motoneurons, respiratory-related neurons, neurons related to the cardiovascular system and interneurons. These neurons are involved in vocalization, respiration, swallowing, emesis and other behaviors associated with the upper aero-digestive tract. As a way of analyzing the functions of some of these neurons, we here describe the results of combining Perievent Time Histograms (PETH) and Spike Triggered Averaging (STA) analyses for study of NA neurons. Neurons were recorded from awake monkeys (Macaca nemestrina) trained to vocalize for a fruit-juice reward. Neurons which gave a burst of activity with vocalization, and sometimes with swallowing also, had a STA with a short latency (5-7ms) excitation in a single muscle and were laryngeal motoneurons. Some neurons had a longer latency (10-20 ms) excitatory or suppressed STA response, and PETH analysis revealed a variety of discharge patterns including: suppressed activity during vocalization, respiratory rhythm, and bursting activity with vocalization along with activity before or after vocalization. These are most likely interneurons or respiratory-related neurons. The general finding is that the latency and form of an STA response corroborates other functional measures of neuronal activity.

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591.11

NEURONS OF THE ANTERIOR CINGULATE MOTOR AREA ARE RELATED TO VOCALIZATION AND OTHER OROMOTOR BEHAVIORS. R. West* and C. R. Larson. Dept. Of Communication Sciences and Disorders, Northwestern University, Evanston, IL 60208.

A large number of anatomical, stimulation and lesion studies have indicated the anterior limbic cortex (perigenual area 24) may play a role in vocal behavior. To further explore this possibility we have recorded neural activity from what we believe is the area of the cingulate sulcus during self-paced conditioned vocal behavior in the awake monkey (Macaca Nemistrina). We also recorded activity while the monkey performed a self-paced jaw opening task to determine whether related units were specific to vocalization. The vast majority of related neurons modulated their activity in the same manner during both tasks, but with different latencies to behavior onset. Vocalization related neurons often had long lead times, and these generally became short lead times during the jaw task. Some cells related to both tasks, however, had drastically different firing patterns. Thus far, a small number of cells related specifically to vocalization and one cell related specifically to jaw opening have been observed. We have also observed cells involved in a variety of other oromotor movements, such as tongue protrusion, lip rounding, and reinforcement consumption. These observations support the earlier findings of other investigators concerning the role of the anterior limbic cortex in vocalization and suggest the neurons of this region play a role in behaviors involving laryngeal, pharyngeal and oral motor systems.

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591.12

DOES SHAPE OR SURFACE AREA INFLUENCE PREHENSION?. P.L. Weir[1] and C.L. MacKenzie[2], Dept. of Kinesiology, [1]University of Windsor, Canada, N9B 3P4, and [2]Simon Fraser University, Canada, V5A 1S6.

MacKenzie & Weir (1991) have shown that when the available contacting surface area is identical between a dowel with a flat gripping surface (force dowel) and one with a curved surface (cylindrical dowel), the actual area contacted is substantially lower on the cylindrical dowel. This difference between dowels is also reflected in a longer deceleration phase for the transport component prior to contact (Weir & MacKenzie, 1991). The purpose of this experiment was to determine if the surface area contacted or the shape of the grasping surface per se was exerting these effects. To do this, it was necessary to equate the force dowel to the cylindrical dowel for the actual area contacted.

Added to the flat gripping plates of the force dowel was a 1 cm strip of light tape, with subjects being instructed to grasp only the tape. In this condition the dowel was referred to as the precision dowel. All three dowels (force, cylindrical, precision) were equated for weight (155 grams). Trials were blocked for the three dowels. Within each block, two tasks (lift versus place) were counterbalanced. Both kinematic and kinetic measures were used, and the movement was divided into four phases: free motion, dowel acquisition, transport and release. If surface area was exerting the effects in Weir & MacKenzie (1991), the precision dowel should take on characteristics similar to the cylindrical dowel.

Kinematic analysis of the arm transport over the free motion phase prior to contact showed that the precision and cylindrical dowels resulted in a longer deceleration phase as compared to the force dowel. The area contacted on the precision and cylindrical dowels was similar, providing further support for the surface area hypothesis. The kinetic measures, load force and grip force, over the acquisition and release phases also revealed differences between the precision and force dowels. These data suggest that surface area does influence all phases of prehension. (Supported by NSERC)

591.13

MODULATION OF CUTANEOUS SENSITIVITY BY ISOMETRIC JAW VERSUS LIMB EXERCISE IN HUMANS. P. Kemppainen*[1,2], H. Leppänen[2] and A. Pertovaara[2]. [1]Dept. of Prosthetic Dent. and [2]Dept. of Physiology, Univ. of Helsinki, Helsinki, Finland.

The effect of isometric exercise on electrotactile thresholds of the skin was studied in human subjects. Exercise consisted of brief (1-10 s) contractions of jaw closing muscles, flexions of the hand or foot against varying loads (10-30 % of the maximum force). A light flash indicated the start and the end of the exercise. The hand exercise produced a load-dependent and rapidly attenuating tactile threshold elevation of the fingers in the exercising hand but not contralaterally. The foot exercise at comparable intensity produced a smaller effect. The threshold elevation was significant also immediately prior to EMG response of the arm but not at the time of light flash (= "go" signal) or prior to it. The jaw exercise induced a significant elevation of tactile thresholds in the lower jaw just prior to and during the early EMG responses of the jaw closing muscles. This threshold elevation was attenuated already before the end of the shortest exercise period (1 s). Thus, isometric exercise produces a phasic, rapidly attenuating threshold elevation to electrotactile stimuli in the exercising limb or jaw but not multisegmentally. A plausible explanation for the threshold increase is the suppression of afferent input due to efferent barrage from motor to sensory areas of the brain.

591.14

CORTICOSPINAL REORGANIZATION AFTER SPINAL CORD INJURY (SCI) IN MAN. **D. Hopkins-Rosseel and B. Brouwer* Dept. of Rehab. Therapy, Queen's Univ., Kingston, ON K7L 3N6.**

It has been suggested that the mature human central nervous system (CNS) has the ability to reorganize following traumatic injury (Levy et al. Brain Research 510:130,1990).

This study used focal transcranial magnetic stimulation (Cadwell Laboratories) to investigate the extent of the motor cortical representation and the activation threshold of each of the biceps, deltoid and triceps muscles in subjects with low cervical lesions of greater than three years duration (n=7) and healthy controls (n=12). Three single stimuli were delivered to the scalp at specific points demarcated with a grid (1.5 cm intervals) centered over the vertex, Cz. Amplitudes were measured from the average of the three evoked compound muscle action potentials (CMAPs).

The number of scalp sites from which CMAPs were elicited and the stimulus intensity required for muscle activation (threshold) did not differ between the two groups ($p > 0.05$) for any of the muscles tested. In addition, the mean relative amplitudes of the short latency CMAPs (normalized to the maximum M-wave elicited by supramaximal electrical stimulation of the muscle nerve) were similar between both groups for all muscles ($p > 0.10$).

These findings do not support the ability of the corticospinal tract to reorganize following longstanding SCI.

591.15

DESCENDING PROJECTIONS OF THE MESENCEPHALIC LOCOMOTOR REGION (MLR) BASED UPON TREADMILL INDUCED C-FOS PROTEIN AND ANTEROGRADE TRACT TRACING. K. Shojania, C.A. Livingston, S. Pylypas, L.M. Jordan* and D.M. Nance. Departments of Pathology and Physiology, University of Manitoba, Winnipeg, MB., R3E 0W3, Canada.

The MLR is operationally defined as the area in the mesopontine region in which low levels of stimulation sustain locomotion in a treadmill. However, variability in effective stimulation sites has hindered the detailed analysis of the projections from the MLR. We report here that exercise on a treadmill induces c-fos protein in the mesencephalon of the rat, and we have used this method to examine the projections from the region of c-fos labeled neurons produced by locomotion. The speed of the treadmill was increased from 0-0.25 m/sec and maintained for 60 min. After perfusion, brain sections were incubated with antibodies to c-fos protein and visualized via the PAP technique. The same or alternate sections were then processed histochemically for NADPH diaphorase (NADPHd). Minimal c-fos labeling was observed in the control animals, but exercised rats showed clusters of c-fos labeled neurons focused primarily in the cuneiform nucleus, ventrolateral central gray as well as the locus coeruleus. Few if any cells in the mesencephalon were positive for both c-fos and NADPHd, but double labeled cells were noted in other areas such as the hypothalamus. Dextran conjugated to rhodamine or biotin was pressure or iontophoretically injected into the mesencephalon, and 7d later the rats were exercised in a treadmill and perfused. Sections were processed for c-fos, dextran and NADPHd. Biotin-dextran labeled cells/terminals were visualized with ABC kits. Injections focused in the area of c-fos labeled cells in the cuneiform nucleus produced terminal labeling in the ventromedial reticular nuclei of the brain stem. Injections located ventral/ventrolateral to the cuneiform nucleus (region of NADPHd positive cells) produced terminal labeling in the ventrolateral medulla. Given the importance of the ventromedial medulla in locomotion, the present results support the idea that the cuneiform nucleus is a functional part of the locomotor pathway. Supported by MRC of Canada, Health Sci. Ctr. Res. Fnd. and National Centres of Excellence.

591.16

EVIDENCE FOR FLEXOR REFLEX ALTERATIONS IN HEMIPARETIC STROKE SUBJECTS. J. P.A. Dewald*, J.R. McGuire and W.Z. Rymer, Sensory Motor Performance Program, Rehab. Institute of Chicago, and Dept. of Physical Medicine and Rehabilitation, Northwestern University, Chicago, IL 60611.

In earlier studies of voluntary muscle activation in hemiparetic stroke subjects, we observed gross alterations in the spatial patterns of muscle activation at the elbow, and a significant increase in stereotypic coactivations of muscles acting at the same or adjacent joints, i.e. elbow and shoulder. In an effort to determine the relation between sensory input and disturbed muscle synergic relations in hemiparetic stroke, flexion reflexes were compared in the impaired and unimpaired upper extremities of 7 hemiparetic stroke subjects. The effects of mildly noxious electrical stimuli (500 Hz; pulse duration 1 ms; train duration 20 ms) delivered to the index finger were measured by recording EMG in 12 arm muscles together with torques measured at the wrist in a plane orthogonal to the long axis of the forearm. Rectified EMG and torque signals from 30-40 responses were analyzed for an interval of 250 ms after stimulus initiation.

Our results reveal an earlier torque and EMG onset in the unimpaired limb relative to the impaired arm. In the unimpaired limb, muscles were recruited in a proximal to distal fashion, with most shoulder muscles, especially shoulder extensors and retractors being recruited approximately 10 ms before elbow flexors. In the impaired limb, the EMG onset was delayed, resulting in simultaneous activation of proximal and distal arm muscles. In addition, the number of muscles activated by the electrical stimulation increased in the impaired upper limb. In spite of these timing differences, torque direction orthogonal to the long axis of the forearm did not vary significantly between arms. Stimulation of the unimpaired limb induced an early and increased EMG and torque response on the impaired side, resulting in an earlier onset of torque response not seen during ipsilateral stimulation.

The convergence of onset times of arm muscle on the impaired side mimics the obligatory coactivation observed under voluntary conditions. The delayed onset and the simultaneous recruitment of all arm muscles could result from an obligatory long loop activation of motoneuron pools via ventromedial multisynaptic pathways. Surprisingly, stimulating the unimpaired limb appears to restore short latency activation of motoneuron pools at the impaired side. Supported by NS 19331 to WZR.

591.17

EVIDENCE FOR CHANGES IN MUSCLE MECHANICAL PROPERTIES AND STRETCH REFLEX CHARACTERISTICS IN SPASTIC HEMIPARETIC STROKE. J.D. Given, J.P.A. Dewald, J.R. McGuire, T.S. Buchanan* and W.Z. Rymer. Sensory Motor Performance Program, Rehab. Institute of Chicago, and Dept. of Physical Medicine and Rehabilitation, Northwestern University Medical School, Chicago, IL.

The primary goals of this study were to quantify joint impedance in spastic limbs of hemiparetic stroke subjects, and to identify the relative contributions from passive and reflex components of spastic muscle. Slow ramp extensions of the elbow, stretching the triceps brachii muscle, were performed on spastic upper limbs (n=6), and compared with a normal control group (n=4). The passive elastic and viscous stiffnesses were estimated using the torque responses recorded in the absence of any significant triceps or biceps brachii EMG activity. A comparison of spastic and normal subjects revealed significant increases in passive elastic stiffness in the impaired limbs in all but one subject. The increases ranged from 30 to 90% of the averaged normal value which indicates that the passive stiffness is potentially a major contributor to the increased resistance observed by the clinician. Intra-subject variability was found to be insignificant within the normal population. However, a significant reduction in passive elastic stiffness (25%) was identified, between repeated protocols for one hemiplegic test subject, presumably because of an intensive course of physical therapy treatment that took place in the intervening period.

In the hemiparetic population, where the stretch reflex is more excitable, the reflex contribution was calculated by subtracting the estimated passive elastic stiffness contribution from the total elbow joint torque. The resulting torque signal exhibited a non-linear transition representing the onset of the stretch reflex followed by a constant stiffness response. The stretch reflex elastic stiffness contribution to the total torque response was approximately 3-4 times greater than that of the passive elastic stiffness contribution in the hemiparetic limb. A direct correlation appears between the identified passive and stretch reflex elastic stiffnesses.

These findings demonstrate the significance of the passive stiffness contribution to the total elbow impedance in many hemiplegic spastic subjects, which until now was mostly attributed to hypersensitive stretch reflexes.

Supported by NS 19331 and AR40245 to WZR

591.18

MOVEMENT PREPARATION IN LEUKOTOMIZED AND UNLEUKOTOMIZED SCHIZOPHRENICS. H. Carnahan*[1], R. Chua[2], D. Elliott[2], V.R. Velamoor[3] and C.J. Carnahan[3]. Dept. of Physical Therapy[1], Univ. of Western Ontario, London, Ont., N6G 1H1 and Dept. of Physical Education[2], McMaster Univ., Hamilton, Ont.,and Victoria Hospital[3], London, Ont. Canada.

The purpose of this study was to investigate the ability of bilaterally leukotomized (L) and unleukotomized (UL) schizophrenic (S) patients to use advance information in the process of movement preparation. Seven LS males, (mean age=66 years), eight ULS males (mean age=70 years), and eight healthy elderly men (mean age=74 years) served as subjects. A manual aiming task was performed in which movements could be defined on the basis of hand used to execute the task, and distance travelled to the target. Subjects were provided with full, partial or no prior information about the upcoming movement by precuing hand, distance, both hand and distance, or by providing no precue. While the leukotomized patients were slower than the control subjects, they showed the same pattern of reaction times (RTs) for the various precue conditions. RTs were not facilitated any more by knowing hand than by knowing distance. Instead RT was influenced simply by the number of stimulus response alternatives. However, the ULS patients did not make use of the advance information that was available. These data are discussed in terms of the role of the frontal lobes in schizophrenia and movement planning. (Supported by NSERC & OMH).

591.19

KINESTHETIC PERCEPTION OF OBJECT ORIENTATION IN 3D SPACE. F. Lacquaniti* and M. Carrozzo. INB, CNR, Milan (Italy)

It has been recently proposed that the transformation into a common reference frame might represent a necessary step to produce the movement required to attain the perceptual target (Soechting and Flanders, 1989). These A showed that, when subjects point with their hand to remembered target locations in 3D space, they make substantial errors in matching target distance, but not in matching target direction (azimuth and elevation). They hypothesized that these errors are due to the specific algorithm used to map target location into the angular coordinates of the arm joints. Object location is essentially a static task, the trajectory of the arm being apparently inconsequential to the performance. We investigated kinesthetic perception of 3D orientation by asking subjects to match the remembered azimuth and elevation of a test bar they had previously explored with their hand. Subjects made consistent errors in both dimensions, undershooting the target outside a linear range of $\pm$ 30°. In addition, a variable amount of offset in matching angle was present dependent on the position of the subject relative to the origin of rotation of the bar. The slope of the regression between matching angles and target angles was affected by experimental manipulation of head-centred references, as obtained by asking the subjects to rotate their head in the horizontal plane or by vibrating their neck muscles at 100 Hz thereby inducing an illusory shift of subjective straightahead. None of these errors were observed in control experiments in which the target was presented visually to the subjects. The postulated transformation from the world coordinates of the target into the arm coordinates might then undergo an intermediate craniotopic stage.

591.20

AN EEG-BASED BRAIN-COMPUTER PARALLEL INTERFACE: THE IMPORTANCE OF FREQUENCY AND SPATIAL RESOLUTION. D.J. McFarland* and J.R. Wolpaw, Wadsworth Labs, New York State Dept of Health and State Univ of New York, Albany, NY 12201.

Individuals can learn to control the amplitude of 8-12 Hz mu rhythm activity in the EEG recorded over sensorimotor cortex and use it to move a cursor to a target on a video screen. Good one-dimensional control (Electroenceph clin Neurophysiol 78:252-259, 1991) and significant two-dimensional (2-D) control have been achieved. We are investigating the hypothesis that 2-D control can be greatly improved by increasing frequency and spatial resolution.

EEG is recorded from scalp at multiple sites in central head regions. Mu rhythm amplitudes in two bipolar channels, one over central sulcus on each side, are evaluated online by fast Fourier transform 5/sec and used to control 2-D cursor movement. EEG from all sites is stored on disk for offline analysis aimed at testing whether greater frequency and/or spatial resolution would have improved movement accuracy and speed.

Initial results are consistent with evidence that mu rhythm activity comprises several separable components, and suggest that individuals achieve 2-D movement by controlling two components independently. This finding supports the hypothesis that increased frequency and topographic resolution can substantially improve cursor control. Thus, online implementation of such resolution may provide an EEG-based brain-computer parallel interface of value to severely disabled individuals.

INVERTEBRATE MOTOR FUNCTION

592.1

CHOLINERGIC EFFECTS ON THE CRAYFISH SWIMMERET CPG. Götz Braun* and B. Mulloney. Department of Zoology, University of California, Davis, CA 95616.

There are two reasons for examining the effects of cholinergic agonists on the crayfish swimmeret CPG: 1) many primary sensory afferents are cholinergic and play a role in modulating the centrally generated motor pattern, 2) cholinergic (muscarinic) agents elicit rhythmic activity in other crustaceean neural systems (walking in crayfish, stomatogastric ganglion in the lobster).

The present study examines the effects of nicotine (nicotinic agonist), pilocarpine (muscarinic agonist), and carbachol (muscarinic and nicotinic agonist). Can they elicit the rhythm in an inactive preparation, and how do they modulate the frequency and pattern of activity produced by proctolin? Motor neuron discharge was monitored extracellularly with pin electrodes on N_1-nerves , drugs were applied to the bath solution.

Pilocarpine reliably elicited rhythmic activity in inactive preparations with a treshold between 1 and 10 μM. Burst frequency increased with concentration up to 100 μM. When pilocarpine was applied to a preparation already activated by proctolin the burst frequency was slightly reduced.

Nicotine normally failed to elicit the rhythm in an inactive preparation, but when applied to a preparation activated by proctolin, nicotine increased the burst frequency considerably.

Carbachol reliably elicited the rhythm in inactive preparations and also modulated the burst frequency in proctolin activated preparations.

The results suggest that cholinergic transmission is used in two ways: as a component of the central circuitry via muscarinic receptors, and as a mediator for the input from primary sensory afferents via nicotinic receptors.

592.2

NEURONS WITH AXONS IN THE MINISCULE TRACT INFLUENCE THE SWIMMERET RHYTHM IN CRAYFISH *PACIFASTACUS LENIUSCULUS* David Murchison* and Brian Mulloney, Dept. of Zoology, University of California, Davis 95616

In crayfish, the swimmeret abdominal appendages display rhythmic movements coordinated between segments. This coordination is the metachronal rhythm and is characterized by a phase lag in the movement cycle of swimmerets in anterior segments relative to posterior segments. This rhythm can be observed in extracellular records of motoneuron bursts from swimmeret nerves in isolated abdominal nerve cords. The metachronal rhythm is mediated by intersegmental coordinating interneurons that are not well described.

Reducing the isolated abdominal nerve cord to chains of two or three ganglia does not disturb metachronal coordination (Paul and Mulloney, 1986), but if the ganglia are hemisected along the midline, the metachronal coordination on both sides is disrupted. This indicates that some bilaterally projecting neurons are involved in the maintenance of the metachronal rhythm.

Some interneurons which have been implicated in the coordination of swimmerets send bilateral processes through the miniscule tract (MT). This tract passes dorsal to the lateral giant neurons in the abdominal ganglia, just beneath the ganglionic sheath (Skinner, 1985). MT can be selectively sectioned by careful surgery. Bilateral section of the MT in one or more ganglia disrupts metachronal coordination. Bilateral coordination still occurs, but motoneuron burst structures, durations and periods are altered. This indicates that neurons of the MT are involved in intersegmental coordination and also participate in structuring motoneuron bursts. Some of the inputs that shape swimmeret motoneuron bursting are intersegmental.

592.3

MOTOR NEURONS OF THE SWIMMERET SYSTEM: MEMBRANE PROPERTIES OF ANTAGONISTS. C.M. Sherff* and B. Mulloney. Department of Zoology, University of California, Davis, CA 95616.

The muscles that move a single crayfish swimmeret are innervated by about 69 motor neurons. When an isolated nerve cord is generating the swimmeret motor pattern, only a few of these motor neurons fire action potentials; the membrane potentials of the rest oscillate but do not produce spikes. These motor neurons may represent pools of similar neurons, which are recruited in order of increasing size by stronger inputs from the central pattern generator to increase the strength of contraction of the swimmeret muscles. Alternatively, these motor neurons may possess different properties and have different functions in the swimmeret motor output.

We have recorded intracellularly from the neuropil or cell bodies of swimmeret motor neurons and recorded their resting membrane potentials, input resistances, and membrane time constants, their active responses to depolarizing current injection, and their effects on the output of the motor program. We have also looked for feedback from these motor neurons onto the central pattern-generating network.

Most of the motor neurons appear to have similar membrane properties and activities in the swimmeret rhythm. No systematic differences were found between power-stroke and return-stroke neurons, excitatory and inhibitory neurons, or spiking and silent motor neurons with regard to passive membrane properties. Most of these neurons also displayed sustained, tonic firing that did not reset the rhythm when they were depolarized by current injection.

A small number of motor neurons display a different electrical profile. These fired phasically with current injection; the membrane fires several action potentials with the onset of depolarization, and then settles to a steady-state depolarization that persists for the duration of the current injection.

592.4

MODELING ESCAPE RESPONSES: ENCOMPASSING IRREGULAR DISCHARGE SEQUENCES. J.L. Johnson*, USD School of Medicine, Department of Physiology/Pharmacology, Vermillion, South Dakota 57069

Recently, a mathematical model was developed describing the function of the neuronal pathway and longitudinal muscle for earthworm head end escape (Johnson, J.L. Internat. J. Neural Systems, 1 & 2 (1991) 79-90). This model equation could not encompass irregular discharge rates in the brief burst of command neuron action potentials needed to induce the rapid head end escape response. In this study, the additional formulations developed permitted an analysis of the impact of irregular discharge sequences upon end organ function. This new model predicts that in any given action potential sequence delivered to the muscle, where the interpulse interval (IPI) between successive impulse pairs is different, the order-effect these different IPIs will produce less than a 20% variation in the net contractile tension. This interesting property of earthworm head end escape is most important, since head end escape is essentially a "one-shot deal." The required repetitive discharge needed must initiate a rapid and powerful response on the first try because of a subsequent profound degree of habituation to further activation.

592.5

DISTRIBUTED INHIBITORY PATHWAYS IN CIRCUITS CONTROLLING HEAD MOVEMENTS IN CALLIPHORID FLIES

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Recordings from frontal nerve motorneurons (FNMs), supplying neck rotator muscles, demonstrate their directionally selective activation by ipsilateral wide-field motion and selective inhibition by stimuli contralaterally. Recordings from premotor descending neurons (DNs) supplying neck motor neuropil also reveal selective inhibition by wide-field stimuli. In the brain, these DNs give rise to specific dendritic branches that extend into an area of neuropil in the lateral deutocerebrum receiving terminals of certain motion-sensitive wide-field neurons originating contralaterally in the lobula plate. The functional significance of such tangential neurons is suggested by immunocytology using an antibody raised against the inhibitory transmitter γ-aminobutyric acid (GABA). 20-25 immunopositive axons extend from the lobula plate, of which at least 8 match Lucifer yellow filled neurons terminating in this deutocerebral area. The relevance of GABAergic pathways to inhibition of frontal nerve motor neurons has been studied using microlesions made while recording from FNMs. Lesions across GABAergic pathways from the lobula plate first abolish the inhibitory component of the FNM response which then returns after minutes. Lesions across ipsilateral DNs abolish the excitatory FNM response which also then recovers. These results suggest distributed routes of pathways controlling head movements. Neurobiotin and cobalt fills into heterolateral ascending and descending neurons, further supports this hypothesis. **Supported by NIH Grant No. R01 EYO-7151**

592.6

CHARACTERIZATION OF MOTOR PROGRAMS AND NEURONS IN THE FRONTAL GANGLION IN THE MOTH *MANDUCA SEXTA*. C.I. Miles* and R. Booker. Section of Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853.

The frontal ganglion (FG) of *Manduca* is involved in feeding and ingestion. Because feeding behavior changes dramatically from the larval stage to adulthood, the role of the FG may be altered during development. As a first step in a study of metamorphosis of this simple ganglion, we describe the larval FG motor program and the identities of some of the neurons which produce it. The FG lies on top of the esophagus anterior to the brain. It is small, measuring about 150 µm in diameter, and contains approximately 30 cell bodies. Two nerves exit the FG; the recurrent nerve, which projects posteriorly along the esophagus, and the frontal nerve, which extends anteriorly from the ganglion. The larval esophagus exhibits rhythmic constrictions and dilations which differ in their timing along its length. The esophageal constrictions anterior to the brain are out of phase with those posterior to the brain. Cutting the frontal nerve eliminates movements of the anterior esophagus, while cutting the recurrent nerve stops the posterior movements. Consistent with this finding, we have recorded rhythmic bursting motor patterns from the frontal and recurrent nerves that are out of phase with each other. This motor program can be recorded in isolated brain-FG preparations. If the FG is isolated from the brain, bursting initially stops, but the motor pattern will resume after several minutes. We have identified motoneurons, interneurons, and neurosecretory cells within the larval FG by cobalt backfills and intracellular recording and staining. The activities and roles of a number of FG neurons in generating the larval motor program will be described.

592.7

ANATOMICAL AND PHYSIOLOGICAL ANALYSIS OF A RAPID STEERING MUSCLE OF THE BLOWFLY. M.S. Tu, and M.H. Dickinson*. Department of Organismal Biology and Anatomy, University of Chicago, 1025 E. 57th ST., Chicago, IL 60637

In addition to the large, stretch-activated power muscles, flies possess a set of 17 direct synchronous muscles that are responsible for elaborate turning maneuvers during flight. Of the steering muscles that have been recorded, The First Basilar (B1) stands out due to its ability to fire each and every wing beat, even at frequencies exceeding 200 Hz, making it among the fastest known synchronous muscles. Its unique physiology makes the B1 a prime candidate for controlling the timing of wing pronation during flight. We have been studying the anatomy and physiology of this muscle in *Calliphora* in order to identify specializations associated with its high rate of activation, and to determine its role in flight control.

The central and peripheral morphology of the B1 motor neuron has been determined using low molecular weight dextran tracers. Both the cell body and the most extensive aborizations are ipsilateral, although one branch of the dendrite crosses the mid-line dorsally. The peripheral morphology of the B1 motor neuron is much more striking: the 250 µm axon branches into a large arborous network that interdigitates throughout the muscle. The axon size and terminal geometry are probably specializations for prolonged high-frequency activation.

Repetitive stimulation of the B1 motor axon at wing beat frequency results in tetanus, although discrete isometric force transients can be resolved at frequencies as high as 100 Hz. However, during flight, the B1 muscle is subjected to cyclic length changes at wing beat frequency. Therefore, we are currently investigating the effects of stimulus phase on the mechanical output during imposed sinusiodal length changes that mimic those which occur naturally during flight.

592.8

A NECESSARY ROLE FOR PROCTOLIN IN MAINTAINING TENSION PRODUCTION BY AN INSECT MUSCLE. JH Belanger*[1] and I Orchard[2]. [1]Arizona Res Lab Div of Neurobiology, U of Arizona, Tucson, AZ 85721, USA and [1,2]Dept of Zoology, U of Toronto, Toronto, ON, M5S 1A1, Canada.

We have been using the neuromuscular substrate underlying oviposition digging in the locust (*Locusta migratoria*) as a model system to try to understand the role(s) played by cotransmitters in the control of insect muscle. The ventral opener muscle is the largest of the ovipositor muscles and produces most of the force used to dig the oviposition hole. We have previously reported the presence and some actions of proctolin in this neuromuscular system (Belanger and Orchard 1988, *Soc. Neurosci. Abs. 14*:934). Here, we report that proctolin is released both by electrical stimulation of nerves and during normal activity of the oviposition digging system, and that this release appears to be necessary for the production of normal amounts of tension by the muscle.

Stimulation of some, but not all, units in the nerve supplying the opener muscle produces a frequency-dependent release of proctolin. Five min of 30 Hz stimulation releases approximately 8% of the total proctolin store of the muscle. During oviposition digging, the opener muscle is driven by a central pattern generator which is located in the terminal abdominal ganglion. In *in vitro* ganglion-muscle preparations which are expressing the digging rhythm, release of about 25% of the muscle proctolin store occurs during the first five minutes of activity. This level declines to below detectability over a period of approximately 20 min. There is a concomitant decline in muscle tension production, even though patterned neural activity is still present and EMGs can still be recorded, implying that conventional synaptic function is still present. Adding exogenous proctolin (10^{-9} M) to the superfusate restores the contractions to their original magnitude.

592.9

LOCAL CALCIUM SPIKES IN THE DENDRITES OF NONSPIKING LOCAL INTERNEURONS IN THE LOCUST. Gilles Laurent*. Biology Div., 139-74, California Institute of Technology, Pasadena CA 91125.

Nonspiking local interneurons directly synapse onto pools of leg motor neurons and are thus well suited to organize leg movement and posture. During a centrally generated rhythm and in the absence of any sensory feedback, their membrane potential undergoes large polarizations (30mV peak-to-peak) centered on a resting potential of ca. -58mV. Depolarization from -60mV leads to outward rectification, which in turn leads to significant changes in membrane time and space constants. Depolarization to -40mV, however, also evokes, in 20% of dendritic impalements, active responses which can take 2 main forms. The first are fast potential oscillations (10-15mV p-t-p) which only occur within a small window of membrane voltages. The second are 30ms-long TTX-resistant action potentials. Whole-cell patch-clamp experiments on cultured nonspiking interneurons indicate the existence of a low-threshold transient Ca^{++} current analogous to T-type currents described in other neurons. These action potentials can be evoked on rebound from IPSP evoked by presynaptic spiking local interneurons. Interestingly, however, such an active response can often be evoked by one only of several IPSPs which the nonspiking interneuron receives from different presynaptic interneurons. This suggests that the channels underlying those action potentials may only be expressed or functional in some, but not all, dendrites of a nonspiking interneuron. Supported by NIH and McKnight, Searle & Sloan Foundations.

592.10

SEGMENT-SPECIFIC FATE OF HOMOLOGOUS MOTONEURONS IN *MANDUCA SEXTA* IS UNCORRELATED WITH DEVELOPMENTAL CHANGES IN MOTONEURON ANATOMY AND PHYSIOLOGY. L. C. Streichert,* D. J. Sandstrom, and J. C. Weeks. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Metamorphosis of the tobacco hornworm, *Manduca sexta*, is accompanied by the dismantling of neural circuits involved in stage-specific behaviors. The loss of the proleg withdrawal reflex at the larval-pupal transformation is associated with dendritic regression of proleg retractor motoneurons, a weakening of afferent input to these motoneurons, and the programmed death of a stereotyped subset of the motoneurons. For instance, the APR motoneurons, which innervate a proleg retractor muscle, undergo programmed cell death on the second day of pupal life (day P2) in abdominal segments A5 and A6, whereas the APRs in A3 and A4 survive and are respecified in the adult. We examined the possibility that these differences in cell fate are correlated with anatomical and physiological changes by comparing the properties of APR motoneurons in segments A3 and A6 during the larval-pupal transformation.

On the third day of the final larval instar (day L3), the structure of APR's dendritic arbor was similar in A3 and A6. Prior to pupation, the dendritic arbors of APRs from both segments regressed similarly such that they were significantly reduced by day P0. There were also no segment-specific changes in the passive electrical properties of the APRs. Electrical stimulation of the nerve containing sensory afferents that synapse upon the APRs evoked compound EPSPs that were of similar mean size in A3 and A6 on day L3. A developmental reduction in the magnitude of the evoked EPSPs was quantitatively similar in APRs from both segments on day P0. Therefore, none of the features that we examined were correlated with the subsequent segment-specific death or survival of homologous motoneurons. Supported by NIH grants HD07244 and NS23208.

592.11

CELLULAR BASIS FOR THE REORGANIZATION OF A PROPRIOCEPTIVE CIRCUIT DURING INSECT METAMORPHOSIS. D.A.Tamarkin* & R.B.Levine. ARL, Div of Neurobiology, Univ of Arizona, Tucson, AZ 85721

During insect metamorphosis, neuronal circuits undergo dramatic reorganization that contributes to the expression of new behavior. We are investigating this reorganization by examining an abdominal proprioceptive circuit during metamorphosis in the hawkmoth, *Manduca sexta*. The effect of activity of a stretch receptor organ (SRO) on intersegmental muscle motoneurons (MNs) was characterized in both the larval and adult stages. The SROs are bilaterally-paired muscle receptor organs that run longitudinally across each abdominal segment. Populations of synergistic and antagonistic MNs are described based on whether contraction of their target muscles would lead to a shortening or lengthening, respectively, of the SRO. In the larva, the synergistic MNs are excited and the antagonistic MNs are inhibited by SRO activation. This pattern of SRO synaptic input changes across metamorphosis, resulting in excitation of both the synergists and antagonists.

Toward understanding this change in the proprioceptive circuit at the cellular level, we have used signal averaging and synaptic latency measurements to examine whether the SRO synaptic input onto individual MNs has a monosynaptic component. Our results suggest that the synergistic MNs that receive the strongest input from the SRO appear to be excited directly in both life stages. Excitatory inputs to other synergistic MNs have a longer latency, but are reliable. The inhibitory SRO synaptic input to the antagonistic MNs in the larva and the new excitatory input to these MNs in the adult is through a purely polysynaptic pathway. The changes in this proprioceptive circuit thus occur through polysynaptic pathways, and implicate the involvement of interneurons in altering synaptic connectivity.

592.12

MONITORING GENE EXPRESSION IN SINGLE NEURONS FROM THE LOBSTER CNS. *E.A.Kravitz, H.Schneider*, B.Brooks+ & J.H.Eberwine+, Neurobiology Dept., Harvard Medical School, Boston, MA 02115 and Pharmacology Dept., University of Pennsylvania School of Medicine, +Philadelphia, PA 19104.*

The physiological properties, transmitter phenotypes and structures of individual neurons are unique, ultimately depending on their differential gene expression. Recently, a method has been developed that allows monitoring of gene expression in single identified neurons following intracellular recording (1).

The pool of mRNA from a single neuron is amplified up to a million fold via cDNA-synthesis within the cytoplasm of the neuron, and the use of the T7-RNA polymerase reaction on the extracted cDNAs. Amplified messages are analysed using expression profiles that enable monitoring of several genes at the same time. In preliminary experiments we have tested the method in the CNS of *Homarus americanus* on large GABA-containing inhibitory motoneurons in the 2nd abdominal ganglion, serotonin-proctolin-containing neurons in the 1st abdominal ganglion and octopamine-containing crotch cells in thoracic ganglia. In all cases successful amplifications have been performed. In expression profiles amplified RNA hybridizes with vertebrate-probes corresponding to neurofilament proteins, c-fos, c-jun, GABA-receptors, glycine-receptors, K^+- Na^+-, Ca^{++}-channels, protein kinase C, and tryptophan hydroxylase. In the long run we plan to study gene expression in single cells in differing developmental and behavioral states. (1) Van Gelder et al. 1990, PNAS 87: 1603-1667. Supported by NIH and DFG Schn 368/1-1.

592.13

MITOCHONDRIAL CONTENT AND TRANSPORT IN PHASIC AND TONIC MOTOR AXONS OF THE CRAYFISH. C.J.Case, and G.A. Lnenicka*. Dept. of Biol. Sci., State Univ. of New York, Albany, N.Y. 12222

Crayfish tonic motor terminals have a greater mitochondrial content than phasic motor terminals (Lnenicka *et.al., J. Neurosci*, 6:2252, 1986). In this study, we examined whether there are similar differences in the mitochondrial content of the phasic and tonic motor axons. After glutaraldehyde fixation, the phasic and tonic motor axons innervating the claw closer muscle were dissected free. Mitochondria were visualized with DIC microscopy, drawn with a *camera lucida* attachment, and their lengths were measured on a digitizing pad. Individual mitochondrial cross-sectional area was determined using TEM. Results indicate that there are differences in mitochondrial volume in the motor axons which are similar to those previously reported for the terminals. The mitochondrial volume per unit volume of axoplasm is 5-fold greater in tonic motor axons than in phasic motor axons. This difference results from more mitochondria per unit volume of axoplasm and a greater cross-sectional area of individual mitochondria in the tonic axon.

Axoplasmic transport of mitochondria was examined in the phasic and tonic axons using video enhanced-contrast DIC microscopy. Consistent with the results from fixed axons, we found that the density of mitochondrial transport in tonic axons was twice that found in the phasic axons.

In order to determine if the differences in mitochondria content and transport are activity-dependent, we have begun to examine tonically stimulated phasic axons. Preliminary results from mitochondrial measurements in fixed axons indicate that tonic stimulation of a phasic axon results in an increase in mitochondrial content. (Supported by NSF grant BNS-9121757.)

592.14

ARE THERE FEEDBACK SUBGROUPS WITHIN CRAYFISH MOTOR POOL? Peter Skorupski and Brian Bush (SPON: Brain Research Association). Department of Physiology, University of Bristol, Bristol BS1 5LS, Bristol, UK.

In the crayfish thoracic ganglia subsets of the leg promotor and remotor pools are excited in positive feedback reflexes resulting from leg movement. Group 1 promotor motoneurons are excited by the thoracocoxal chordotonal organ, which signals leg promotion, and group 1 remotor motoneurons are excited by the thoracocoxal muscle receptor organ, which signals leg remotion (Skorupski et al (1992), J. Neurophysiol. 67, 648-663). Other members of these motor pools lack these inputs and are excited by the opposite directions of movement, which correspond to negative feedback.

The amine octopamine, which is thought to be an inhibitory transmitter or neuromodulator in the crayfish swimmeret system (Mulloney et al (1987), J. Neurophysiol. 58, 584-597) also has inhibitory effects in thoracic ganglia. These effects are selective, however, and correlated with subdivisions of the motor pool on the basis of reflex input. At low concentrations ($1\text{-}10\times10^{-6}$M) octopamine disrupts or slows down any rhythmic activity and selectively depresses positive feedback reflexes. In the case of promotor group 1s, excitatory TCCO input may reverse to become inhibitory. At high concentrations ($50\text{-}100\times10^{-6}$M) octopamine hyperpolarizes some group 1 remotors by 15 mV or more, coupled with a reduction in input resistance of 60-70%. Group 2 remotors, at the same concentration, are depolarized by 1-5 mV, with little or no change in input resistance.

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592.15

GROWTH AND THE PASSIVE INTEGRATIVE PROPERTIES OF NEURONS. D.H. Edwards*. Dept. of Biology, Georgia State University, Atlanta, GA 30302-4010

Study of the response properties of the lateral giant interneuron in large and small crayfish suggests that its PSPs are increasingly low-pass filtered as the cell grows. To test the effects of size increases on a neuron's passive integrative properties, the responses to current steps and conductance-increase synaptic inputs were calculated for small and large (10X) equivalent cylinder models of neurons. Response rise-times and the attenuation of steady-state responses were significantly greater in the larger model, whereas the input resistance was 50-fold smaller. Increases in the injected current and the maximum postsynaptic conductance restored the peak responses of the large model to the values of the small model, but did not affect the response attenuation or rise-times. Several-fold increases in the specific membrane resistance increased the large model's input resistance and reduced the steady-state attenuation, but also greatly slowed its responses. The response properties of the large model were restored to those of the small model when increases in specific membrane resistance were matched by similar decreases in the specific membrane capacitance.

592.16

LOCOMOTION IN THE MEDICINAL LEECH, *Hirudo medicinalis*: ANALYSIS OF A COMPLEX RHYTHMIC BEHAVIOR. A. Baader* and W.B. Kristan Jr., Department of Biology, UCSD, La Jolla, CA 92093-0322.

Locomotor performances are often generated by rhythmic activities of the central nervous system. Leech swimming is a robust rhythmic pattern with a period length of a cycle in the range of 0.5 to 1 sec, whereas crawling is much more variable: a single step can last between 3 and 15 seconds, elongations and contractions of the body may involve some or all segments, and the temporal organization of a complete step is sustained by the additional activity of head and tail suckers. We are determining the neuronal mechanisms by which the leech nervous system generates such a complex behavior.

Tethered animals are suspended in an apparatus in which they perform various behaviors (e.g., swimming, crawling, or shortening), while extracellular and intracellular recordings are obtained. Behaviors and electrophysiological data are video-monitored simultaneously to evaluate the contribution to individual neurons on behavior. Systematic intracellular recordings in midbody ganglia showed that few interneurones are modulated during crawling and their selective activation did not affect the behavior. Recordings from the tail brain revealed many different units to be activated in all phases of crawling. Cutting nerves to various parts of the body showed that sensory input from either one of the suckers is necessary to produce normal crawling behavior. Supported by the NIH (WBK, AB) and the Deutsche Forschungsgemeinschaft (AB).

592.17

RECOVERY OF CONSUMMATORY FEEDING BEHAVIOR AFTER BILATERAL CNS LESIONS IN *APLYSIA*. M.L. Scott* and M.D. Kirk Div. Biol. Sci., Univ. Missouri-Columbia, Columbia, MO 65211.

Consummatory feeding behavior in *Aplysia californica* is generated in the buccal ganglia under the control of neural elements in the cerebral ganglion. Consummatory feeding is selectively abolished by cutting the cerebral-buccal connectives (CBCs) bilaterally and does not recover during the subsequent three weeks. We have used the feeding system in *Aplysia* as a model for studies of functional neural regeneration following bilateral CBC crushes. Normal feeding parameters (Rosen et al., '89, J. Neurosci., 9:1562) were tested preoperatively. CBCs of anesthetized subjects (130-300 g) were crushed bilaterally with #5 forceps through a dorsal incision. Subject behavior was tested postoperatively on day 2, then every 4th day through day 30. Subjects were fed a total of one strip of seaweed (dried laver, 1 x 15 cm strip) every 2 days. Appetitive behaviors were not affected by the lesions. Rhythmic biting was initially abolished, and reappeared in all lesioned animals by day 14. Although bite magnitude and bite frequency were initially less than preoperative levels or sham controls, both increased toward control values with additional recovery time. Differences in neuromuscular activity and connections are being explored at various timepoints during recovery using *in vivo* and *in vitro* recordings.

To initially assess the progress of neural regeneration, cobalt backfills into the cerebral and buccal ganglia from the CBCs were performed on days 3, 9, 13, 21, and 30 postlesion. Staining of cell bodies was consistently seen beginning on day 9, and the return of cobalt staining parallels the timecourse of behavioral recovery. Therefore, the feeding system of *Aplysia* will be an ideal model system to study functional neural regeneration *in vivo*. Supported by NIH.

592.18

ORGANIZATION AND FUNCTION OF A MULTIFUNCTIONAL NEUROMUSCULAR SYSTEM DURING FEEDING IN HELISOMA TRIVOLVIS. B.C. Arnett* and A.D. Murphy Department of Biological Sciences, University of Illinois at Chicago, Chicago, Illinois 60680

A feeding cycle in Helisoma is characterized by dynamic stages of protraction, retraction, and hyper-retraction of the buccal mass, radula, and odontophore cartilage. Feeding behavior is generated by a buccal pattern generator comprised of three neuronal subunits (S1, S2 and S3). Each subunit is an independent oscillator but can be linked temporally to either one or two of the other subunits to produce diverse motor patterns. The large intrinsic supralateral radular tensor (SLRT) muscle of the pharyngeal buccal mass plays a role in retraction, tensing, and hyper-retraction of the radula. The SLRT is innervated by at least 8 motoneurons. Two motoneurons driven by S3, and two motoneurons driven by S2 have been identified. Each motoneuron innervates a distinct region of the SLRT. Muscle contractions in different regions of the SLRT and at different times during the feeding cycle confer multifunctionality and allow flexibility during feeding behaviors. The SLRT neuromuscular system is therefore an excellent model to study the contributions of polyneuronal innervation to complex behaviors.

592.19

TWO B (OR NOT TO BE) A *THIRTYSOMETHING* CELL IN *APLYSIA*. I. Hurwitz, R. Goldstein*, L. Cleary & A.J. Susswein. Dept Life Sci, Bar Ilan Univ, Ramat Gan, Israel 52 900, and Dept Neurobiol & Anatomy, U Texas Med School, Houston, TX 77025.

The B31/B32 cells in the buccal ganglia of *Aplysia californica* were previously shown to have many unusual electrophysiological features. The somata of these strongly coupled cells do not sustain conventional action potentials. Depolarization of the soma produces a sustained, long-lasting regenerative response, followed by a hyperpolarization. The hyperpolarization is correlated with a patterned burst expressed in most of the neurons of the buccal ganglia. In an attempt to understand the function of these unusual features, we have examined the morphology of the B31/B32 cells. The cells have a rich neuropil with varicosities close to the soma, and a proximal axon that is ~20% of the soma diameter. They also have peripheral varicosity-bearing axons which innervate the I2 muscle of the buccal mass, a major protractor muscle, via fine branches of the radular nerves. However, the cells can be distinguished morphologically. 1) The cells innervate different regions of the muscle. 2) The rich neuropil of one cell arises directly from the soma, while that of the other arises from the proximal segment of the axon. 3) Only one cell has significant neuropil in more distal axonal regions. One such region is at the origin of the buccal commisure, close to neurons B20 and B4. These data suggest that the soma and axon of the B31/B32 may be functionally compartmentalized. Information processing near the soma is accomplished by slow, regenerative potentials, while information processing in the axon and I2 muscle is via conventional action potentials. The neuropil close to the soma should be unaffected by fast, conventional action potentials that occur in the axon, while more distal neuropil should be unaffected by slow potentials in the soma. We are performing electrophysiological experiments to test this possibility.

592.20

AFTERDISCHARGE ACTIVITY OF MOTONEURONS INVOLVED IN FEEDING BEHAVIOR IN PTEROPOD MOLLUSC. T.P.Norekian* and R.A. Satterlie. Dept.of Zoology, Arizona State Univ., Tempe, AZ 85287 and Friday Harbor Laboratories, Friday Harbor, WA 98250.

The pteropod mollusc *Clione limacina* is a highly specialized carnivore which feeds on shelled pteropods and uses, for their capture, three pairs of oral appendages, called buccal cones. Contact with the prey induces rapid eversion of buccal cones, which then become tentacle-like and grasp the shell of the prey. A large group of electrically coupled, normally silent cells (A motoneurons) has been described in the cerebral ganglia of *Clione*, whose activation induces opening of oral skin folds and extrusion of buccal cones.

Brief intracellular stimulation of an A neuron can produce prolonged firing (afterdischarge), lasting up to 40 seconds, in the entire population of A neurons. Afterdischarge activity is based on a slow depolarization evoked by an initial strong burst of A neuron spikes. Obtained data suggest that the afterdepolarization underlying afterdischarge activity is a slow excitatory synaptic input from an unidentified neuron or neurons which in turn recieve excitatory inputs from A neurons, thus organizing positive feedback. The main functional role of this positive feedback is the spread and synchronization of spike activity among all A neurons in the population. In addition, it transfers brief excitatory inputs to A neurons into a prolonged and stable firing pattern, which is required during certain phases of feeding behavior.

592.21
PERIPHERAL MODULATION OF SWIM MUSCULATURE IN THE PTEROPOD MOLLUSC CLIONE LIMACINA. R.A. Satterlie* Dept. of Zoology, Arizona State Univ., Tempe, AZ 85287-1501.

Neuromuscular organization in Clione includes two distinct types of muscle fibers, fast-twitch fatigable and slow-twitch fatigue-resistant, and two types classes of pedal motoneurons separable based on soma diameters and innervation fields. In addition, four serotonin-immunoreactive pedal neurons send axons to the swim musculature. Serotonin-immunoreactive terminals are found associated with slow-twitch fibers only. Activity in the serotonin-immunoreactive neurons does not produce a motor response in the absence of ongoing swimming activity, but does enhance motoneuron-induced electrical activity in slow-twitch muscle cells. This modulatory action is restricted to the periphery, and is reversibly blocked by 10^{-5}M mianserin suggesting that serotonin is the modulatory transmitter. Since bath-applied serotonin produces both central and peripheral modulation of swimming, independent central and peripheral modulatory pathways are suggested.

592.22
EFFECTS OF NEUROTRANSMITTERS ON THE ELECTRICAL COUPLING OF ASCARIS SOMATIC MUSCLE CELLS. S. Solórzano, J. Serrato and A. Rivera*. Dept. of Physiology, Centro de Investigación del IPN, México 07000 DF, MEXICO.

Somatic muscle cells from the nematode Ascaris are electrically coupled and receive modulatory neural input from excitatory and inhibitory motorneurons. We have suggested that one factor involved in contractile wave propagation is the pattern of electrical coupling observed between somatic muscle cells. Since several neurotransmitters have been implicated in gap junction channel modulation, it seemed of interest to test whether ACh or GABA had any effect on the gap junction channels of Ascaris muscle cells. Substances were bath applied while recording input resistances and coupling coefficients in pairs of well coupled somatic muscle cells from anterior portions of the animal. Other compounds were also tested, including heptanol and octanol, which have been shown to close junctional channels in many preparations. GABA, 10 μM, hyperpolarized the cells, decreased the coupling coefficient and decreased the input resistance; ACh, 1, 10 and 100 μM, depolarized the cells and caused variable small changes in the coupling coefficient and input resistance; heptanol, 1 mM, induced a small decrease in the coupling coefficient and increased the input resistance, while octanol had negligible effect. The data suggest that gap junction channels from Ascaris somatic muscle are not modulated by the natural transmitters, and close in response to heptanol.

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OTHER SYSTEMS OF THE CNS: HYPOTHALAMUS

593.1
BED NUCLEUS OF THE STRIA TERMINALIS DIRECTLY INNERVATES CRF NEURONS IN THE LATERAL HYPOTHALAMUS. T.S. Gray and D.J. Magnuson,* Dept. of Cell Biology, Neurobiology and Anatomy, Loyola University Stritch Sch. of Med, Maywood, Il. 60153

The bed nucleus of the stria terminalis (BST) projects to the lateral hypothalamus. Numerous CRF immunoreactive neurons are located in this region. The present study investigated the distribution of BST efferents in the lateral hypothalamus and the possibility that BST terminals innervate CRF expressing neurons in this region.

The BST of Long-Evans, black-hooded male rats was injected with *Phaseolus vulgaris* leucoagglutinin lectin (PHA-L) tracer. After 10-16 days rats were overdosed with sodium pentobarbital and their brains were fixed using 4% paraformaldehyde. Tissue was processed using brown DAB staining of PHA-L followed by glucose-oxidase blue immunostaining of CRF.

The heaviest projections to the lateral hypothalamus were observed after injections of PHA-L in ventral and lateral parts of the BST. Most of the BST terminals were located caudally and extended dorsally and ventrolaterally around the fornix. BST terminals contacted numerous CRF immunoreactive neurons in the lateral hypothalamus and the paraventricular nucleus. Fewer CRF neurons were innervated after PHA-L injections in the medial and dorsal parts of the BST. Thus, the BST directly innervates CRF neurons in the lateral and paraventricular hypothalamus. Previous studies have demonstrated that CRF lateral hypothalamic neurons project to the central grey, parabrachial nucleus and dorsal vagal complex. Thus, the BST can alter the activity of CRF neurons that project to the median eminence and to a separate population of CRF cells that innervate autonomic regions of the brainstem. Supported by NIH NS 20041.

593.2
THE EFFERENT CONNECTIONS OF THE DORSOMEDIAL NUCLEUS OF THE HYPOTHALAMUS: A PHAL STUDY IN THE RAT. R.H. Thompson, N. S. Canteras, L.W. Swanson.* Dept. Biol. Sci., University of Southern California, Los Angeles, CA 90089.

PHAL was injected stereotaxically into 25 rats to characterize the efferent projections of the DMH. No differences in the pattern of labeled projections were apparent in experiments with injections centered in any of the 3 major parts of the nucleus. DMH projections may be divided into ascending and descending components, with the former divided into periventricular (PVP) and lateral (LP) pathways. The ascending pathways are predominantly intrahypothalamic and contain the vast majority of fibers arising in the DMH. The PVP courses rostrally to end mainly in the parvicellular paraventricular, parastrial, median preoptic, anterodorsal preoptic, preoptic suprachiasmatic (PSCh) nuclei and in the region surrounding the PSCh. Some fibers also innervate the paraventricular nucleus of the thalamus. The LP runs through dorsomedial and ventromedial parts of the medial forebrain bundle. It contributes fibers to the medial preoptic area and nucleus, and to the anteroventral preoptic nucleus; some of these fibers turn medially, joining those from the PVP to innervate the PSCh. LP fibers also project to ventral parts of the bed nuclei of the stria terminalis and lateral septum. Descending fibers from the DMH innervate the posterior hypothalamic nucleus, tuberomammillary and supramammillary nuclei, periaqueductal gray, specific parts of the reticular formation, Barrington's nucleus, parabrachial nucleus, and nucleus of the solitary tract. In summary, DMH projections remain primarily within the hypothalamus, innervating structures that mediate endocrine and autonomic responses. To a lesser extent, they also project to premotor nuclei that may influence voluntary movements.

593.3
DIENCEPHALIC ORIGINS OF MCH-IMMUNOREACTIVE (MCH-ir) PROJECTIONS TO THE PERIAQUEDUCTAL GRAY (PAG). C.F. Elias and J.C. Bittencourt*. Lab. of Immunohistochemistry of Peptides and Neuronal Tracers, Dept. of Anatomy ICB/USP, Sao Paulo - SP 05508 - BRASIL.

The PAG has been implicated in many functions, like: analgesia, vocal expression of emotion, blood pressure control, control of reproductive behavior. For this reason, the afferent projections to this midbrain region are extensive, such as the lateral hypothalamic area, zona incerta, anterior cingulate cortex. Bittencourt et al (1992) described an extended MCH-ir terminals in the CNS of the rat, including the PAG. In order to address the origins of those projections, we have used retrogradely transported fluorescent dye (5% of crystalline deposits of True-Blue in the lateral-ventral portion of the PAG) and immunohistochemical techniques. The results suggest that the MCH-ir cells projecting to the PAG are concentrated in the following regions: medial aspect of the zona incerta, dorsolateral hypothalamic area, dorsomedial aspect of the tuberomammillary nucleus, caudal part of the anterior hypothalamic area an surrounding the fornix.

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593.4
QUANTIFICATION OF NORADRENERGIC VARICOSITIES IN APPOSITION TO VASOPRESSIN-IMMUNOREACTIVE NEURONS IN THE MONKEY PARAVENTRICULAR NUCLEUS. S.D. Ginsberg[1]*, P.R. Hof[1,2], W.G. Young[3], and J.H. Morrison[1,2]. [1]Fishberg Res Ctr for Neurobiology and [2]Dept of Geriatrics and Adult Development, Mt Sinai School of Medicine, New York, NY 10029, [3]Dept of Neuropharmacology, Scripps Clinic, La Jolla, CA 92037.

Previous investigation of dopamine-ß-hydroxylase-immunoreactive (DßH-ir) innervation of monkey hypothalamus has revealed a high density of noradrenergic varicosities throughout the paraventricular nucleus (PVH). In order to further characterize DßH-ir varicosity density on specific transmitter-identified postsynaptic targets within the primate PVH, double label immunofluorescence combined with confocal laser scanning microscopy was employed. In this study, DßH-ir varicosities and vasopressin-immunoreactive (VP-ir) perikarya were scanned confocally and the resultant images were superimposed. In this double label scheme, DßH-ir varicosities that were in apposition to (less than 1 μm away) VP-ir profiles were counted, whereas varicosities not in apposition to VP-ir profiles were not quantified. This procedure yielded a varicosity to neuron ratio which gave one measure of transmitter innervation. In addition, the distribution of DßH-ir/VP-ir appositions was assessed to determine the cellular structures on which the majority of contacts occur (e.g. dendrites or perikarya). Preliminary observations indicate that the DßH-ir varicosities are distributed primarily on or around VP-ir dendrites, and may be one of the dominant inputs to this subclass of PVH neurons. Supported by the MacArthur Foundation, and NIH MH45212.

593.5

GLUTAMATE- AND ASPARTATE-LIKE IMMUNOREACTIVITIES IN CHEMICALLY IDENTIFIED HYPOTHALAMIC NEURONS.

B. Meister*, A.P. Nicholas and T. Hökfelt, *Dept. of Histology and Neurobiology, Karolinska Institute, P.O. Box 60400, 104 01 Stockholm, Sweden.*

Recent evidence suggests that the amino acid glutamate (GLU) may be the dominant excitatory transmitter in neuroendocrine regulation. Both GLU and aspartate (ASP) have been demonstrated in presynaptic hypothalamic axons. In the present study we have by means of indirect immunofluorescence histochemistry examined the distribution of GLU- and ASP-immunoreactive (-IR) cell bodies within the hypothalamus. Of special interest was to explore the presence of GLU- and ASP-like immunoreactivity (-LI) in chemically identified neurons of hypothalamic nuclei. GLU/ASP-LI was demonstrated in cell bodies of the suprachiasmatic (SCN), periventricular (Pe), supraoptic (SON), paraventricular (PVN) and arcuate (Arc) nuclei. In the SCN, GLU/ASP-LI was demonstrated in many vasopressin (VP)-containing cells in the dorsomedial aspect of the nucleus. Few GLU/ASP-IR cells in the SCN were also somatostatin (SOM)-IR. In the Pe, single SOM-IR cells were GLU/ASP-positive. Magnocellular neurons in the SON and PVN contained GLU/ASP-IR and after double-labelling it was shown that there was colocalization with both VP and oxytocin. Within the Arc, GLU/ASP was mainly found in the ventromedial aspect of the nucleus, however, single neurons were also distributed in the ventolateral aspect. Double-labelling revealed that most of the GLU/ASP-IR cells also were neuropeptide Y (NPY)-containing. Few GLU/ASP-IR cells in the ventrolateral aspect of the Arc showed tyrosine hydroxylase (TH)-LI. The TH-containing neurons within the A13 cell group of the zona incerta were to a large extent GLU/ASP-positive. The results suggest that the excitatory amino acids GLU and ASP are colocalized with several peptides and TH in hypothalamic neurons.

593.6

IN VITRO DEPOLARIZATION INDUCED CHANGES OF PITUICYTE MORPHOLOGY ARE NOT DEPENDENT ON EXTRACELLULAR CALCIUM. B.-G. Zhao[1] and P. Cobbett, Dept Pharmacol. & Toxicol. and Neuroscience Prog., Michigan State Univ., East Lansing, MI 48824, USA.

Morphological plasticity in the neurohypophysis following certain changes in physiological state is well established. We have used cultures of adult pituicytes (Bicknell et al., Brain Res Bull 22, 379-388, 1989) to examine the involvement of extracellular Ca in the control of pituicyte morphology. Pieces of neurohypophysis were cultured for 14 days, when culture medium was replaced by a balanced salt solution (with 2mM Ca and 2mM Mg) for 90min and the fraction of cells that were stellate was determined. In control medium, this fraction was 0.09±0.01 (mean±SEM, n= 5 dishes); in medium containing 50mM K, it was significantly increased independent of Ca concentration (0.77±0.04, n=8, Ca 2mM and Mg 2mM; 0.72±0.04, n=8, Ca 50μM and Mg 4mM). In medium containing the β-adrenergic receptor agonist isoproterenol (10μM) or isoproterenol (10μM) and reduced Ca (Ca 50μM and Mg 4mM), it was also increased (0.69±0.03, n=5, and 0.78±0.04, n=5, respectively). We conclude that Ca influx is not necessary for changes of pituicyte morphology to occur.

Supported in part by NIH grant NS28206. [1]Permanent Address: Shanghai Institute of Planned Parenthood Research, 2200 Xie Tu Road, Shanghai, PRC.

593.7

SYNCHRONOUS NEURONAL ACTIVITY IN THE SUPRACHIASMATIC NUCLEUS (SCN) INDEPENDENT OF CHEMICAL SYNAPTIC TRANSMISSION. Y. Bouskila and F. E. Dudek*. Mental Retardation Res. Ctr., UCLA Sch. of Med., Los Angeles, CA 90024

The SCN, which contains the mammalian biological clock, exhibits a circadian rhythm of firing rate. Since these data are derived from neuronal populations they also imply that neuronal activity in the SCN is synchronized. To examine possible mechanisms of synchronization, coronal hypothalamic slices (350-500 μm) were prepared 2 h before the dark phase (12:12 L:D) from male rats. Simultaneous multiple-unit recordings showed that the circadian rhythm of neuronal activity level was correlated between different locations within the SCN ($0.63 \le r \le 0.99$, $p<0.0015$) and thus confirmed the neuronal *synchronization* (n=6). To study mechanisms of synchronization in the SCN, extracellular Ca^{2+} was replaced with Mg^{2+} (+ 0.1 mM BABTA), which blocks chemical synaptic transmission and increases membrane excitability. This treatment produced periodic bursts of action potentials which were synchronized throughout the SCN (n=11), but were not synchronized with similar bursts in the contralateral SCN (n=9). Furthermore, a mixture of NMDA, non-NMDA and $GABA_A$ receptor antagonists (AP-5 - 100 μM, DNQX - 50 μM and bicuculline - 50 μM) had no effect on burst synchrony (n=6). Whole-cell patch-clamp recordings confirmed that the Ca^{2+}-free medium blocked evoked postsynaptic potentials (PSPs) (n=4) and that the mixture of antagonists blocked the remaining spontaneous PSPs (n=6). These results indicate that synchronous neuronal activity can occur in the SCN without active chemical synapses, suggesting that a different mechanism of communication exists; a similar mechanism(s) of neuronal synchronization may coordinate the cellular elements in the SCN responsible for circadian rhythms in mammals. Supported by AFOSR.

593.8

NMDA receptor-linked channels in primary hypothalamic supraoptic punch cultures M.C. Curras,* J.N. Hayward and R.B. Meeker. Department of Pharmacology, Neurology and the Neurobiology Curriculum, University of North Carolina at Chapel Hill, North Carolina

Although glutamate binding to NMDA receptors is low in the hypothalamus (Monaghan et al, 1983), responses to NMDA have recently been reported (Hu and Bourque, 1991; Van den Pol et al., 1991). However, these studies disagree on the abundance of NMDA receptors. To characterize supraoptic (SON) NMDA receptors we used the high resolution of single-channel patch clamp. Timed pulses of NMDA (100 μM) in combination with added glycine (1-10 μM) were applied to outside-out patches from large neurons in embryonic (ED16) SON explant cultures in nominally-free Mg solutions at -60mV. Before excising patches most neurons were filled with 0.4% biocytin for 3-7 minutes. The mean unitary conductance of NMDA-activated channels was 46.7±4.0 pS (n=9) and raising the glycine concentration from 1 to 10 μM increased the frequency of openings (n=2). 1mM Mg^{2+} blocked channel openings at negatve potentials in 4 out of 4 patches. To test for the presence of GluR1-7 non-NMDA receptor phenotypes at this resolution we exposed the same outside-out patches to 300 μM kainic acid (KA) and 100 μM Amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) sequentially. Current responses to KA were much greater than to NMDA/glycine indicating a relative abundance of KA receptors. AMPA currents were rapidly desensitizing (n=2) and KA currents were either desensitizing (n=1) or non-desensitizing (n=1). These findings confirm an earlier report that NMDA, KA, and AMPA channels are present on individual SON neurons (Hu and Bourque, 1991). In addition, these results suggest the presence of GluR1-4 and functional GluR5 (Herb et al., 1992) glutamate receptor subunits in the hypothalamus. Supported, in part, by NIH Grant NS-13411.

593.9

Whole-cell patch clamp recordings of spontaneous synaptic currents and their block by amino acid neurotransmitter antagonists in medial preoptic slices from immature male rats. N. W. Hoffman*, J.-P. Wuarin, and F. E. Dudek, Mental Retardation Res. Cetr. UCLA Sch. of Med., Los Angeles, CA. 90024

Inhibitory and excitatory amino acid neurotransmitters mediate fast synaptic potentials in several discrete hypothalamic nuclei (e.g., van Den Pol, A.N., et al., Science, 250: 1276, 1990). In the medial preoptic area, a more diffuse hypothalamic region implicated in diverse biologic functions, electrically evoked IPSPs are due to activation of $GABA_A$ receptors (Hoffman, N.W., et al., Soc. Neurosci. Abstr., 16: 574, 1990). The present investigation used whole-cell patch-clamp recordings in slices (400 μm) from immature rats to examine the pharmacology and development of spontaneous synaptic currents in medial preoptic neurons. These currents, particularly the spontaneous IPSCs, were prominent in recordings (n=12) from animals 8 to 33 days of age. Bath application of the $GABA_A$ receptor antagonist, bicuculline (10 μM, n=6), blocked the spontaneous IPSCs (15-50 pA), and the non-NMDA glutamate receptor antagonist, CNQX (3 μM; n=4), blocked the spontaneous EPSCs (3-10 pA). Tetrodotoxin (0.5 μg/100 ml) abolished most spontaneous postsynaptic currents, indicating their dependence on Na^+ spikes; however, some tetrodotoxin-resistant miniature postsynaptic potentials persisted during this treatment. These results support the hypothesis that amino acid neurotransmitters mediate all fast synaptic events in the medial preoptic area, and these chemical synapses are functional when medial preoptic subregions are undergoing sexual differentiation.

593.10

ASYMMETRICAL DIFFERENCES IN THE ELECTROENCEPHALOGRAPHIC (EEG) ACTIVITY OF THE PREOPTIC AREA-ANTERIOR HYPOTHALAMIC AREA (POA-AHA) OF THE FEMALE RATS. **M.A.Sánchez,J.Manjarrez,R.Alvarado,R.Tapia*, and R.Domínguez. Escuela Nacional de Estudios Profesionales Zaragoza, UNAM, Instituto Nacional de Neurología y Neurocirugía, and Instituto de Fisiología Celular, UNAM.**

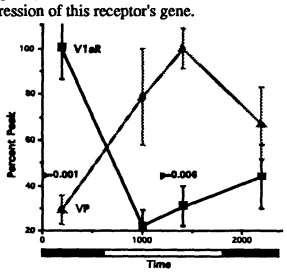
The hypothalamus presents neurochemical and functional asymmetries. In this report we studied the EEG activity in both sides of POA-AHA during the estrous cycle of the rat. The record was performed with bipolar electrodes, and analized with the Rhythm program (Stellate System). Left and right sides of POA-AHA showed differences both in amplitude and power in the EEG activity. Four rats had more amplitude and power in the right side, and three animals showed an inverse pattern. These differences correlated with the position adopted by the animals when they were anaesthetized and mounted in the stereotaxic apparatus. Neither correlation nor coherence between left and right recordings were observed. These results indicate that differential EEG activity in both sides of the POA-AHA is related with an intrinsic behavioral asymmetry.

593.11

CIRCADIAN RHYTHM OF EXPRESSION OF VASOPRESSIN V1a RECEPTOR GENE IN THE SCN. W.S. Young, III, N.L. Ostrowski*, A-M. O'Carroll, and S.J. Lolait Lab. of Cell Biology, N.I.M.H., Bethesda, MD 20892.

The suprachiasmatic nucleus (SCN) is thought to be the generator of circadian rhythms in mammals. Diurnal variations in the expression of several neuropeptide genes, including the one encoding vasopressin (VP), exist in the SCN, although the roles of these neuropeptides are not understood. VP receptors (of the V1a type that is present in kidney and liver and linked to phosphoinositol turnover) are also present in the SCN. Our cloning of the rat V1a receptor (V1aR; Nature 356 523, 1992) enabled us to look for a diurnal rhythm in the expression of this receptor's gene.

Four groups of adult male Sprague-Dawley rats (4 per group) were entrained for 10 days (lights on 0600-1800) prior to sacrifice at 0200, 1000, 1400, and 2200. 12μ frozen sections through the SCN were processed for hybridization histochemistry for V1aR (Endocrinology 131, 1992) or VP (Mol. Brain Res. 1 231, 1986) mRNA. We confirmed the diurnal rhythm for VP expression with highest levels at 1400. V1aR mRNA had just as large a swing in levels, but peaked at 0200. (statistical comparisons are with respective peak values).



We are currently performing double-labeling experiments to see if the V1aR and VP transcripts are co-localized and are examining Brattleboro rats to see if the rhythm in V1aR expression is maintained.

593.12

CONNEXIN 32 mRNA LEVELS IN THE SUPRAOPTIC NUCLEUS PRIOR TO AND DURING LACTATION. G.I. Hatton* and P.E. Micevych, Dept. of Neuroscience, Univ. California, Riverside, CA 92521 and Dept. Anatomy & Cell Biology, Univ. California, Los Angeles, CA 90024

Recent evidence suggests that there is elevated direct intercellular communication in the supraoptic nucleus (SON) during lactation. The major evidence for this is an increase in the incidence of dye-coupling in the SON of nursing mothers compared with virgin rats. Dye-coupling is thought to be mediated by direct intercellular channels of connexin protein, which in neurons has been shown to be connexin 32 (Cx32). To test whether the increased coupling is related to elevated Cx32 mRNA in the SON, virgin rats, mothers sacrificed before the start of lactation and mothers that had been lactating for 14 days were processed for Cx32 *in situ* hybridization histochemistry with a ^{35}S-labeled riboprobe (Micevych and Abelson, *JCN* 305:96-118, '91). The labeling ratio was designated as density of silver grains over a structure ÷ density of silver grains over the background. Cx32 cRNA hybridization was observed over scattered cells throughout the SON in virgin rats (labeling ratio = 3.98 ± 1.20). No distinct dorso-ventral gradient was noted but most of the hybridization was observed in the rostral to middle portions of the nucleus. Mothers that were sacrificed after parturition but before lactation had the highest labeling ratio (10.61 ± 1.51) in the SON. Mothers that had been allowed to lactate for 14 days had a SON labeling ratio (6.62 ± 2.50). These results indicate that the Cx32 mRNA increases prior to lactation and then is reduced during lactation and suckling. Thus, the neural and endocrine events at the time of parturition may be responsible for the elevated levels of Cx32 mRNA which code for gap junction proteins that account for increased dye-coupling between SON neurons. (Supported by NS21220 and NS09140).

593.13

EXPRESSION AND REGULATION OF A HUMAN PROENKEPHALIN FUSION GENE IN THE HYPOTHALAMUS OF TRANSGENIC MICE D. Borsook*, A. Dauber, R. Burstein, A. Strassman, K. Herrup, M. Comb, S Hyman Molecular Neurobiology Laboratory, Mass. Gen. Hospital, Boston MA 02114

Proenkephalin (ENK) is expressed in many nuclei of the hypothalamus where it is regulated by a variety of physiological stimuli. For example, estrogen increases ENK expression in the ventromedial nucleus and stress produces increases of ENK expression in the paraventricular nucleus. However, the trans-synaptic regulation of ENK expression in the hypothalamus is not well understood. In order to investigate developmental and physiological influences on enkephalin expression in the hypothalamus, we have produced transgenic mice expressing a human ENK ß-Galactosidase fusion gene containing 3kb of 5' flanking sequences and 1kb of the 3' flanking sequences from the human proenkephalin gene and studied the expression of the fusion gene in both fetal and adult animals under a variety of conditions. This construct confers basal expression of the reporter gene ß-Gal in the adult and fetal hypothalamus. In the adult high levels of expression are observed in the paraventricular, ventromedial, supraoptic and mammillary nuclei, moderate levels in the anterior, perifornical, lateral, periventricular and low levels in the suprachiasmatic nucleus. In In the fetal hypothalamus expression was seen in the periventricular region and in regions destined to become specific nuclei, particularly those with high levels of expression viz. the paraventricular and ventromedial nuclei. ß-Gal expression correlated with the endogenous gene based on *in-situ* hybridization. Regulation of the transgene was produced by (a) Injections (i.p.) of hypertonic saline produced an increase of ß-Gal in the supraoptic and paraventricular nuclei at 3, 6 and 12 hours with no change in controls (max at 6 hrs); (b) A noxious stimulus (electrical stimulation of the maxillary nerve) in urethane anesthetized animals, produced enhanced expression in the lateral nucleus; (c) pregnancy increased levels in the lateral ventromedial nucleus. In order to identify activation of identified enkephalin neurons by such stimuli, we have double-labelled ß-Gal positive cells with the IEG c-fos. These results show that the transgenic model may be useful for investigating mechanisms of proenkephalin regulation in the hypothalamus.

ASSOCIATION CORTEX AND THALAMOCORTICAL RELATIONS

594.1

PATTERN AND COLOR ENCODING NEURONS WITH OCULOMOTOR PROPERTIES IN THE MACAQUE PULVINAR. J.D. Port, E. F. Castillo and L. A. Benevento*. Dept. of Anatomy, University of Maryland College of Dental Surgery, Baltimore, MD 21201, and Dept. of Anatomy and Cell Biology Univ. of Illinois, Chicago, IL 60612.

The dorsal portion of the lateral pulvinar and the adjacent portion of the medial pulvinar (PL/PM) are interconnected with cortical and subcortical regions which encode information as to the form or location of visual objects. We recorded from single cells in PL/PM of awake behaving monkeys (Macaca mulatta). We found a class of neurons that responded differentially to 9 different forms or colors. Such cells responded to several of the patterns or colors, but usually they responded best to only one or two of them. These responses were said to be pattern or color preferential if a certain pattern or color gave a response which was statistically different than the responses to the other patterns or colors. Of the 83 cells tested for pattern selectivity to date, 37% responded with excitatory peaks to the presentation of at least one of the pattern stimuli. The 400 visual cells tested had response latencies falling cleanly into three latency classes identical to those found in a previous study with anesthetized animals (Benevento et.al., Exp. Brain Res., 1992). Class A cells had response latencies of < 75 msec; class B cells had latencies of 76 - 140 msec, and class C cells had response latencies > 140 msec. Interestingly pattern/color responsive cells could be found in all latency classes. Visually responsive cells also had a variety of oculomotor properties. Seventy-six percent of the pattern preferential cells possessed some oculomotor property such as presaccadic and postsaccadic discharges to targets placed in diagonally opposed quadrants and a suppression of discharge during the saccade. Eye movements could enhance the response to a pattern or color. The results suggest that the pulvinar contains cells which have sensory and oculomotor properties which are usually segregated in other cortical and subcortical regions.

594.2

ASYMMETRIES IN BEHAVIOR FOLLOWING UNILATERAL MEDIODORSAL THALAMIC LESIONS IN RATS. J.M. Vargo*, T.L. Callahan and P.J. Best. Dept. of Psychology, Miami Univ., Oxford, OH 45056.

Bilateral lesions of either the medial precentral prefrontal cortex (AGm) or mediodorsal thalamic nucleus (MD) produce spatial context recognition deficits evidenced by a failure to respond to familiar but out-of-place stimuli (K.A. Stokes & P.J. Best, 1987, Neurosci. Abs., 13:1067; J.M. Vargo & P.J. Best, 1990, Neurosci. Abs., 16:1095). Asymmetries in the behavior of rats with left vs right AGm lesions have been reported (J.M. Vargo et al., 1988, Exp. Neurol., 102, 199). This study investigated the possibility that MD, the primary thalamic input to AGm, may be lateralized. Rats with left MD lesions demonstrated more asymmetrical rotational behavior compared to right MD, bilateral MD, or control animals. Left MD animals also demonstrated greater contralateral spatial context recognition deficits in a unilateral version of the "out-of-place" paradigm. However, neither left nor right MD lesions produced hemi-inattention as is seen following unilateral AGm lesions.

594.3

SEROTONINERGIC, NORADRENERGIC AND DOPAMINERGIC INNERVATION OF THE PRIMATE MEDIODORSAL THALAMIC NUCLEUS M.L. Schwartz* and L. MrzljakSect. of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Since many of the modulatory transmitter systems originating from the brainstem nuclei have similar actions on the activity of thalamocortical relay cells (McCormick & Pape, 1990, J. Physiol., *431*), we examined the distributions of serotonin (5-HT), noradrenaline (NA) and dopamine (DA) immunoreactive fibers in the mediodorsal (MD) thalamic nucleus of the adult rhesus monkey. Our goal was to determine whether these systems have overlapping or distinct anatomical distributions. Immunoreactive axons of each of these fiber systems exhibited a different pattern of distribution. The densest innervation was found for 5-HT labeled axons. These were present at all levels of the MD, although their density was greatest in caudal portions of the nucleus. The most striking feature of the 5-HT fiber distribution was the irregular mosaic-like arrangement of areas containing higher and lower fiber densities. This mosaic-like organization was evident throughout the nucleus. In contrast to the innervation by 5-HT fibers, both the DA and NA innervations of the MD exhibited striking regional variations in their rostral-caudal and medial-lateral distributions. NA fibers were sparsely distributed throughout the nucleus, but were more dense in the medial magnocellular division. DA immunoreactive fibers were rare in rostral portions of the MD. In caudal portions of the nucleus DA fibers were concentrated and numerous in ventral and lateral regions of the parvicellular division. Comparisons of adjacent DA and 5-HT immunoreacted sections revealed that these two transmitter systems exhibited an interdigitating and complimentary pattern of innervation in the posterior MD. These data indicate that modulatory transmitter systems originating in the brainstem have distinctive regional patterns of innervation in the MD which may contribute to the functional diversity of the subdivisions of this nucleus and its cortical targets. Supported by NS 22807-06 and MH 44866.

594.4

GLUTAMATE IS THE THALAMOCORTICAL TRANSMITTER IN PROJECTIONS FROM THE MEDIAL DORSAL THALAMIC NUCLEUS TO THE PREFRONTAL CORTEX IN THE RAT: AN IN VIVO IONTOPHORETIC STUDY. J. Gigg*, A.M. Tan and D.M. Finch. Department of Neurology and Brain Research Institute, University of California, Los Angeles, CA 90024.

Anatomical and physiological studies have shown projections from the medial dorsal thalamus (MD) to anterior cingulate cortex (CG). We used an iontophoretic technique to identify the neurotransmitter used in this thalamic projection. Rats were anesthetized and stimulating and recording electrodes were placed stereotaxically. Extracellular responses from CG neurons were recorded after electrical stimulation of the MD thalamus and vicinity. Recordings were made with 5-30MΩ "piggy-back" electrodes (single recording pipettes attached to seven-barreled iontophoretic assemblies). Twenty-eight percent of all cells showed an excitatory response to MD stimulation. Sixty-five cells that showed no excitation were tested with the $GABA_A$ antagonist bicuculline methiodide (BMI). BMI produced a short latency excitatory response in 37 (57%) of these cells.

Application of the AMPA antagonists CNQX or DNQX selectively decreased the excitatory responses. These data indicate that: (a) the thalamic projection to prefrontal cortex is glutamatergic; and (b) this projection is regulated by GABA. Supported by NIH Grant NS 16721.

594.5

CHEMOARCHITECTONIC STUDY OF THE MEDIODORSAL THALAMIC NUCLEUS IN THE MACAQUE MONKEY. C. Cavada*, T. Compañy, A. Hernández-González, and F. Reinoso-Suárez. Dept. Morfología, Fac. Medicina, Univ. Autónoma de Madrid, 28029 Madrid, Spain.

Histochemical and immunohistochemical compartments were uncovered in the mediodorsal (MD) thalamic nucleus of macaques by analyzing the distributions of acetylcholinesterase (AChE) and cytochrome oxidase (CO) activities, and of serotonin (5-HT) and tyrosine hydroxylase (TH) immunoreactive (ir) fibers. Adjacent sections through the thalamus of adult *Macaca nemestrina* were stained to reveal Nissl substance and myelin, and processed for AChE and CO histochemistry, and for 5-HT and TH immunohistochemistry. Two AChE-poor, but CO-rich, sectors were observed along most of the rostro-caudal extent of MD: the medial third, and the ventral rim adjacent to the centromedian nucleus. Within and between these sectors lie patches of moderate AChE staining. The lateral two-thirds of MD show prominent AChE activity, which is unevenly distributed: poor- and very rich-AChE patches are intermingled with larger AChE-rich zones. CO staining in this region is less intense than in the medial third, and is distributed in dark and light zones. Many of the CO-rich zones appear to overlap with zones of AChE-moderate or rich staining. The rostral end of MD is predominantly AChE-poor, whereas the dorsal and caudal ends of the nucleus are predominantly AChE- and CO-rich. The 5-HT innervation of MD is among the densest within the thalamus. The distribution of 5-HT-ir fibers is heterogeneous, with denser aggregates usually coextensive with AChE-moderate or rich zones. TH-ir fibers in MD are relatively sparse, making it difficult to characterize heterogeneities in their distribution.

The present findings show that MD is a mosaic of biochemically diverse zones. This diversity is likely to have a bearing on the functional interactions of MD with the association areas of the frontal, parietal and temporal lobes.

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594.6

CALCIUM BINDING PROTEIN IMMUNOREACTIVITY IN GABA NEURONS OF CAT ANTERIOR INTRALAMINAR NUCLEI. M.Molinari*, M.E.Dell'Anna, E.Rausell, T.Hashikawa, M.G.Leggio, G.Macchi, E.G.Jones. Exp. Neurology Lab, Catholic University, Rome (Italy) and Neural System Lab, FRP, Riken, Wako (Japan).

Recent data have stressed the importance of certain calcium binding proteins (CBP) as markers for thalamo-cortical neurons. In particular Parvalbumin (PV) and 28 kd-calbindin (CB) have been shown to be expressed in projection neurons in the monkey thalamus, and to distinguish separate classes according to their input-output connections. In the monkey thalamus, relatively few GABA neurons, mainly those of the reticular nucleus, show PV immunoreactivity (IR) (Jones & Hendry, Europ. J. Neurosci., 1, 222-246, 1989). We have observed that PV is more widely distributed in the GABA neurons of cat thalamus, which suggests certain interspecies variability in terms of neuronal specificity of CBPs. Little is known about the distribution of the CBPs in neurons of the intralaminar nuclei (IL). The aim of the present study was the immunocytochemical characterization of GABA-IR neurons of the cat anterior IL nuclei according to the CB and PV-IR. Single-labeling immunoperoxidase and double-labeling immunofluorescence techniques have been used for the localization of the three antigens. GABA, PV and CB-IR was distributed in a patchy fashion within the IL nuclei. In superimposed adjacent sections processed for GABA, PV or CB, some degree of complementarity was observed, i.e., rich CB zones overlap with PV poor zones and viceversa. The distribution of GABA-IR is similar to that of PV-IR although the overlap was not complete. Double labeling experiments demonstrated that the majority of PV-IR neurons within IL (up to 90%) were also GABA-IR, while only a small number of GABA-IR cells (10 to 20%) did not show PV-IR. Less than 30% of the total CB-IR neurons showed GABA-IR, while CB-IR was present in about 70% of the GABA-IR neurons. The present findings demonstrated that the majority of IL GABA cells contain at least one of the two CBPs PV or CB and possibly that CB and PV might coexist in the same GABA cells. Thus, GABA cells of the cat IL form a heterogeneous population in terms of their expression of CBPs, and present significant differences in comparison with monkey thalamic GABA neurons. This diversity may be related to the functional role of the IL nuclei in the two species.

594.7

ELECTROPHYSIOLOGICAL PROPERTIES OF CAT ASSOCIATION NEOCORTICAL CELLS. A. Nuñez*, F. Amzica and M. Steriade. Lab. of Neurophysiology, Laval Univ. Sch. of Med., Quebec, Canada G1K 7P4.

Intrinsic properties and synaptic responses of association cortical neurons were studied in vivo, by means of intracellular recordings in areas 5 & 7 of cats under urethane anesthesia. Cells were identified by ortho- and antidromic activation from LP and CL thalamic nuclei and from contralateral cortex. A group of physiologically identified neurons were intracellularly stained with Lucifer Yellow and found to be pyramidal-shaped elements.

Two neuronal types, regular spiking and intrinsically bursting cells, were detected. Regular spiking cells were further differentiated according to the shape of their AHP and to their adaptation properties. High-threshold spike-bursts were rhythmically fired at 4-7 Hz.

A subpopulation of cortical cells displayed oscillations at 20-50 Hz. This rhythmic activity was voltage-dependent: it was triggered by depolarizing pulses, was blocked by hyperpolarization, and was not observed after intracellular injection with QX-314. The cells expressing 20-50 Hz oscillations were antidromically activated from the homotopic contralateral cortex and from the thalamus, thus suggesting that the fast oscillation can be synchronized in corticocortical and corticothalamic networks.

Thalamic as well as cortical stimuli evoked EPSPs with an earlier and a late component. The former increased with hyperpolarization, whereas the latter component decreased and eventually disappeared with membrane hyperpolarization. The late EPSP component was strikingly potentiated at frequencies above 3-4 Hz.

All these intrinsic and synaptic properties may assist cortical neurons in their rhythmic activities during sleepy and alert states.

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594.8

ANATOMIC ORGANIZATION OF THE HUMAN ORBITOFRONTAL CORTEX. P.R. Hof*[1], E.J. Mufson[2], N. Archin[1], A. Edwards[1], W.G. Janssen[1] and J.H. Morrison[1]. [1]Fishberg Res Ctr for Neurobiology, Mount Sinai Sch of Med, New York, NY 10029, [2]Tech 2000, Rush Presbyterian-St Luke's Med Ctr, Chicago, IL 60612.

The primate orbitofrontal cortex (OFC) is a component the paralimbic belt and consists of several distinct areas. Studies of the monkey OFC indicate that these areas are characterized by a complex array of intrinsic and extrinsic connections. To define the chemoarchitectonic organization of the human OFC, we have used antibodies to nonphosphorylated neurofilament protein (SMI32) and to the calcium-binding protein parvalbumin (PV). Immunohistochemistry revealed complementary labeling patterns corresponding to the cytoarchitecture defined by Nissl preparations. SMI32-immunoreactive (ir) pyramidal neurons were located only in layers V-VI in the dysgranular caudal OFC, whereas they were distributed in both layers III and V-VI in the lateral and polar granular regions. Interestingly, the density of PV-ir neurons and neuropil staining paralleled that of SMI32-ir cells in layer III. Neuropil labeling exhibited a high degree of regional specialization in that a dense plexus of PV-ir fibers was observed in layer I of the lateral OFC, while the medial dysgranular OFC displayed dark fiber patches in layer III. These patches were less densely labeled in areas with low SMI32-ir neuron counts in layer III. PV staining in the polar area of the OFC showed the typical features of granular cortex with labeled neurons predominating in layers III and IV. These region-specific staining patterns may represent a possible anatomical substrate for the distribution of select SMI32-ir corticocortical efferent, and PV-ir thalamocortical and intrinsic systems in the human OFC.

594.9

CHEMOARCHITECTURE OF THE MONKEY AND HUMAN CINGULATE CORTEX. B.A. Vogt*[1], E.A. Nimchinsky[2], P.R. Hof[2] and J.H. Morrison[2]. [1]Dept of Physiology and Pharmacology, Bowman-Gray Sch of Med, Winston-Salem, NC 27103, and [2]Fishberg Res Ctr for Neurobiology, Mount Sinai Sch of Med, New York, NY 10029.

Physiological studies have demonstrated that the cingulate cortex contains a variety of subareas involved in the integration of sensorimotor functions. In order to characterize the complex anatomic organization of these regions, we have studied the chemoarchitecture of the monkey and human cingulate cortex in its full rostrocaudal extent. Immunostaining with antibodies to nonphosphorylated neurofilament protein (SMI32) and to the calcium-binding protein parvalbumin (PV) revealed striking regional differences and clear delineation of subareas in both species. For instance, layer III of areas 24b and 24c was practically devoid of SMI32-immunoreactive (ir) pyramidal cells, whereas the posterior areas 23b and 23c displayed an increasing density of these cells in both layers III and V. PV staining showed a bilaminar neuropil pattern anteriorly coinciding with layers III and V, and a more homogeneous labeling posteriorly as layer IV emerges in area 23. Interestingly, an area possibly corresponding to a cingulate motor region (area 24c') appears to be located at the transition zone between typical anterior and posterior staining patterns and is characterized by the presence of numerous large SMI32-ir pyramidal cells in layers III and V. In addition, in the human, large PV-ir pericellular baskets were observed in layer V of area 24c'. Thus, SMI32 and PV exhibit regionally coordinate staining patterns that may represent anatomic equivalents of physiologically defined elements of the cingulate cortex.

594.10

SUBDIVISION OF MACAQUE ORBITAL AND MEDIAL PREFRONTAL CORTEX. S. T. Carmichael* and J. L. Price, Dept of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

To analyze the cortical structure of the orbital and medial prefrontal cortex (OMPFC), we studied immunohisotochemical staining with four antibodies in three macaque species. These can be correlated with histochemistry and axonal connections to provide multiple structural criteria for the recognition of cortical areas. These antibodies included: an antibody directed against a non-phosphorylated neurofilament epitope (SMI-32), an antibody directed against a membrane bound proteoglycan derived from motor cortex extracts [8b3 (Guimaraes et al., *Soc. Neurosci. Abst.* 16:240)] and two antisera directed against the calcium binding proteins parvalbumin and calbindin. Parvalbumin immunoreactivity shows areal variation in the density and laminar position of stained cell bodies and the presence of a horizontal plexus of neurites. Medial prefrontal areas have relatively few parv-ir cells, whereas the orbital areas 13l, 11m and the gustatory cortex have a dense parv-ir plexus. With calbindin immunostaining, areas differ in the relative density of a population of stained cells that forms a band in layers V and VI and in the extent of vertical neurite staining. SMI-32-ir pyramidal cell bodies and proximal dendrites are prominent in specific medial orbital areas (e.g. 13l and lad), but nearly absent in areas on the medial wall (24, 32) and the gyrus rectus (14c, 14r). 8b3 antibody staining within the OMPFC varies in the laminar distribution of reactive cells and the presence of a band of neuropil staining in the deep layers. In particular, area 12l is the only OMPFC area that has a significant population of densely immunoreactive cells in layer II.

In all, 22 areas have been distinguished within the OMPFC. The axonal connections of these areas, examined in a parallel study, indicate that these provide a complex substrate for the integration of limbic and sensory inputs. Supported by NIH grant DC00093 and training grant 5T32NS07057.

594.11

STRIPE-LIKE INTRINSIC CONNECTIONS MADE BY PYRAMIDAL NEURONS IN MONKEY PREFRONTAL CORTEX (AREAS 9-46). J.S. Lund*, J.B. Levitt, D.A. Lewis, and T. Yoshioka. Departments of Psychiatry and Neurobiology, Anatomy and Cell Science, University of Pittsburgh, Pittsburgh, PA 15261.

We have used biocytin injections in macaque prefrontal cortex (anterior to the arcuate sulcus between principal sulcus and mid-line) to examine pyramidal neuron intrinsic connectivity as a possible model for prefrontal cortical organization in the normal human. Iontophoretic (core size: 150-300μm) and pressure (300-400μm) injections made into the superficial layers give wide-ranging lateral projections within the same area of cortex. Projections local to a small iontophoretic injection form a narrow band of terminals in layers 1-3 (300-400μm wide, 2-4mm long) centered on the injection site. Collateral fibers spread orthogonally from this terminal band, making frequent bifurcations, to establish a series of parallel bands of terminals with uninervated bands between, spaced fairly regularly across the cortex (center to center 650-800μm). The whole pattern of terminal label is stripe-like, with occasional cross-links between the bands, and insertions of narrow interbands and can reach 7-8mm across the cortex. The projections arise from neurons in layers 2-3 and 5 and terminate in layers 1-3. The stripe-like pattern contrasts with patch-like patterns in other cortical regions (V1, V2, V4, motor, somatosensory) and is smaller in scale than stripe-like zones of corticocortical afferent terminals (300-750μm wide, 1.0-1.5mm apart; Goldman-Rakic and Schwartz, '82). However we find the intrinsic stripe width to match gaps in the distribution of callosal efferent neuron populations: it is therefore possible that the intrinsic system samples inputs from several sources of afferents but relays only to selected efferent destinations from any one point across the region. (Supported by NIMH grants MH00519 and MH45156, NEI EY05282, EY08098, & NRSA EY06275).

594.12

INTRINSIC CONNECTIONS OF PRIMATE PREFRONTAL AND PARIETAL CORTEX REVEALED BY MICROINJECTIONS OF CHOLERA TOXIN. M.F. Kritzer* and P.S. Goldman-Rakic. Section of Neurobiology, Yale. Univ. Sch. Med, New Haven, CT 06510.

Physiological studies in primates show that neurons in prefrontal and parietal cortices selectively respond in complex ways to visuospatial stimuli and, as a population, represent all portions of the visual field. As in sensory areas, intracortical connections are likely to participate in scultping these responses. In order to gain insight into the layer-by-layer organization of intrinsic connections in these areas, we plotted neurons retrogradely labeled by microinjections (5-15nl) of gold-conjugated cholera toxin (B-subunit) in specific layers of prefrontal and parietal cortex in rhesus monkeys.

The intrinsic circuits were similar in prefrontal and parietal cortices and the laminar location of injections predicted the pattern of labeling in each area. Supragranular injections produced widespread labeling in layers II/III, with labeled neurons extending as far as 3mm laterally and often grouped in clusters some 0.5-0.7mm wide; labeled cells in layers IV-VI mainly occupied narrow columns (~1mm across) directly beneath these same injection sites. Injections in infragranular layers produced nearly inverse patterns; cells in layers II/III formed one or two clusters above injection sites, while more uniform labeling in layers V/VI extended up to 3mm laterally. A striking feature of labeling in deep layers was marked asymmetry, e.g., for some injections, labeled cells extended millimeters in some directions and only a few hundred microns in others. Finally, injections centered in layer IV yielded single columns of labeling (0.5-1mm across) passing through all layers. This layer-by-layer diversity in intracortical connections in prefrontal and parietal cortices may support the varied computational processing carried out in these areas. Supported by MH44866-04.

594.13

HEMISPHERIC ASYMMETRY OF DARPP-32 IN RAT CINGULATE CORTEX. C.S. Toomim*[1], P. Greengard[2] & P.S. Goldman-Rakic[1] [1]Section of Neurobiology, Yale Sch. Med., New Haven, CT 06510 & [2]Lab. Molec. Cell. Neurosci., Rockefeller Univ. NY, NY.

Immunohistochemistry of rat rostral forebrain for 32 kdalton-dopamine regulated phosphoprotein (DARPP-32) at sub-maximal staining levels revealed consistent lateral asymmetries in several nuclei and functionally identified cortical areas, with each animal having its own pattern of staining. The most easily discriminable areas were medial frontal (Fr) cortex and striatum. Because of dopamine D1 receptor involvement in memory tasks (Sawaguchi & Goldman-Rakic Sci '91) and the importance of medial frontal cortex in memory tasks (Kolb & Tees Cerebral Cortex of the Rat '90), we analyzed cortical areas cingulate (Cg) 1, 2 & 3 and Fr 2. In Cg1 & 2, DARPP is lateralized in 14 out of 22 rats studied. The laterality is consistent throughout Cg1 & 2, but may show abrupt changes at the Cg 2 - 3 junction, the cingulate - retrosplenial junction, and at the Cg 1 - Fr 2 interface. The pattern is also independent of laterality in the striatum. Staining of adjacent sections for Calbindin and cAMP regulated phosphoprotein (ARPP) shows that any asymmetry of these proteins is not correlated with that of DARPP-32. The lack of correlation between the antigens indicates that the pattern is not due to perfusion artifact. The constancy of the pattern in assays done on different days rules out the possibility of staining artifact as a cause of the laterality. We are currently investigating the relationship between staining asymmetry and behavioral asymmetries.

594.14

SPATIAL DEFICITS AND HEMISPHERIC ASYMMETRIES IN THE RAT FOLLOWING UNILATERAL AND BILATERAL LESIONS OF POSTERIOR PARIETAL OR MEDIAL AGRANULAR CORTEX. V. King and J.V. Corwin*. Depts. of Psychology, Univ. of Wisconsin, Madison, WI 53706, and Northern Illinois Univ., DeKalb, IL 60115.

Previous research (Kesner et al., 1989) has indicated that the posterior parietal (PPC) and medial agranular (AGm) cortices play a prominent role in spatial processing in rodents. Bilateral (bi) PPC lesions produce allocentric spatial deficits and biAGm lesions produce egocentric spatial deficits.

To further examine the roles of AGm and PPC in spatial processing in rodents the current study examined the effects of unilateral (uni) PPC or AGm lesions to determine if space is represented in rodents in a hemispatial fashion as is the case in humans. Eight groups of rats received either uni or bi PPC, AGm, or cortical control lesions. The rats were tested in either an adjacent arm (egocentric) task, or a cheeseboard (allocentric) maze

As found in previous studies, biPPC operates were impaired in learning the allocentric task but not the egocentric task, while the AGm operates demonstrated the opposite pattern. The uniPPC operates demonstrated lateralized deficits in the allocentric task. Right hemisphere PPC operates were significantly impaired relative to left hemisphere operates. UniAGm operates demonstrated a deficit in the egocentric task intermediate to the controls and the biAGm operates. Analysis of the pattern of errors in the uni lesion groups indicated no evidence of hemispatial deficits in either the egocentric or allocentric tasks. Thus, unlike humans with PPC lesions rats do not demonstrate hemispatial deficits. The present results do indicate that, as in humans, there is a hemispheric asymmetry for allocentric spatial processing.

594.15

ROSTRAL-CAUDAL TOPOGRAPHY OF SUBCORTICAL EFFERENT PROJECTIONS OF THE MEDIAL AGRANULAR CORTEX IN THE RAT. J.V. Corwin , R.L. Reep*, V.King, and J. Leslie. Depts. of Psychology, Northern Illinois University, DeKalb, IL 60115, and Univ. of Wisconsin, Madison, WI 53706; and Dept. of Physiol. Sciences, Univ. of Florida, Gainesville, FL 32610.

Our previous research has indicated that unilateral destruction of the rostral or caudal medial agranular cortex (AGm) produces dissociable behavioral components of the neglect syndrome. Given the behavioral differences resulting from rostral vs. caudal lesions it is of some interest to examine differences in the anatomical connectivity of these components of the AGm. In the present study we have focused on the rostral-caudal (r-c) topography of the subcortical projections of AGm.

Autoradiography, Fluoro-Ruby, and WGA-HRP were used to make spaced microinjections throughout the r-c extent of AGm. A distinct r-c topography was noted for projections to caudate-putamen (c-p) and thalamus. Rostral injections produced terminal fields in the dorsal central core of the c-p rostral to the anterior commissure, injections in middle AGm at the level of the septum labeled the dorsolateral rim of c-p from the level of the genu to the fornix. Caudal AGm injections produced sparse terminal fields in the dorsal c-p caudal to the genu. In thalamus, rostral AGm projects to the centralis lateralis (CL), ventral lateral (VL), gelatinosis, and ventromedial (VM) nuclei; middle AGm to the dorsal CL, and posterior (PO) nuclei; and caudal AGm to PO and the ventral laterdorsal nucleus. Thus, the trend through r-c AGm is to demonstrate a rostro-ventral to caudo-dorsal pattern of thalamic labeling. The behavioral significance of these r-c differences in the efferents of AGm remain to be determined.

594.16

AREAL SUBDIVISIONS AND THALAMIC CONNECTIONS OF THE CAT'S INSULAR CORTEX. F. Clascá, A. Llamas* and F. Reinoso-Suárez. Dept. of Morphology, Autonomous Univ. of Madrid, Sch. of Med., 28029 Madrid, SPAIN.

The thalamic connections of the cortex surrounding the sylvian, ectosylvian, orbital and anterior rhinal sulci of the cat's brain were mapped by making small unilateral injections of WGA-HRP and serial reconstructions of the labeling. These results are part of a study aimed to elucidate the cortical territory of the cat's brain with cytoarchitectonic and connectional characteristics comparable to those reported on the insular areas of the old world monkey.

The main anatomofunctional subdivisions of the insula (Granular -IG-, Dysgranular -IDg-, and Agranular -IA- areas) were recognized in the cat. These newly defined insular areas are situated in the orbital gyrus and the orbital and anterior rhinal sulci. IG thalamic connections are stablished with somatosensory regions, such as the anterior suprageniculate and ventral posteroinferior nuclei. IDg is linked to the visceral, gustatory and premotor thalamus (ventral periphery of the ventral posteromedial nucleus, and the ventral lateral nucleus). IA is connected with the mediodorsal and parafascicular thalamic nuclei.

The connections of the classical cat's "insular" cortex (anterior sylvian gyrus and sylvian sulcus) are not comparable with those of the primate insula. Therefore we propose to name these zones anterior sylvian (SA) and parainsular (Pal) areas. SA is related with the caudal suprageniculate and ventromedial nuclei, and the most medial portion of the lateral posterior-pulvinar complex. Pal is mainly connected with the dorsal division of the medial geniculate complex. The connections of these two areas are reminiscent of the visual-auditory fields of the caudal superior temporal sulcus, and of the the parainsular cortex (Brodmann's area 52) of old world monkeys.

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594.17

Functional anatomy of matching and discrimination of shape in man. R.Kawashima, P.E.Roland* and B.T.O'Sullivan Lab. for Brain Research and PET, Karolinska Institute, S10401,Stockholm, Sweden

The purpose of this study was to compare the activation patterns in the human brain of 1) discrimination versus matching as psychophysical procedures and 2) intra-modal (i.e. tactile) matching versus cross-modal (i.e. tactile-visual) matching. The regional cerebral blood flow was measured with ^{15}O-butanol and positron emission tomography in 9 normal volunteers during 1) a control state, 2) a tactile discrimination task, 3) a tactile matching task and 4) a tactile visual matching task. The right hand was used for all tasks. The shape stimuli were spherical ellipses (Roland &Mortensen, Brain Res. Rev. 12: 1-42,1987). The contralateral postcentral gyrus and supplementary motor area, ipsilateral anterior lobe of cerebellum and anterior insula were activated in all four tasks. Discrimination exclusively activated the prefrontal cortex and contralateral head of the caudate nucleus. The tactile-visual matching activated visual association areas: the posterior inferior. parietal lobule bilaterally, the ipsilateral inferior temporal cortex and the ipsilateral pulvinar. The tactile-tactile matching task activated the ipsilateral posterior inferior temporal cortex and the contralateral precuneus. The results indicated that the comparison of two stimuli, in contrast to matching, require the participation of the prefrontal cortex. That the remote visual association areas are of importance for the conversion of tactile shape into visual representations and the representation of visual shape.

594.18

VISUAL EVOKED MAGNETIC FIELD RESPONSES IN PARIETAL CORTEX IN MAN

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It is well Known that Parietal Cortex(PC) disorder induces a constructive apraxia and other visuo-spatial symptoms in man.Otherhand,in monky,neural responses to visual and other stimuli were recorded in the same neuron of PC. The function of PC seems to be related to integrating and processing visual information.But little is reported about the direct recording PC responses to visual stimuli in man. In the present work,we recorded magnetic field responses to visual stimulation in order to find out where the sources of the responses were in PC.

Four healthy adults were studied in the magnetically shieled room.Stimulation was provided randomly(1-2 sec) using 3 LED(light duration 0.5 or 1 sec fixed) arranged in center or 20°right or left to the subject,50 cm away from the eye.The magnetic field over PC was recorded with a 37-channel SQUID magnetometer system(BTi).128 responses were averaged and from the distribution of field responses a current dipole source was estimated.The spacial locations of the source were mapped on the MRI of each subject brain.

Magnetic field responses were found at latency 100-140, 190-220 and 260-290 msec.The dipole sources of their responses plotted on MRI suggested that they were located in intraparietal sulcus.

594.19

ENDOGENOUS POTENTIALS IN THE HUMAN ORBITOFRONTAL CORTEX EVOKED BY RARE AND REPEATED STIMULI. [1,3,4]E. Halgren*, [2]P. Baudena, [1]J. Clarke, [2,5]G. Heit. [1]INSERM CJF90-12, Neurology, CHRU Pontchaillou, 35033 Rennes, France; [2]INSERM U97, Paris; [3]Wadsworth VAMC; [4]Psychiatry and Brain Res., UCLA; [5]Neurosurgery, Stanford.

Evoked potentials were recorded from 15 patients with electrodes chronically implanted into the orbitofrontal cortex in order to localize their epileptogenic zones prior to surgical treatment. Electrodes were positioned perpendicular to the interhemispheric plane, from the inferior frontal g. to the g. rectus. P3-like potentials up to 200μV in amplitude were observed during sensory discrimination and recent memory tasks, with two patterns of response. The first was a triphasic negative-positive-negative waveform evoked at about 200-285-350ms latencies by rare target and distractor visual and especially auditory stimuli, but usually not by repeated words or faces. These peaks resembled the scalp N2/P3a/SW, but occurred up to 100ms earlier than the peaks at the scalp (Pz), and often with equal latency to distractor versus target stimuli. This waveform was widespread in the orbital cortex, with steep voltage gradients and occasional inversions in the most medial lead (g. rectus). The second pattern was a broad positivity with peak latency of about 350ms, approximately equal to that of the scalp P3b. This potential was generally larger and more focal than the triphasic waveform, was not recorded by more dorsal electrodes, and polarity-inverted in the most lateral cortex in one case. It was evoked by rare tones and symbols, and especially by repeated words and faces. In summary, the human orbitofrontal cortex appears to generate cognitive evoked potentials corresponding to the scalp N2/P3a/SW, and P3b. Whether orbital generators propagate significantly to the scalp requires additional study.

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594.20

ORIGIN AND CHARACTERISTICS OF COHERENT THALAMO-CORTICAL 40-HZ OSCILLATIONS IN THE HUMAN BRAIN.

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A 14- and a 37-channel MEG system (BTi) were used, in order to determine the origin and the spatio-temporal dynamic properties of 40-Hz oscillatory activities in the human brain.

MEG data was recorded over an entire hemisphere (from 35-37 positions) before and during auditory processing. Magnetic 40-Hz responses were obtained from adult healthy subjects and from Alzheimer's patients. Magnetic Field Tomography (MFT) localized the underlying distributed activities within the brain (Ribary et al., Proc. Natl. Acad. Sci. 88, 11037-11041, 1991). This technique comprises a mathematically weighted source space which allows the spatial resolution of simultaneous sources localized at different brain depths. The MFT solutions were superimposed onto three-dimensional reconstructed brain images (MRI). Well-defined 40-Hz coherence was found between cortical-subcortical sites with a time shift that is consistent with thalamo-cortical conduction times. Similar data were also found in single recording epochs before and during auditory processing, indicating that the oscillatory avtivity in the 40-Hz range is continuously generated by the central nervous system, and is resetted by the auditory input. In addition, these single epoch recordings confirm the existance of a sweep-like rostro-caudal progression of activity at thalamic and at cortical level. These activities were altered in Alzheimer's patients, where a similar activity pattern was present, but the cortical component was reduced.

Our study indicates the possibility to analyze, non-invasively, the functional properties underlying higher frequency oscillations in the human brain and to evaluate its changes during pathological states.

595.1

EFFECTS OF PERIRHINAL CORTICAL LESIONS ON EXPLORATORY BEHAVIOUR AND SPATIAL MEMORY IN THE RAT. K.A. Wiig and D.K. Bilkey*. Department of Psychology, University of Otago, Dunedin, New Zealand.

The perirhinal cortex, situated between neocortical areas dorsal to the rhinal sulcus and entorhinal areas ventral to the rhinal sulcus, is considered to be a nodal point in the exchange of information between cortical and limbic regions. Behavioural results from primate studies suggest that the perirhinal cortex may play an important role in memory, as lesions of this area result in a severe memory impairment (Zola-Morgan et al *J. Neurosci*, Vol 9(12) 1989). To date, there have been no studies investigating the function of the perirhinal cortex in the rat. In order to determine whether damage to this region produces a memory related deficit, bilaterally lesioned and control rats were tested in two paradigms. The initial procedure investigated the rats' response to novelty and environmental changes. In this experiment, perirhinal lesioned rats spent significantly more time exploring both neutral and novel objects than control animals. In addition, lesioned animals showed an increased rate of rearing relative to control rats. Lesioned rats spent significantly less time grooming than control animals, whereas locomotor activity remained unchanged. Rats were also trained in the Morris Water Maze in order to test for deficits in spatial memory. It was found that rats with bilateral damage to the perirhinal cortex were not impaired at this task. In fact, latencies to find the platform were significantly lower than those of control animals. These results suggest that the perirhinal cortex, unlike other temporal lobe or limbic system structures, is not critically involved in spatial memory. Rather, it appears that this region may play some role in habituation or attentional processes.

595.2

MEDIAL SEPTAL LESIONS IMPAIR SPATIAL REVERSAL LEARNING. J.J. Boitano*, T. Small, M.M. Fiorini, S. Belanger, T. Savinelli and C.P.J. Dokla. Depts. of Psychology, Fairfield Univ., Fairfield, CT 06430 & Saint Anselm College, Manchester, NH 03102.

Reversal learning in F-344 rats that had sustained electrolytic lesions of the medial septal area (MS) was examined using a water task version of a T-maze. Rats had to locate a stable, hidden platform and avoid a collapsable, hidden platform positioned in either the NW or NE quadrants of a 1.5 m pool from a fixed starting position (S). Side preference was determined over 5 adaptation days using two stable, hidden platforms. Acquisition testing followed with 10 trials/day to the preferred side using a criterion of 9 out of 10 correct trials. Finally, 10 reversal problems were conducted using the stable and collapsable platforms. The MS rats were not significantly different from the sham-operated controls (CONT) during acquisition on a days-to-criterion measure. However, MS rats were highly impaired on the reversal problems compared to the CONT rats (5.4 vs. 2.7 days; $p < 0.002$). These results confirm and extend earlier findings on the importance of the MS-cholinergic pathway in spatial reversal learning.

595.3

SPATIAL MEMORY DEFICITS FROM LESIONS OF THE CHOLINERGIC BASAL FOREBRAIN USING IBOTENATE, QUISQUALATE, AND AMPA. J.J. Waite* and L.J. Thal. Dept. of Neuroscience & Neurology, UCSD & VAMC, San Diego, CA 92161.

Rats were lesioned in both the medial septum and bilateral nucleus basalis with 3 excitotoxins which interact with different subsets of glutamate receptors: ibotenic acid (Ibo), quisqualic acid (Quis) and AMPA. Animals were trained in a Morris water maze to escape to a submerged platform. All 3 groups of lesioned animals were deficient in their ability to acquire the spatial memory task in comparison to sham-operated controls. Three weeks later, Ibo and AMPA groups were tested on their ability to learn a new platform location. Neither of the 2 lesioned groups were able to incorporate knowledge of the new platform location as well as the control group, based on the overall latency to escape to the platform and quadrant analysis of individual search strategies. The activity of choline acetyltransferase (expressed as percent of control) was assayed from rats sacrificed 2 months after completion of surgeries:

	Frontal Cortex	Posterior Cortex	Hippocampus	Globus Pallidus
Ibo	62.1%	78.8%	56.0%	98.6%
Quis	52.5%	43.3%	57.9%	99.5%
AMPA	33.5%	16.3%	43.5%	89.1%

Each lesioned group exhibited spatial learning and memory deficits in comparison to controls in the water maze. The behavioral deficits measured did not correlate with the degree of depletion of ChAT activity assessed in regions of cholinergic terminal fields.

595.4

THE EFFECTS OF ENTORHINAL LESIONS ON SPATIAL MEMORY IN TWO VERSIONS OF THE WATER MAZE TASK. A.H. Nagahara*, T.A. Otto, and M. Gallagher. Department of Psychology, University of North Carolina, Chapel Hill, N.C., 27599.

Entorhinal cortex provides a major link between the hippocampus and neocortical systems. The present experiment examined the role of entorhinal cortex in spatial memory, a function that depends on the integrity of the hippocampus.

Long-Evans rats underwent either entorhinal lesion by aspiration or sham operation. Three weeks after surgery, rats were trained to locate a submerged platform in the Morris water maze (6 trials/day for 3 days; ITI 30s) followed by a free swim probe trial. Three weeks later animals received the same protocol for reversal training with the platform placed in the opposite quadrant. Animals were then trained on a single-trial place learning task involving one acquisition trial and a retention trial in a daily session (with the platform randomly relocated each day). After initial training, rats were repeatedly tested at delays of 30 s or 5 min.

Histological analysis showed extensive damage to entorhinal cortex with limited damage to surrounding regions; Timm stain confirmed extensive loss of the entorhinal projection to the outer molecular layer of the dentate gyrus. Entorhinal lesioned animals showed only modest impairment on the multiple-trial place learning task. However, on the single-trial place learning task, there was a pronounced delay-dependent impairment in the lesioned rats. These results suggest that memory demand (delay interval) substantially increases the sensitivity of a spatial task to the effects of entorhinal damage. Supported by NIA grant PO1 AG09973 and a NIMH RSDA to MG (K02-MH00406) and a NIMH fellowship to AHN (F32-MH10246)

595.5

Spatial Memory Impairment following Adrenalectomy-Induced Dentate Gyrus Volume Reduction in Rats. C.D. Condon* and E.J. Roy. Neuroscience Program and Dept. of Psychology, Univ. of Illinois, Champaign, IL 61820.

Long-term adrenalectomy (ADX) has previously been shown to cause selective cell loss in the hippocampal dentate gyrus of the rat. Using stereological estimates, we quantified the volume of the dentate gyrus and investigated whether damage to the dentate gyrus disrupts learning in two spatial memory tasks (Morris Water maze and 8-arm radial maze).

Rats were trained on the Morris Water Maze and radial maze (4 arms baited) 12 and 21 weeks after ADX respectively. Rats were sacrificed 7.5 months after ADX. Water maze performance was significantly impaired in ADX rats (n=8) relative to SHAM and partial ADX (n=8) rats. However, all rats were able to learn the maze. Radial maze learning (both working and reference memory) and reversal did not differ between groups. Dentate gyrus volume was greatly reduced in ADX rats (10 - 80%, mean 41%). Pyramidal cell regions CA1, CA2, CA3 and CA4 were unchanged. To account for the near-normal performance of ADX rats, we hypothesize that under conditions of long-term ADX (1) the dentate connectivity is redundant (2) the dentate is capable of sprouting and reorganization or (3) another brain region is functioning in place of the dentate gyrus.

595.6

THE EFFECTS OF LESIONS OF THE ENTORHINAL CORTEX AND THE HORIZONTAL NUCLEUS OF THE DIAGONAL BAND OF BROCA ON PERFORMANCE OF A SPATIAL LOCATION ORDER RECOGNITION TASK. D.L. Johnson*, A.A. Chiba and R.P. Kesner. Dept. of Psychology, Univ. of Utah, Salt Lake City, Utah 84112.

Rats were trained on an 8-arm radial maze to perform an order recognition memory task for spatial location information. Each day, rats were given a study phase followed by a test phase. In the study phase, rats were allowed to visit all 8 arms in a predetermined sequence that varied on a daily basis. During the test phase, the rats had to choose which of two arms was visited earlier in the study sequence of 8 arms, in order to receive additional food reinforcement. Temporal distance refers to the number of spatial locations that are visited between test items. In this study, performance for temporal distances of 0,2,4, and 6 served as the dependent variable.

After extensive training, percent correct performance increases as a function of temporal distance with excellent performance for distances of 2, 4, and 6 but chance performance for distance of 0. After rats displayed better than chance performance on tests of the 2,4, and 6 distances, they received electrolytic lesions of the entorhinal cortex (ENTO), ibotenic or quisqualic acid lesions of the horizontal nucleus of the diagonal band of Broca (HNDB), or saline injections into the HNDB or sham operations (CONTROL).

CONTROL rats continued to perform well for the longer temporal distances. However, ENTO and HNDB lesioned groups showed a performance deficit for all temporal distances. The quisqualic acid HNDB lesion group displayed a greater performance deficit than the ibotenic acid HNDB lesion group. These findings suggest that the ENTO and the HNDB play an important role in memory for the sequential ordering of spatial information.

595.7

EFFECTS OF PARIETAL CORTEX AND HIPPOCAMPUS IN MEMORY FOR DISTANCE INFORMATION. J.M. Long*, R.P. Kesner, Dept. of Psychology, Univ. of Utah, Salt Lake City, UT 84112.

It has been postulated that rats form an internal representation of the environment as a spatial cognitive map. Of the many cues (e.g.distance, direction, spatial location) available to an animal in spatial tasks, it is not known which cues are represented in memory. In order to test for the contribution of the hippocampus and parietal cortex to memory for allocentric spatial cues, Long-Evans rats were trained on a go/nogo task which required the animal to remember the distance between two identical objects. After acquisition, rats were given either hippocampal or parietal cortex lesions, which resulted in impaired performance. In a different experiment rats were trained to discriminate the distance between two identical objects. Distance discrimination performance was impaired in rats with parietal cortex, but not hippocampal lesions. It appears that rats can represent allocentric distance information within their spatial cognitive maps. Furthermore, the hippocampus might be involved in temporary representation of allocentric distance information, whereas the parietal cortex might be the site of a more permanent representation of allocentric distance information.

595.8

THE EFFECTS OF LESIONS OF THE DORSAL HIPPOCAMPUS OR THE VENTRAL HIPPOCAMPUS ON PERFORMANCE OF A SPATIAL LOCATION ORDER RECOGNITION TASK. A.A. Chiba*, D.L. Johnson, and R.P. Kesner, Department of Psychology, University of Utah, Salt Lake City, Utah 84112.

Each rat was trained on an eight-arm radial maze task examining memory for the temporal order of spatial location as a function of temporal distance. During the study phase of each trial, rats were allowed to visit each of eight arms once in an order that was randomly selected for that trial. During the test phase of each trial, rats were required to choose which of two arms occurred earlier in the sequence of arms visited during the study phase. The arms presented in the test phase varied according to temporal distance (0,2,4,6), or the number of arms in the running sequence of the study phase that occurred between the two test arms. Once the rats reached a criterion of 75% correct performance for the temporal distances of 2, 4, and 6 (rats continued to display chance performance for the temporal distance of 0), electrolytic brain lesions of the dorsal hippocampus, the ventral hippocampus, or the cortex immediately above the dorsal hippocampus (control lesions) were made.

Following surgery, control lesioned rats continued to perform at chance for the temporal distance of 0, but performed at or above 75% correct for the temporal distances of 2, 4, and 6. The performance of rats with ventral hippocampal lesions did not significantly differ from that of control lesioned rats. The performance of rats with dorsal hippocampal lesions was at chance for both the temporal distances of 0 and 2, but did not significantly differ from that of control animals for the temporal distances of 4 and 6. These data suggest that memory for the temporal order of spatial location across all temporal distances is not dependent on the integrity of the ventral hippocampus, whereas memory for shorter temporal distances is dependent on the integrity of the dorsal hippocampus. However, previously reported data (Chiba & Kesner, 1989) revealed a performance deficit across all temporal distances (0,2,4, and 6) in rats with total hippocampal (dorsal and ventral) lesions. Thus, it appears that the effect of total hippocampal lesions in rats on memory for the temporal order of spatial location is not equivalent to the combined effects of independent dorsal and ventral hippocampal lesions in rats.

595.9

PHTHALIC ACID LESIONS OF THE RAT NUCLEUS BASALIS MAGNOCELLULARIS (NBM) IMPAIR MEMORY IN A DOUBLE Y-MAZE. **P.E. Mallet*, R.J. Beninger, K. Jhamandas and R.J. Boegman. Depts. of Psychol. and Pharmacol. Toxicol., Queen's Univ., Kingston, Canada, K7L 3N6.**

The differential effects of intra-NBM injections of excitotoxins on amygdalar choline acetyltransferase (ChAT) rather than cortical ChAT, may explain the differential mnemonic effects observed. The present study evaluated the mnemonic effects of intra-NBM infusions of phthalic acid (1,2-Benzenedicarboxylic acid), an excitotoxin that strongly decreases amygdalar ChAT activity. Sprague-Dawley rats were trained to traverse a double Y-maze for food reinforcement until performance exceeded 88% correct. Successful performance depended on accuracy in both a working and a reference memory component. Rats were then given either unilateral phthalic acid (300 nM, 0.5 µl) or sham (0.9% saline, 0.5 µl) lesions of the NBM, and were again tested on the maze. Biochemical analysis revealed that phthalic acid lesions produced a large decrease in amygdalar ChAT, but had little effect on cortical ChAT. Behavioral results showed an increase in working but not reference memory errors in the phthalic acid lesioned rats, and no effect in the sham operated controls. These results suggest that the cholinergic projections from the NBM to the basolateral amygdala play an important role in mnemonic functioning.

595.10

Amygdala Lesions Impair and Fornix Lesions Enhance Acquisition of Information about Spatial Locations: Dependence on Method of Reinforcer Presentation. R.J. McDonald* and N.M. White. Department of Psychology, McGill University, Montreal, Québec, Canada.

We have previously shown (McDonald and White, Neuroscience Abs. 17, 54.1) that lesions of the amygdala, but not of the hippocampus or dorsal striatum, impair acquisition/expression of a conditioned *cue* preference using 2 arms of an 8-arm radial maze in which the arms were distinguished from each other by the presence or absence of a light at the entrance. In the present experiment we tested the effects of these lesions on a conditioned *place* preference using 2 randomly chosen arm locations (excluding adjacent locations) for each subject on a radial maze. The maze was physically rotated before each daily trial so the arms were distinguished solely by their spatial locations. Each pairing consisted of a session in which a rat was confined to one arm that contained food and a session in which the rat was confined to the other arm with no food, in a counterbalanced manner. On the test day, each rat was given free access to arms in both locations for 20 min and the amount of time spent on each arm was recorded. Control rats showed a preference for the location associated with food after 3 pairings, but not after 1 or 2 pairings. Rats with bilateral damage to the lateral amygdala showed no significant preference for either location. Rats with damage to the fornix showed significant preferences for the food-associated location after 1, 2 or 3 pairings. These results suggest: 1) the method of reinforcer presentation may be a factor determining which neural substrate mediates acquisition of a memory; 2) when rewards are presented passively, acquisition of information about their relationship to neutral stimuli may be mediated by a memory system that includes the amygdala, 3) when rewards are presented passively, hippocampal processing may interfere with acquisition even when the task involves learning about spatial locations.

595.11

CUE FAMILIARITY REDUCES SPATIAL DISORIENTATION FOLLOWING HIPPOCAMPAL DAMAGE. J.E. Holden* and B. Therrien. The University of Michigan, Ann Arbor, MI 48109

When the hippocampus (HPC) is damaged, place navigation is disrupted and spatial disorientation results. A single cue marking a hidden goal has decreased disorientation in rats. However, they were impaired when compared to controls. We hypothesized that cue familiarity would enhance the ability of rats with HPC damage to locate a cued hidden platform in the Morris water test. After preoperative training with the cue (n = 21) or handling only (n = 17), rats were given electrolytic bilateral HPC (BHPC) lesions or sham surgery for controls. All rats were then tested for four days, six trials per day, with the cue marking the platform location. One way ANOVA showed that rats with BHPC lesions familiar with the cue (FB) were significantly more efficient than rats unfamiliar with the cue (UB) in swim time (X = 10.31 ± 2.20 vs 46.72 ± 7.51 seconds, $p<.05$) and in directional heading error (X = 31.98 ± 3.40 vs 57.92 ± 4.02 degrees, $p<.05$) on Day 1. These differences occurred across the four test days. No significant differences were found between FB animals and controls familiar with the cue (FC). A second experiment tested the effect of cue familiarity when a competing cue was present. Cue familiar animals were tested with a distractor in the testing environment postoperatively. No significant differences were found between FB (n = 7) and FC (n = 4) animals. We conclude that: 1) cue familiarity improves performance on a spatial memory task following HPC damage; and 2) the effect of cue familiarity remains in the presence of an environmental distractor.

595.12

A ROLE FOR THE LATERAL STRIATUM IN THE MEDIATION OF VISUOSPATIAL COGNITION. JCS Furtado* and MF Mazurek McMaster University Medical Centre, Hamilton, Ontario, CANADA

Recent studies have emphasized the importance of parallel pathways in the cortico-striato-pallido-thalamic circuit. In this model, the medial striatum is understood as being involved in "cognitive" processing while the lateral striatum is regarded as selectively mediating "motor" function. We have studied the "cognitive" and "motor" abilities of rats with bilateral quinolinate-induced lesions of the medial (MED) or lateral (LAT) striatum. Compared with saline-injected controls, the experimental groups were significantly impaired in the place task version of the Morris Water Maze. No group differences were found when the platform was visible, suggesting that the observed impairment in the water maze reflects an impairment, on the part of the MED and LAT groups, to use visuospatial cues. By contrast, when the same animals performed two tests of motor behaviour (tongue extension and food manipulation) only the LAT group was impaired. This study confirms the selective role of the lateral striatum in the control of specialized motor behaviours, but suggests that both the medial and lateral striatum are involved in the mediation of visuospatial cognition.

595.13

HEMIDECORTICATION, PRIOR TRAINING AND LEFT-RIGHT RESPONSE LEARNING IN THE RAT: EVIDENCE FOR A LEFT-HEMISPHERE SPECIALIZATION FOR THE LEFT-RIGHT SENSE. M. Noonan* and S. Axelrod, Canisius College and SUNY/Buffalo, Buffalo, New York 14208.

We hypothesized that unilateral cortex removal has two opposite effects pertinent to learning left-right response tasks: (1) provision of a pronounced neural asymmetry which facilitates acquisition, but also (2) induction of a unilateral response bias so strong that the facilitatory effect is masked. We trained hemidecorticated and sham-operated rats on two water-escape T-maze tasks, predicting that, in the hemidecorticates, mastery of the first task should break the induced response bias, thereby allowing the facilitation to appear on the second. The prediction was confirmed, but only for the right operates, who were indeed worse than the shams at learning either task when it was first, but better than the shams at either task when it was second. By contrast, left operates were worse than the shams at both tasks, irrespective of testing order. (Morris-test performance was unaffected by either left or right hemidecortication.) The results confirm the hypothesis that induced neural asymmetry can facilitate left-right learning, and appear to reveal a left-hemisphere specialization in rats, parallel to that in humans, for the ability to tell left from right.

595.14

DISSOCIATING THE EFFECTS OF HIPPOCAMPAL AND AMYGDALAR LESIONS IN RATS WITH A BATTERY OF NONSPATIAL MEMORY TASKS. D.G. Mumby*, J.P.J. Pinel, & T.J. Kornecook. Dept. of Psychology, University of British Columbia, Vancouver, B.C. Canada V6T 1Z4.

We tested normal rats on a battery of nonspatial memory tests that resemble some of those used in studies of brain-damage-produced amnesia in monkeys: (1) Object discrimination and reversal, (2) concurrent object discriminations, (3) delayed nonmatching-to-sample (DNMS) with various retention delays (4, 15, 60, and 120-s) and list lengths (1, 3, 5, and 7 items), and (4) temporal-order recognition. Each task made use of the same apparatus and, like the monkey versions of these tasks, each one used objects as test stimuli. The performance of intact rats on these tasks was compared to that of rats that received either bilateral aspiration lesions of the hippocampal formation and posterior parietal cortex (HF) or posterior parietal cortex alone (PPC), or bilateral electrolytic lesions of the amygdala (AM). All testing was conducted postsurgery.

The results revealed several dissociations. Rats with HF or PPC lesions were impaired in learning the object discrimination; rats with HF lesions were impaired on the reversal. Rats with AM or HF lesions were impaired in learning the concurrent object discriminations, and those with HF lesions were significantly worse than those with AM lesions. Rats with AM lesions were impaired in learning DNMS, but after all of the rats had mastered this task at a 4-s retention delay, those with AM lesions performed as well as the intact rats at longer delays, whereas those with HF or PPC lesions were impaired. There were no significant differences among the groups on the DNMS-with-lists task or on the temporal-order recognition task. The resemblance that the present test battery bears to the nonspatial test batteries that are often used in studies of brain-damage-produced amnesia in monkeys should enable direct comparisons between the present results and those of similar experiments in monkeys.

595.15

THE ROLE OF POSTERIOR CINGULATE CORTEX (AREA 29) IN LEARNING AND MEMORY IN THE RAT. R. J. Sutherland*, J. M. Hoesing, R. Kornelson and J. E. Evanson. Depts. of Psychology and Physiology, Univ. of New Mexico, Albuquerque, NM 87131-1161.

Area 29 is extensively connected with the hippocampal system (HPC), cortical association areas, and dorsal thalamus. Clinical studies and experimental work with animal models suggest that area 29 damage can produce amnesic symptoms. Our experiments were designed to explore the characteristics of the memory disorder and to clarify the anatomical basis for these effects. Prior work (J. Neurosci. 1988, 8(6): 1863-1872) with rats revealed that area 29 aspiration produces a long-lasting impairment of place learning using the Morris water task. We now demonstrate that: 1. destruction of area 29 intrinsic neurons using multiple microinjections of the cytotoxin, quisqualic acid, produces an impairment of place navigation which is indistinguishable from nonspecific aspiration lesions, 2. limiting the neurotoxic damage to either primarily area 29c or 29d produces similar, but less dramatic, impairments than total removal, 3. using a combined lesion strategy including neurotoxic HPC, electrolytic anterior thalamic, and area 29 aspiration lesions, unilateral crossed lesions of area 29 + HPC or area 29 + anterior thalamus produce impairments comparable in magnitude to destruction of these structures bilaterally, 4. increasing the interval between place navigation training and area 29 damage does not affect the impairment, 5. posterior parietal cortex, but not perirhinal, aspirations produce anterograde and retrograde impairments comparable to area 29 damage, and 6. area 29 aspiration impairs the ability to resolve a nonspatial, negative patterning discrimination in a discrete trial bar-pressing task. Thus, area 29 circuitry is important in certain forms of memory, acting in concert with HPC, thalamic, and posterior parietal circuitry.

595.16

SELECTIVE DAMAGE OF FOREBRAIN-CORTICAL PATHWAYS: COMPARATIVE EFFECT ON SPATIAL LEARNING AND CORTICAL CHOLINERGIC RELEASE. M. Ammassari-Teule* G.L. Forloni, D. Amoroso and S. Consolo. Inst. of Psychobiology , C.N.R. 00198 Rome Italy and Mario Negri Inst., 20157 Milan, Italy.

Rats were assigned to one of the following treatments: mechanical deafferentation of the basal forebrain-cortical projections (DEAFF), ibotenic (IBO) or quisqualic (QUIS) acid lesions of the nucleus basalis magnocellularis (NBM) and sham operations (SHAM). They were trained to perform radial eight-arm maze tasks with all the paths or only four paths baited. Cortical cholinergic release measured by microdialysis in vivo and choline acetyltransferase (ChAT) activity were then assessed in the four lesion conditions. The results show that, in the full baited maze task, only the DEAFF group showed severe spatial learning deficits. In the four-baited path task, the deafferented group was still more impaired but some defects emerged in rats with neurotoxic lesions of the NBM. ChAT activity was reduced by 30% after IBO lesions and by more than 50% after DEAFF or QUIS lesions. Cholinergic release, however, was unaffected by IBO lesions but dropped in the same fashion after DEAFF or QUIS lesions. Thus, no correlation between cortical cholinergic depletion and spatial learning impairment was evident. The similar behavioral deficit produced by DEAFF and fornix sections suggests that, among the basal forebrain-cortical pathways, descending fibers projecting towards the septo-hippocampal system could exert a strong control on spatial learning.

595.17

MEMORY FOR OBJECTS, SPATIAL LOCATIONS AND MOTOR RESPONSES: TRIPLE DISSOCIATION AMONG THE HIPPOCAMPUS, CAUDATE NUCLEUS AND MEDIAL EXTRASTRIATE VISUAL CORTEX. R. P. Kesner*, M. Dakis, Dept. of Psychology, Univ. of Utah, Salt Lake City, UT 84112.

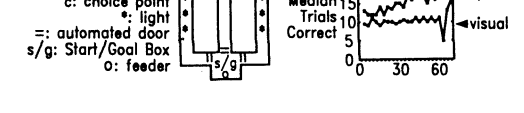
Rats were first trained in a matching or non-matching-to-sample task for spatial location, response and visual object information aimed at measuring working or data-based memory for allocentric spatial, response (egocentric spatial) and sensory-perceptual (visual object) attributes. After training, rats received lesions of either the hippocampus, caudate nucleus or medial extrastriate visual cortex. After recovery from surgery they were retested. Results indicated that there was only a memory deficit for spatial location information even at the shortest delays for rats with hippocampal lesions, only a memory deficit for response information even at the shortest delays for rats with caudate nucleus lesions, and only a memory deficit for visual object information even at the shortest delays for rats with medial extrastriate visual cortex lesions. Thus, there appears to be a triple dissociation among the hippocampus, caudate nucleus and medial extrastriate visual cortex in mediating allocentric spatial, response (egocentric spatial) and sensory-perceptual (visual object) attributes of memory. The results support the neurobiology of an attribute model of memory.

595.18

DIFFERENCES IN SPATIAL AND VISUAL WORKING MEMORY PERFORMANCE OF RATS IN A PHI(ϕ)-MAZE. W. J. Wilson*, N.C. Steinbronn, J.C. Lopshire, P.H. Galloway, S.A. Kellenberger, A.M. Hartman, & R.L. Bennett. Dept. of Psychological Sciences, Indiana University – Purdue University, Fort Wayne, IN 46805, USA.

Working memory in the rat has been studied extensively with tasks that involve spatial cues, often using the radial-arm maze or the T-maze. We compared working memory for spatial cues with that for visual cues in the phi (ϕ)-maze, a maze modeled loosely after the "automated T-maze" designed by Berger (1977), in which the rat is reinforced upon its return to the Start Box for having chosen the correct arm.

Rats were run in one of two reinforcement conditions in the ϕ-maze. In the Spatial task, rats were reinforced for alternately selecting the left and right arms. All 5 rats in this task reached the criterion of 15 or more correct trials on two successive daily 20-trial sessions within 16 sessions. In the Visual task, rats were reinforced for alternating between the lighted and dark arms. None of the 6 rats in this task reached the criterion within 60 sessions; they performed at near chance levels throughout, yet all readily learned a visual discrimination task in the ϕ-maze thereafter. Thus rats were able to perform a task that required working memory of spatial information, but were unable to use visual working memory, despite being able to discriminate based on the same visual cues. Explanations include the possibilities that rats differ in their capacity to encode spatial (proprioceptive, kinesthetic?) and visual information in working memory, or that the omni-present spatial cues interfered with learning of the Visual task.



596.1

RECOVERY OF SPATIAL NAVIGATION AND LEARNING IN THE MORRIS WATER MAZE AFTER BILATERAL TRANSECTION OF THE PERFORANT PATH IN RATS. R.W. Skelton* & R.K. McNamara. Dept. Psychology, Univ. Victoria, Box 3050, Victoria, British Columbia, CANADA, V8W 3P5.

Knife cuts to the perforant path (PP) disrupt acquisition of spatial (place, locale) learning in the Morris water maze (Skelton & McNamara, 1992 Hippocampus, 2, 78). The present study examined retention and recovery of both spatial navigation and place learning.

Rats were trained to locate a submerged platform, then given bilateral knife cuts of the PP, either in the angular bundle (1.7 mm ant. to EBZ) or 2 mm further anterior (to dennervate only the dorsal hippocampus). Starting 48 hrs after surgery, rats were tested with 4 submerged platform trials, 1 probe trial and 1 visible platform trial daily for 14 days, then weekly for 6 weeks. Rats with cuts at either level of PP were initially impaired on submerged platform and probe trials, but not on visible platform trials. Performance on probe trials reached control levels after about 10 test days, and continued to improve over the 6 weekly tests. A residual deficit was revealed when the platform was then moved to a new location: rats with cuts at either level took 3-4 days to reach the level of probe trial performance reached by controls in 1 day. These results suggest that 1) damage to the PP innervation of the dorsal hippocampus may be as debilitating as total PP transection 2) the PP is required for accurate navigation and place recall, 3) substantial recovery or retraining of spatial navigation occurs within 10 days, and 4) recovery of place learning remains incomplete for up to two months. (Supported by research grant from NSERC.)

596.2

PERFORMANCE OF ISCHEMIC RATS ON WIN-STAY AND WIN-SHIFT TASKS. E.R. Wood,* T.J. Bussey and A.G. Phillips. Department of Psychology, University of British Columbia, Vancouver, BC, Canada V6T 1Z4.

The present study was designed to assess in rats the effects of ischemia-induced damage on the performance of two 8-arm radial maze tasks: one (spatial win-shift) sensitive to lesions of the hippocampus, and the other (cued win-stay) sensitive to lesions of the caudate. Male Wistar rats received either sham surgery or 20-min transient forebrain ischemia induced by a combination of bilateral carotid occlusion and hemorrhagic hypotension. Following an 8 week recovery period the rats were tested on one of the two tasks. Each trial of the win-shift task consisted of two phases. In phase 1, 4 target arms were blocked and rats were allowed to enter and retrieve food from the 4 unblocked arms. In phase 2, all arms were unblocked and the previously blocked target arms were baited. Rats were allowed to explore the maze until all 4 target arms had been visited. Each rat received daily trials with a delay of 4-s between phase 1 and phase 2, until they reached the criterion of 4 target-arm entries in the first 5 arm entries during phase 2, for 3 consecutive trials. Rats then received 15 trials with a 5-min delay, and 6 trials with a 2-h delay. A random set of 4 target arms was used on each trial. Ischemic rats were significantly impaired at the 2-h delay, but not at the 4-s or 5-min delays, indicating that ischemia affects performance of this spatial win-shift task only at long delays.

In the cued win-stay task, all 8 arms were accessible, with light cues at the distal ends of 4 target arms. On each daily trial, rats were allowed to explore the maze until they had entered and retrieved food from each lit arm twice (arms were rebaited after the first entry). After a rat retrieved food from a lit arm for the second time, the light was turned off. A random set of 4 target arms was used on each trial. Ischemic rats were not significantly impaired on this cued win-stay task.

These findings of ischemia-induced impairments on a win-shift task at long delays, but not on a cued win-stay task suggest that this model of ischemia causes functional damage to the hippocampus, but not to the striatum.

596.3

AGED C57 MICE DECLINE IN SPATIAL LEARNING PERFORMANCE AND ASSOCIATED HIPPOCAMPAL PROTEIN KINASE C ACTIVITY. J.M. Wehner* and D.E. Fordyce. Institute for Behavioral Genetics, The University of Colorado at Boulder, Boulder, CO. 80309-0447

C57BL/6 (Ibg and Nia) mice were tested on the Morris water maze task for 6 days followed by 12 days of testing on the place learning-set task (8 trials/day with each task). Mice were tested at 3, 14, and 25 months of age. A decline in learning performance was observed in mice 15 and 25 months of age compared to mice 3 months of age. Mice 25 months of age were significantly impaired in both the Morris and place learning-set task probe trial performance compared to 3 months of age mice ($p<.02$) with no significant alteration in swim speed. These aged mice also demonstrated a significant reduction in membrane-bound hippocampal protein kinase C activity ($p<.05$) with no significant change in cytosolic or loosely-bound protein kinase C activity. The effects of aging in C57 mice on spatial learning performance and hippocampal protein kinase C activity were found in both the Ibg and Nia substrains. These data, therefore, indicate that the age-induced decline in spatial learning performance in C57 mice may be associated with an age-induced alteration in hippocampal protein kinase C activity.

Supported by NSF BNS-882 and NIH HD-07289 postdoctoral training grant.

596.4

PHYSICAL ACTIVITY ENHANCES SPATIAL LEARNING PERFORMANCE WITH AN ASSOCIATED ALTERATION IN HIPPOCAMPAL PROTEIN KINASE C ACTIVITY IN C57 AND DBA MICE. D.E. Fordyce* and J.M. Wehner. Institute for Behavioral Genetics, The University of Colorado at Boulder, Boulder, CO. 80309-0447

The effects of physical activity on spatial learning performance and associated hippocampal functioning were examined in C57BL/6 and DBA/2 (Ibg) mice. Previously, we observed a marked enhancement in spatial learning performance and associated alterations in hippocampal cholinergic function of rats exposed to physical activity (Fordyce and Farrar, 1991, Beh. Brain Res.46:123-133). Because of genetic analyses afforded by using inbred strains of mice, it was of interest to examine if this physical activity effect could be achieved in two strains of mice differing in learning performance (Wehner et al., 1987, Brain Res. 523:181-187). C57 and DBA mice (3 months of age) were subjected to an eight week physical activity protocol consisting of moderate pace (0.4 mph) treadmill running 5 days/week and 60 min/day. Mice were then tested on the Morris water task for 6 days followed by the place learning-set task for 12 days (8 trials/day with each task). Hippocampal protein kinase C activity was measured in cytosolic, loosely-bound and membrane-bound homogenate fractions. Mice subjected to the physical activity protocol were compared to age-matched sedentary controls from the same set of litters. Physical activity enhanced performance on both the Morris and place learning-set task probe trials ($p<.02$). DBA mice, which characteristically perform poorly in comparison to C57 mice, were enhanced to perform similarly to C57 mice. These alterations in performance were accompanied by alterations in membrane-bound protein kinase C activity ($p<.05$). The data from this study, therefore, indicate that the protein kinase C second messenger system, as well as cholinergic function, in the hippocampus may be involved in the physical activity-induced enhancement of spatial learning performance.

Supported by NSF BNS-8820076 and NIH HD-07289 postdoctoral training grant.

596.5

MOUSE STRAIN DIFFERENCES IN LEARNING SET SPATIAL NAVIGATION PERFORMANCE

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Swiss Webster (SW); DBA; and Deer Mice (DM) were tested for acquisition and retention of a learning set place task in the Morris water maze. The learning set consisted of daily placing the hidden platform sequentially at 1 of 4 separate locations in the pool. All animals were swum for 63 days in this version of the water task. SW mice were unable to reliably find the platform. The time taken by DBA and DM in escaping the pool declined rapidly, reaching asymptote within 21 days, with DM showing the ability, throughout the study, to reach the platform significantly faster than either SW or DBA. Analyses of swim path selection used by the 3 strains clearly indicated that DM were the most systematic in the selecting and sequencing, from a variety of potential strategies, the appropriate methods necessary for the most efficient solution of the problem. The present results suggest, that in light of the strain differences observed in swimming behaviors investigation of strain differences in the neuroanatomic structures believed to be related to the solving of spatial problems, might be a fruitful area of investigation.

596.6

SPATIAL LEARNING IN DEER MICE: EFFECTS OF SEX AND REPRODUCTIVE STATUS. M. Kavaliers,* L. A. M. Galea, E. L. Hargreaves, D. L. Innes and K. -P. Ossenkopp. Neuroscience Prog., Univ. Western Ontario, London, ON, Canada.

Sex difference in spatial ability have been proposed to be related to mating systems, space use, and reproductive condition. Deer mice, Peromyscus maniculatus, are polygynous rodents with reproductive males displaying greater range sizes than females. The present study considered the effects of reproductive status on spatial learning by males and females of two different populations of deer mice. The performance of the two populations of adult breeding and non-breeding deer mice was examined in a Morris water maze. Latency of the animals to reach a hidden platform was measured over 6 blocks of 4 trials. All groups of mice were able to learn the spatial task, with the mice derived from a population originally present in an arid interior region acquiring the task more slowly than mice that were descendants of a population inhabiting a small island. In both populations, breeding males showed significantly faster acquisition and better retention of the spatial task than did breeding females. In contrast, non-breeding mice of both populations displayed no significant sex differences in their spatial performance. These findings indicate that reproductive and hormonal condition have significant effects on the display of sex differences in spatial learning.

596.7

ACQUISITION, LEARNING-SET, AND LONG-TERM RETENTION DEFICITS OF SPATIAL INFORMATIONS IN AGED MICE. C. Lebrun, I.Koenig*, and R.Jaffard. Laboratoire de Neurosciences Comportementales et Cognitives, URA 339 and *Laboratoire de Neurobiologie Cellulaires, Univ. Bordeaux I et II, Avenue des Facultés, 33405 Talence, France.

Ability in learning and memory during aging in mice has been investigated using two-choice discrimination tasks in a 8-arm radial maze. Each discrimination consisted of presenting two adjacent arms (pairs) with only one always the same, baited across trials. On the first phase, animals were concurrently trained on two pairs (Acquisition 1) until they reached a criterion of 13 correct responses out of 16 successive trials. On the second phase (Acquisition 2), animals learned to discriminate the two other pairs. To acquire the task, the animals had the possibility to use either extra-maze (place) or intra-maze cues (black and white visual cues constantly placed on each arm across the trials).

Results showed that, as compared to young mice (4-5 months), aged animals (24-25 months) exhibit i) slower acquisition on the first two pairs (Acquisition 1), ii) weacker positive transfer from Acquisition 1 to Acquisition 2, iii) a long-term retention deficit (over 20 and 40 days) of previously acquired informations (Acquisition 1 and 2).

An additional experiment was conducted in order to verify the contribution of intra-maze cues to discrimination performance in young and aged mice. Results showed that young mice use preferentially extra-maze cues, whereas old animals learn by using both intra- and extra-maze cues. Supported by CNRS, URA 339, and Fondation "France Alzheimer".

596.8

MEMORY DEFICITS AND ASSOCIATED DECREASES OF CEREBRAL 2-DG LABELLING INDUCED BY CHRONIC ALCOHOL CONSUMPTION IN MICE: REVERSAL BY METHYL β-CARBOLINE-3-CARBOXYLATE ADMINISTRATION. B. Bontempi, D.J. Beracochea, C. Destrade* and R. Jaffard. Lab. Neurosci. Comportementales et Cognitives, URA CNRS 339, Univ. Bordeaux 1, Av. Facultés, 33405 Talence, France.

We previously showed that long term (17 months) chronic alcohol consumption by mice produced significant reduction in cerebral energy metabolism as measured by 2-DG labelling in the diencephalon, in particular the mammillary body (MB) (Soc. Neurosci., 1991, Abs. 17, 481). Here, we investigated the effects of two shorter durations (6 and 12 months) of alcohol consumption by Balb/c mice on 2-DG uptake patterns observed following a memory test in a T-maze. 2-DG was injected into the jugular vein immediately before a 35 min period of testing for sequential alternation in a T-maze and the subjects were sacrificed for autoradiographic analysis using the relative 2-DG method. Although no significant effects on either 2-DG labelling or memory performance were observed after 6 months of alcohol consumption in comparison to controls, mice of the 12 month group showed significantly increased susceptibility to interference in the alternation task. This mnesic deficit was associated with a concomitant decrease in testing-induced 2-DG labelling intensity of the MB. Administration of β-CCM (0.5 mg/Kg s.c.) to subjects of the 12 month alcohol group reversed the memory impairment of these mice as compared to saline-injected controls. Parallel studies of the effect of β-CCM on 2-DG labelling patterns in these mice are being conducted in an attempt to establish an eventual correlation between memory performance in the alternation task and MB metabolic activity.

596.9

LITTER SEX-RATIOS AFFECTS ADULT PERFORMANCE IN A SPATIAL TASK. L.A. M. Galea*, K.-P. Ossenkopp, & M. Kavaliers. Neuroscience Prog., Univ. of Western Ontario, CANADA.

Previous research in this laboratory has shown that breeding adult meadow voles *Microtus pennsylvanicus* acquire a spatial task in a sexually-dimorphic manner, favoring males, and that no sex differences are shown in performance in immature voles. The present study addressed the question of whether there would be sex differences in performance on a spatial task in breeding adult voles which had previously not demonstrated a sex difference when tested as juveniles. Sixteen litters of adults were re-tested in a hidden platform Morris water-maze six weeks after being initally tested at either day 10, 15, 20, or 25 after birth. (Juvenile voles tested at days 20 or 25 acquired the task faster than voles tested at days 10 or 15). Adult voles that had previously learned the task at day 20 or 25 retained the task and showed no sex differences in performance. However, adult breeding voles previously tested at days 10 or 15 did demonstrate a sex difference in performance favoring males but **only** in litters with a female-biased sex-ratio (♀ > ♂). Thus, females in male-biased litters acquired the task as rapidly as males. This suggests that meadow voles retained the spatial task better if they had initially showed faster acquisition or learned the task at an older age, and that the relative amount of androgens *in utero* may influence the development of spatial ability.

596.10

EFFECTS ON SIMPLE ASSOCIATION AND SPATIAL MEMORY OF NEUROTOXIN LESIONS TO THE AVIAN HIPPOCAMPUS. H.N. Rice and D.F. Sherry*. University of Western Ontario, London, Ont., N6A 5C2, Canada.

Black-capped chickadees (*Parus atricapillus*) (n=11) were trained on a simple cue association and a spatial task in an indoor aviary with 6 artificial trees, each with 10 food sites. The spatial task required birds to find 6 seeds placed in the same sites from trial to trial. The cue task required birds to find 6 seeds at cued sites randomly assigned to locations on each trial. After reaching a criterion of 3 correct choices in the first 6 attempts, birds were randomly assigned to one of three groups: kanic acid (0.2μg/μl) lesion, colchicine (4.0μg/μl) lesion, or control. Two min infusions of 0.25 μl of solution were made at four sites in the hippocampus through a stereotaxically placed 10 gauge cannula. Following recovery, birds were tested on acquisition of novel simple association and spatial tasks. Both lesion groups were impaired on the spatial task, regardless of the neurotoxin. Preliminary results suggest no impairment on the simple cue task. Previous findings showed, in contrast, that black-capped chickadees with bilateral aspirations of the entire hippocampus do not re-acquire this spatial task.

596.11

EFFECTS OF HIPPOCAMPAL LESIONS ON VISUAL SPATIAL ATTENTION IN PIGEONS. T. Fremouw,* P. Jackson-Smith, C. P. Shimp, and R. P. Kesner. Dept. of Psychology., Univ. of Utah, Salt Lake, UT 84112.

We explored the role of the hippocampus in determining reaction times of pigeons (Columba livia) in a task somewhat analogous to the classic Posner spatial attention paradigm. Brief cues preceded targets to which pigeons responded for food. Most cues were valid in the sense that the cue appeared in the same location as the subsequent target. On some trials, however, cues were invalid and appeared in the opposite location as the target. Hippocampal lesions do not affect location specific attention as reflected in the "validity effect." In other words, reaction times were faster after valid than invalid cues. Hippocampal lesions may, however, affect the length of time over which pigeons can sustain non-location specific attention to the task, as reflected in an increase in overall reaction times on both valid and invalid trials in contrast to corresponding results from control animals. Thus, the hippocampus may be required for sustained global attention, but not spatial specific att ntion.

596.12

CALLOSOTOMY BUT NOT EARLY EXPERIENCE INFLUENCES SPATIAL LEARNING IN THE RAT. S.E. Maier* & D.P. Crowne, Dept. Psychology, Univ. Waterloo, Waterloo, ON, Canada, N2L 3G1

For a number of years, research on the effects of early experience in the form of infantile 'handling' revealed propitious treatment effects on measures of spatial directionality and avoidance learning. In this experiment, we wondered whether handling would facilitate spatial learning and safeguard against the disruptive effects of corpus callosum transection on spatial learning. Rats were given or denied 'handling' between birth and weaning. As adults they underwent complete section of the corpus callosum and were tested in the Morris water maze and open field. Handled rats were not different from non-handled rats at learning the spatial task. Cutting the corpus callosum interfered with spatial learning even though it had no adverse effect on open field ambulation, thus ruling out a motor impairment to explain the spatial outcome. The results suggest that the early experience manipulation may be specific to certain types of spatial behaviors and learning tasks.

596.13

CONTINGENCIES CONTROLLING WIN-SHIFT ALTERNATION IN A SPATIAL TASK.

A.G. Gittis* and M. McHaddon, Psychology Department, Westminster College, New Wilmington, PA 16172.

Two choice spatial alternation procedures have been variously called "win-shift" tasks or delayed non-match to sample. Variants of this procedure are used to assess functioning of subcortical memory mechanisms under the assumption that working memory is needed to remember a prior response and that shifting is an acquired habit. Lesion (Chudik and Gittis, *SN Abstr.*, 1991) and ontogenetic studies (Gittis, et.al., *AL&B*, 1988) question these assumptions. This experiment investigates the task by evaluating the role contingencies play in maintaining performance.

Hooded rats were trained to an 80% acquisition criterion on a win-shift task in a Y-maze. "Winning" is a forced component of a trial in which one arm of the Y is blocked with the rat finding food at the end of the open arm. By choosing the alternative arm, "shifting", the rat is subsequently rewarded. After acquisition, subjects were assigned to groups in which, for an additional 100 trials, reward for winning, shifting or both was withheld. Results showed that presence or absence of reward on the forced component plays little role in task performance, whereas removal of reward for shifting is highly disruptive. These results place in doubt that memory for reward at the prior response (the foraging model) plays any role in alternation. The fact that reward for shifting is of critical importance makes the results enigmatic in light of observations that alternation requires little prodding when it appears ontogenetically. A resolution is offered by interpreting alternation through the "dead reckoning" model proposed by Gallistel, (*Organization of Learning*, 1990).

596.14

NEONATAL HANDLING: EFFECTS ON SPATIAL MEMORY, BASAL CORTICOSTERONE LEVELS, AND HIPPOCAMPAL FUNCTION. D.M. Hayden-Hixson*[1], N. White[2], J.C. Ritchie[2], and C. B. Nemeroff[3]. [1]Toxicology Progr. and [2]Psychobiology Res. Lab, Duke University Medical Center, Durham, NC 27710, [3] Dept. Psychiatry, Emory University School of Medicine, Atlanta, GA 30322.

Accumulated data indicate the neurobiological concommittants of neonatal handling include increased resistance to the deleterious consequences of chronic stress and normal aging on brain function. This study examined the effects of neonatal handling on spatial memory/ learning and basal plasma corticosterone levels in male and female Long Evans rats. Animals were either handled 5min/day (days 1-21: H1; days 5-21: H5) or left undisturbed. At $2^{1/2}$ months of age, animals were trained on an 8-arm radial arm maze (RAM) for 21 days. All manipulations were performed during the first 4 hrs of the dark phase of the light:dark cycle. Food access was not restricted at any time.

Overall, handled males were significantly (P<.05) more accurate (higher first arm to repeat scores, and fewer total errors to complete task scores) and more efficient (lower total time and arms to complete task scores) than both nonhandled males and nonhandled females. Handled females were also significantly more accurate and efficient than nonhandled males, but not nonhandled females. Plasma corticosterone levels were significantly lower in handled compared to nonhandled males. Handled and nonhandled females had comparable corticosterone levels. Hippocampal glucocorticoid receptor autoradiography and 11-hydroxysteroid dehydrogenase bioactivity studies on the brains of these animals and their appropriate controls are ongoing at this time.

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596.15

EFFECTS OF LEARNING PLACE SIGNIFICANCE IN RAT HIPPOCAMPAL PLACE CELLS

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Rats implanted with lateral hypothalamic electrodes for rewarding intracranial self-stimulation (ICSS) were trained to explore a circular open-field, in which ICSS was delivered whenever the animals' exploration behavior met certain experimenter defined criteria. Complex spike activity from the dorsal hippocampus CA1 was recorded during the task. The following three conditions were examined for naive and well-trained animals. (1) ICSS was delivered after the animal entered a randomly located circular area in the field. (2) ICSS was delivered when the animal entered a circular area corresponding to the place field, or remained in it for 2s; after a previous visit to a similar area outside the place field. (3) ICSS was delivered only when the animal entered a circular area outside of the place field, after a previous visit to a circular area corresponding to the place field. Some hippocampal cells have place field responses during homogeneous exploration. These place fields could be moved, after several trials, to other positions where reward was newly delivered. This change was gradual in successive trials. The naive rats could learn task (2) after several trials. Some hippocampal cells acquired clear place fields during learning. The results suggest plasticity of place cells in the hippocampus, and their importance in learning and memory of place.

596.16

SPATIAL MEMORY PERFORMANCE OF RATS ON A WORKING MEMORY TASK DOES NOT REQUIRE THE PRESENCE OF VISUAL DISTAL CUES. L.S. Janis*, C.W. Weaver, M.A. Kocot, T.L. Mosher, & G.L. Dunbar. Institute of Animal Behavior, Rutgers University, Newark, N.J. 07102*& Central Michigan University, Mt. Pleasant, Mi. 48859.

A popular construct in recent theories of spatial performance is that rats use visual distal cues to create a "cognitive map" of the maze surroundings. Previous research has indicated that rats will either use a distal-cue (spatial) strategy or a pattern-response (non-spatial) strategy to solve a working memory task in the radial arm maze. In the present experiment we used a novel testing paradigm to test the hypothesis that rats which use the spatial strategy require the presence of distal cues. Sixteen male rats were first trained on a working memory task on an eight-arm radial maze with all visual distal cues present. Following the attainment of an 85% accuracy criterion for 5 consecutive days, the rats were then retested on the maze with all the visual distal cues blocked by a black curtain. Results indicated that of the 7 rats that used a spatial strategy during the initial training phase, 4 of these rats continued to use an apparent spatial strategy during the retest phase although no visual cues were present. Our results indicate that spatial strategy need not be derived exclusively from distal cues. Additionally, one of the rats who initially used a response strategy during the training switched strategy in the retest phase. These results suggest that rats probably use complex response strategies to solve difficult spatial tasks and that distal visual cues may not be critical.

596.17

PROBE TESTS TO ASSESS SPATIAL MEMORY IN THE WATER TANK. A.L.Markowska*, J.Long, C.Johnson, and D.S.Olton, Department of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

The present experiment introduces a variable interval (VI) probe trial to assess spatial memory in the water maze, and demonstrates a substantial influence of the response/reinforcement contingencies during probe trials on performance. For platform trials, a platform was in its raised position, accessible to the rat. Three groups differed in their probe trials. Two groups had the standard no-platform probe (NP) trials in which the platform was unavailable for the entire trial: NP6,12 had the NP probe trial in Sessions 6 and 12, NP1-12 had the NP probe trial once each session for all 12 sessions. The third group, VI1-12, had the VI probe trial once each session for all 12 sessions. During the VI probe trial, the platform was in its lowered position, unavailable to the rat, for a variable interval of time, and then raised so that the rat could escape from the water, maintaining the same response/reinforcement contingencies on probe trials that were present on platform trials. On platform trials, VI1-12 had more accurate memory of the platform location than NP6,12 or NP1-12, as indicated by heading angle. On probe trials, VI1-12 showed better and more stable performance as indicated by time in the platform location, and number of times the platform location was crossed. These effects were due to the lack of extinction produced by the VI probe trial as compared to the NP probe trial, and to the acceleration of spatial learning induced by the VI probe trial. Because the VI procedure produced better performance than the NP procedure in both platform trials and probe trials, it can be used for repeated measurements of spatial memory, a characteristic that is not readily attainable with the NP probe trial.

596.18

LONG-TERM EFFECTS OF NEONATAL LIMBIC LESIONS ON SPATIAL RECOGNITION IN RHESUS MONKEYS. L. Malkova*, J. Bachevalier and M. Mishkin. Lab. of Neuropsychology, NIMH, Bethesda, MD 20892.

Three monkeys with neonatal amygdalo-hippocampal lesions (AH), 3 with neonatal cortical area TE lesions (TE), and 5 normal controls (N) were trained between 4-9 years of age in a series of cognitive tests to assess the long-term effects of neonatal lesions on memory. Earlier we reported that monkeys with neonatal limbic lesions were severely impaired in both visual and tactile recognition (Bachevalier et al., Soc. Neurosci. 14:1, 1988, Malkova et al., Soc. Neurosci. 17:338, 1991). Here, we report the results of additional behavioral testing of the same monkeys on delayed nonmatching-to-sample for location (DNMS). The animals in group TE were unimpaired, reaching criterion on 10-sec DNMS in 360 trials and averaging 77% across 30-120 sec delays, as compared to scores of 286 and 81%, respectively, in the normal controls. By contrast, none of the animals in group AH reached the criterion in 1000 trials, though two attained 90% and 86% with additional correction training. On 30-120 sec delays, the performance scores of these two animals dropped sharply, averaging 66%. The results confirm and extend our previous findings indicating that early damage to the limbic system (i.e. amygdala, hippocampus, and adjacent cortical areas) yields a permanent and global memory loss.

597.1

FOURTH VENTRICLE BOMBESIN INJECTIONS SUPPRESS SUCROSE INTAKE IN DECEREBRATE RATS. F.W. Flynn.* Dept. of Psychology and Neuroscience Program, Univ. of Wyoming, Laramie, WY.

Systemic injections of bombesin (BN) suppress sucrose intake in decerebrate rats. Also, 4th ventricle injections of BN in intact rats inhibit feeding. These studies do not *simultaneously* demonstrate that BN-produced afferent signals are both detected and integrated by caudal brainstem (CBS) neural circuitry to control intake. To evaluate whether the CBS, in isolation of the forebrain, contains the requisite neural systems to mediate the effects of 4th ventricle BN injection on ingestive behavior, intraoral sucrose (0.1 M) intake was measured in control and decerebrate rats (n=11/group) following 4th ventricle injections of 1, 5, 10, 20, and 50 ng BN. Fourth ventricle BN injections produced the same reliable dose-dependent reductions of sucrose intake in both groups. This result, taken with a previous report that systemic injections of BN suppress ingestive behavior in decerebrate rats, provides compelling evidence that local CBS neural circuitry mediates the sensory-motor integrative aspects that underlie bombesin's effects on ingestive behavior. (supported by NIH NS24879)

597.2

MICROINJECTION OF BOMBESIN INTO MIDLINE THALAMIC NUCLEI ELEVATES BLOOD GLUCOSE, FREE FATTY ACIDS, AND CORTICOSTERONE IN RATS. M.W. Gunion,* M.J. Rosenthal, S. Miller, M. Hoyt, and D. Yonson. Research Service, Sepulveda VAMC, Sepulveda, CA 91343; Dept. Medicine, U.C.L.A. Los Angeles, CA 90024.

Several midline thalamic nuclei are implicated in autonomic, particularly cardiovascular, regulation. They have not been evaluated for roles in metabolic fuel or glucocorticoid regulation. Some of these nuclei strongly bind bombesin; bombesin microinjections into other autonomic nuclei cause elevated blood levels of glucose, free fatty acids, and corticosterone. Under methoxyflurane anesthesia, male Sprague-Dawley rats (300–350) received chronic guide cannula aimed at various midline thalamic nuclei. After a minimum of 7 days recovery, microinjections of bombesin (15, 50, 150 ng/300nl, in 0.9% NaCl+0.1% BSA) were made. Blood samples (120 ul) taken from the tail tip 0, 15, 30, 60, 90, and 120 min postmicroinjection were assayed for serum glucose, free fatty acids, and corticosterone. Positive sites were found in these thalamic nuclei: interanteromedial, paraventricular, rhomboid, centromedian. In the thalamic paraventricular nucleus, positive sites were clustered in the anterior subdivision of the nucleus. In the centromedian nucleus, positive sites were grouped on the midline, and not found in the lateral extensions of the nucleus. This pattern of active sites corresponds well with the previously demonstrated midline distribution of bombesin-binding sites. [Supported by PHS NS20660 (MWG), AG04793 (MJR), and Veterans Administration funds (MWG, MJR).]

597.3

GALANIN ANTAGONISTS M40, C7, AND M32, MICROINJECTED INTO THE PVN OF THE HYPOTHALAMUS, BLOCK GALANIN-INDUCED FEEDING IN RATS. R.L. Corwin*, J.K. Robinson, U. Langel+, T. Bartfai+, and J.N. Crawley. NIMH, Building 10, Room 4N214, Bethesda, MD 20892, + Dept. of Neurochemistry and Neurotoxicology, Stockholm Univ., Sweden.

Exogenous galanin (GAL) microinjected into the paraventricular nucleus of the hypothalamus (PVN), increases consumption of high fat foods in nondeprived rats. Potent galanin receptor antagonists have recently been developed which are chimeric peptides with GAL 1-13 at the N-terminus and another peptide sequence at the C-terminus. We tested three of these antagonists (M40, C7, and M32) in a feeding paradigm previously used to assay GAL-induced feeding. Male Sprague-Dawley rats were surgically implanted with bilateral stainless steel cannulae, aimed just dorsal to the PVN. Approximately 1 week after surgery, free-feeding rats were trained to consume a mash consisting of 2 vanilla wafers soaked in 10 ml deionized water during a 30-min test. On test days, Ringer's vehicle or antagonists, and GAL 1-29 or vehicle were injected separately and bilaterally into the PVN. GAL 1-29 (0.5 nM) significantly increased mash consumption. M40 (0.5 nM), C7 (0.1 nM) and M32 (0.25 nM) significantly blocked GAL-induced feeding. None of the antagonists had a significant effect on mash consumption when given alone. These new receptor antagonists appear to be effective blockers of feeding stimulated by exogenous galanin microinjected into the PVN, indicating their potential usefulness in investigating the behavioral actions of endogenous galanin.

597.4

HYPOTHALAMIC GALANIN GENE EXPRESSION IS ASSOCIATED WITH PREFERENTIAL FAT INTAKE, INSULIN LEVELS AND BODY WEIGHT. S.J. Tucker, M. Jhanwar-Uniyal, S.C. Chua Jr, J. Grinker* and S.F. Leibowitz. The Rockefeller Univ., New York, NY 10021, Univ Michigan, Ann Arbor, Michigan.

The self-selection feeding paradigm enables rats to select the macronutrients of choice. Studies conducted with neuropeptide galanin (GAL) show that GAL selectively induces ingestion of fat when injected into the hypothalamus. In this study, Sprague-Dawley rats (n=13) were given pure macronutrient diets (carbohydrate, protein and fat). Using quantitative Northern blot analysis, we have measured the levels of hypothalamic GAL messenger RNA (GAL mRNA). Circulating levels of insulin (INS) were assessed by RIA, and glucose (GLU) levels were estimated by YSI glucose analyzer. Body mass index (BMI) and 3 different fat pad weights, namely, retroperitoneal, inguinal and epididymal, were also recorded. The results, in rats consuming a minimum of 20 Kcal/24 hrs of fat, displayed that: 1) a significant positive correlation exists between daily fat ingestion and hypothalamic GAL mRNA ($r=0.61$; $p<0.05$); 2) GAL gene expression is significantly correlated with INS levels ($r=0.58$; $p<0.05$) and INS/GLU ratio ($r=0.60$; $p<0.05$); 3) GAL mRNA is positively correlated with body weight ($r=0.66$; $p<0.05$) and BMI ($r=0.63$; $p<0.05$), but not correlated with any of the individual fat pad weights. The results of this study demonstrate that increased hypothalamic GAL synthesis may be related to greater fat ingestion, INS secretion, as well as body weight.

597.5

[Leu^{31}-Pro^{34}] Neuropeptide Y (NPY), but not NPY 20-36, has discriminative stimulus effects similar to NPY and induces food intake. D. C. Jewett*, J. Cleary, D. W. Schaal, T. Thompson & A. S. Levine University of Minnesota and VA Medical Center Minneapolis, MN 55455 and 55417

Previously we demonstrated that the stimuli associated with NPY can be discriminated from those stimuli associated with an injection of vehicle. In the present study, we compared the discriminative stimulus effects of a Y1 [Leu^{31}-Pro^{34}] (LP-NPY) and Y2 agonist [NPY 20-36] to those of NPY. Five rats were maintained at 80% of their free-feeding weights and trained to discriminate a 1.2 nmol intracerebroventricular (icv) NPY injection from no injection. Responses on one lever following injection with NPY were reinforced, while responses on the other lever following no injection were reinforced. Responses prior to the first reinforcer or timeout were a measure of the discriminative stimulus effects of the agent. Following acquisition of the discrimination, rats were administered icv saline, and 1.2, 2.4, 3.5 nmol LP-NPY and NPY 20-36. Our findings suggest LP-NPY has stimulus effects similar to NPY. 3.5 nmol LP-NPY completely generalized to 1.2 nmol NPY. Lower doses of LP-NPY partially generalized to the training dose. Approximately 50% of the responses following 1.2 nmol and about 75% of the responses following 2.4 nmol were made on the NPY-appropriate lever. No NPY 20-36 doses tested resulted in NPY-appropriate responding. Saline administration also did not engender NPY-appropriate responding. LP-NPY also increased food intake. Rats were injected with the same agents and doses as above, and food intake was measured over 2 hours. All doses of LP-NPY significantly increased food intake relative to saline controls ($p<.0001$). Rats ate 4.7, 6.7 and 5.2 g after 1.2, 2.4, 3.5 nmol LP-NPY. No significant increases in food intake were observed following NPY 20-36 administration relative to saline control.

597.6

INCREASED FOOD INTAKE FOLLOWING MORPHINE OR NPY ADMINISTRATION INTO THE VENTRAL TEGMENTAL AREA OF RATS. J. M. Rudski*, M. K. Grace, C.J. Billington and A.S. Levine. University of Minnesota, Minneapolis MN 55455 and VA Medical Center, Minneapolis MN 55417

Previous studies in our laboratory indicate that administration of Neuropeptide Y (NPY) or *mu* opioid agonists into the lateral ventrical in rats produces naloxone reversible increases in food intake at times of day when feeding is usually not observed. Feeding following opiate administration typically requires repeated injections, suggesting that tolerance to sedation produced by opiates must develop before feeding can occur. It has been suggested that morphine applied to the VTA produces motivational without motoric effects (Noel & Wise, *Society for Neuroscience Abstracts* 1990). In the present studies, morphine (0.1, 1.0 or 10.0 n mol), NPY (1 µg), or NPY and naloxone (50µg) were administered into the VTA 2 hours into the light cycle and 4 hour cumulative food intake was recorded. Increases in food intake were not evident following the first injection and required repeated administration. Feeding was not increased over the first 2 hours following morphine injections, and casual observation of the rats indicated some motor impairment. However, 4 hour cumulative food intake was positively related to dose ($F_{(3,25)}=8.14$, $p<0.001$). NPY administered into the VTA produced feeding at 1 ($F_{(2,15)}=16.17$, $p<0.005$), 2 ($F_{(2,15)}=13.93$, $p<0.005$) and 4 ($F_{(2,15)}=7.77$, $p<0.005$) hours after injections. NPY's effect was blocked by naloxone (50µg). These results demonstrate that morphine and NPY administered into the VTA increase food intake.

597.7

NALOXONE AND FLUOXETINE BLOCK EATING IN A PYY-MODEL OF BULIMIA NERVOSA. M. M. Hagan and D. E. Moss*. Laboratory of Psychobiochemistry, University of Texas at El Paso, Texas 79968.

Peptide YY (PYY) has been implicated in the neurobiology of bulimia (Kaye et al., 1990; Morley et al., 1987). Naloxone (NAL) and fluoxetine (FLU) have been proposed as anti-bulimic agents (Enas, 1989; Mitchell, 1989). Little is known about the effect of NAL and FLU on PYY-induced eating.

Female rats with cannulae in the fourth ventricle were pretreated with NAL 100ug/3ul i.c.v., NAL 10mg/kg sc, FLU 3-30ug/3ul i.c.v., FLU 5-10mg/kg ip, clomipramine (CLO) 3-30ug/3ul i.c.v., CLO 5-10mg/kg ip, or vehicle (VEH). At least 13 rats served in each condition. Twenty minutes after pretreatment, all rats received 5ug/5ul PYY i.c.v. every hour for a total of 15 ug PYY. Cumulative 4h food intake for PYY alone was 8.02g. VEH intake was 3.22g. Both central and peripheral injections of NAL blocked PYY-induced eating (2.58g and 3.21g, respectively). FLU ip also suppressed PYY-induced eating (3.29g). Central injection of FLU, however, did not affect PYY-induced eating (6.30g). Both central and peripheral CLO also had no effect on PYY-induced eating (8.35g).

Our results with NAL suggest opioids mediate PYY-induced eating. The resuls with FLU and CLO indicated that a serotonergic mechanism may be involved but at a site distant from the fourth ventricle and via FLU-sensitive 5-HT receptors. Further research is being directed at other neurochemical locations.

[Fluoxetine gift from Eli Lilly. Supported by MIRDP 47167].

597.8

THE MU AND KAPPA OPIOID ANTAGONISTS, ß-FUNALTREXAMINE (ß-FNA) AND NOR-BINALTORPHIMINE (nor-BNI), REDUCE NEUROPEPTIDE Y-INDUCED FEEDING. C.M. Kotz, M. K. Grace, C.J. Billington and A.S. Levine*, VA Medical Center, Minneapolis MN 55417 and University of Minnesota, Minneapolis MN 55455.

Intracerebroventricular (ICV) injection of naloxone has been reported to decrease the robust feeding observed after ICV injection of Neuropeptide Y (NPY). In the present study we evaluated the effects of ß-FNA and nor-BNI, two opioid antagonists selective for the mu and kappa opioid receptors, on NPY-induced feeding. In the first study we pre-injected (ICV) male Sprague Dawley rats with 50 nmol of nor-BNI 60 min before ICV administration of NPY (5µg). Nor-BNI reduced NPY-induced feeding 2 and 4 hours after NPY injection (table). ß-FNA acts as a kappa opioid agonist immediately after injection and as a mu antagonist 20-24 hours after injection. We found that when 50 nmol of ß-FNA was injected (ICV) 22 hours before NPY (5 µg ICV) injection, food intake (g) was decreased.

	0-1 Hour	0-2 Hour	0-4 Hour
Vehicle	0.1 ± 0.1	0.1 ± 0.1	0.7 ± 0.3
NPY	0.9 ± 0.3	1.9 ± 0.5	3.6 ± 0.7
NPY + nor-BNI	0.3 ± 0.3	0.6 ± 0.5	1.1 ± 0.5
Vehicle	0.6 ± 0.3	1.2 ± 0.4	1.7 ± 0.4
NPY	3.4 ± 0.5	4.5 ± 0.8	5.5 ± 0.8
NPY + ß-FNA	1.1 ± 0.4	2.3 ± 0.6	3.7 ± 0.8

These data indicate that blockade of the mu or kappa opioid receptors decreases NPY-induced feeding. This suggests that NPY's ability to increase food intake is dependent upon opioid action.

597.9

PEPTIDE CCK RECEPTOR ANTAGONIST INCREASES REAL FEEDING AND ATTENUATES SUPPRESSION OF SHAM FEEDING BY EXOGENOUS CCK-8 IN RATS. Brenner*, L.A. & Ritter, R.C. Dept. of VCAPP, Washington State University, Pullman, WA 99164

Non-peptide CCK receptor antagonists abolish suppression of real and sham feeding by exogenous CCK and some intestinal nutrients. They also increase real feeding when administered in the absence of exogenous CCK. t-Boc-Tyr(SO_3H)-Nle-Gly-D-Trp-Nle-Asp-2-phenylethylester (D-Trp-JMV) is a potent, heptapeptide, CCK receptor antagonist. Since this antagonist is similar in size, molecular form and weight to CCK-8, we reasoned that it should reach receptors accessible to exogenous CCK-8 and not receptors protected by blood-neural barriers. Therefore, we have examined the ability of D-Trp-JMV to increase real feeding and to attenuate suppression of sham feeding by exogenous CCK-8 or intraintestinal oleate. Intraperitoneal injection of 500 ug/kg of this compound abolished CCK-8 (2ug/kg) induced suppression of sham feeding or real feeding. A 1.0 mg/kg dose of D-Trp-JMV significantly increased real feeding. Suppression of sham feeding by intraintestinal oleate was not reliably attenuated by D-Trp-JMV at doses up to 4.0 mg/kg. The fact that oleate-induced suppression of sham feeding was not abolished by D-Trp-JMV may indicate that this effect is mediated at a different receptor site than that mediating suppression of feeding by exogenous CCK-8. Alternatively, levels of CCK released by oleate during sham feeding may have been too high to permit effective competition by the doses of D-Trp-JMV administered. Finally, our results indicate that both exogenous CCK-8 and endogenous CCK, released during real feeding, reduce food intake by acting at a site(s) accessible to peripherally administered peptides. Supported by NIH NS20561.

597.10

STUDIES ON THE DEVELOPMENT OF TOLERANCE TO THE ANORECTIC ACTIONS OF CCK-8 IN RATS. L. Bednarz, K.E. Asin and A.M. Nadzan*, Neuroscience Research Div., Pharmaceutical Discovery, Abbott Labs, D-47U, Bldg-AP10, Abbott Park, IL 60064.

Although acute injections of CCK-8 suppress food intakes in rats, when the compound is administered on a daily basis, progressively higher doses are required to affect intakes as animals become tolerant to its anorectic actions. We examined in more detail the development of this tolerance.

Four groups of male rats were placed on a restricted feeding schedule where they were allowed access to a liquid diet for 60min in the AM and for 30min in the PM until intakes had stabilized. During the subsequent **training period,** rats were injected (ip) daily with either vehicle (V) or CCK-8 (10nmol/kg) 10min prior to the AM feeding period and again 2h after it. Administration of CCK-8 prior to feeding suppressed ($p<.05$) 60 min intakes about 25% on the first injection day but had no effect by Day 3. Ten min prior to feeding on Day 4 **(test day),** one group of rats was administered V, while the other 3 were given CCK-8. Analysis of 60min intakes indicated that CCK-8 on the **test** day suppressed feeding in rats that had received it **after** the feeding period during training, but not in those that had received it **prior to** the feeding period during training. Thus, although these 2 groups had received CCK-8 the same number of times, "tolerance" to its anorectic effects developed only in the group which had had the drug injected prior to the feeding period. These results suggest that alterations in drug metabolism or receptor mechanisms cannot fully account for the development of tolerance to CCK-8. In other studies, we found that the development of tolerance was slower in 4h deprived rats than in rats maintained on the feeding schedule described above.

Our results suggest that learning mechanisms and the animal's motivational state may be involved in the development of tolerance to the anorectic effects of CCK-8.

597.11

EFFECTS OF CCK-8 AND VARIOUS ANORECTIC DRUGS ON THE LICKING BEHAVIOR OF RATS. K.E. Asin[1]*, J.D. Davis[2] and L. Bednarz[1], Neuroscience Res. Div., Pharmaceutical Discovery, Abbott Labs, D-47U Bldg-AP10, Abbott Park, IL 60064 and [2]Univ. IL-Chicago, Dept. Psychol., Chicago, IL 60680.

The licking behavior of rats can be reduced to its component parts which can then be described qualitatively and quantitatively. Detailed analysis of these behaviors may prove to be useful for comparing how different classes of anorectic drugs act to suppress intakes. In the present studies we examined the licking behavior of rats drinking sucrose following the injection of various clinically used anorectic drugs and CCK-8.

Male, Sprague-Dawley rats were trained to consume a 0.4M sucrose solution over a 30 min period in cages equipped with lickometers. On test days, rats were injected (ip) with various doses of either the catecholamine (CA) agonists d-amphetamine sulfate (AMPH)(0-1 mg/kg) or phenylpropanolamine (PPA)(0-16 mg/kg), or with the indirect serotonergic (5HT) agonists d-fenfluramine (FEN)(0-5 mg/kg) or fluoxetine (FLU) (0-10 mg/kg) 30 min prior to drinking. Other rats were treated with CCK-8 (0-10 nm/kg) 10 min prior to the drinking session.

AMPH and PPA suppressed intakes primarily by reducing the number of bursts of licking, perhaps reflecting the stimulant properties of these drugs. In contrast, FEN and FLU reduced intakes by reducing the size of the burst and by increasing the number of licks/ml (ie: reducing lick efficiency). Burst size was also reduced by CCK-8. Reductions in burst size have been reported to occur in response to decreases in the palatability of the solution being consumed (Davis 1989). Thus, as a class, the CA anorectics reduced intakes by affecting mechanisms distinct from those altered by the 5HT drugs. The effects of CCK-8 were most similar to those of FLU and FEN; more detailed analyses will be presented.

These studies demonstrate that the microstructural analysis of licking behaviors better characterizes how anorectic drugs act to suppress intakes than does simple measurement of volume consumed.

597.12

CIS-FLUPENTHIXOL DOES NOT REVERSE THE INHIBITION OF FOOD INTAKE PRODUCED BY CCK-8 L.H. Schneider*, J. Dokko, J. Gibbs and G.P. Smith. Dept. of Psychiatry, NY Hospital-Cornell Medical Center, Bourne Laboratory, White Plains, NY 10605

PURPOSE We wanted to investigate the report that a dose of the dopamine (DA) antagonist cis-flupenthixol (FLU) reversed the inhibition of food intake produced by CCK-8 (Linden, *Acta Physiol. Scand.*, 137, 1989).

METHODS Male S-D rats (n=7) had 30-min access to a 10% sucrose solution following a 6 h pellet deprivation and ip injections at -15 min (saline or FLU) and -5 min (saline or CCK-8), using a crossover design.

RESULTS Baseline test intake (ml/30 min) was 11.4 (± 0.8) [mean (± SE)]. CCK-8 (4 ug/kg) decreased intake significantly [5.1. ± 0.8; $p < .001$)]. Pretreatment with FLU (0.1 mg/kg) did not change the inhibitory effect of CCK-8 [5.7 (± 0.7)]. This dose of FLU did not inhibit test intake [12.3 (± 1.6)].

CONCLUSION Under our conditions, FLU did not reverse the inhibition of food intake produced by CCK-8. Thus, we did not find the effect reported by Linden. Possible reasons for this disparity include the test diet and period she used (1 h access to rat chow pellets). Further work is required to demonstrate that the satiating effect of CCK-8 on food intake may be modulated by dopaminergic mechanisms.

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597.13

EFFECTS OF ISLET AMYLOID POLYPEPTIDE (IAPP) ON FOOD INTAKE IN RATS. J.E. Blevins*, T.E. Adrian, R.D. Reidelberger. Veterans Administration Medical Center and Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE 68105.

IAPP (also called amylin) inhibits feeding when injected intrahypothalamically in rats and intraperitoneally in mice. Because amylin is secreted from pancreatic islets into the circulation in response to feeding, we examined the effects of intravenous administration of IAPP on food intake. Rats (n=9-14) with chronic jugular vein catheters received bolus injections of vehicle and either rat or human IAPP (5 and 10 nmol/kg) or continuous infusion of vehicle and rat IAPP (2 nmol/kg-h for 2 h) at the beginning of the dark period after a 5-h fast. Ingestion of ground chow was measured 1, 2, 4, and 24 h later. ***Results***: Human IAPP did not inhibit feeding. Rat IAPP suppressed feeding at each dose. Cumulative food intake was inhibited by 5 nmol/kg at 1 h (50%, $P<0.001$) and 2 h (35%, $P<0.01$); by 10 nmol/kg at 1 h (42%, $P<0.05$), 2 h (49%, $P<0.01$), and 4 h (20%, $P<0.05$); and by 2 nmol/kg-h at 2 h (24%, $P<0.001$) and 4 h (17%, $P<0.01$). ***Conclusions***: 1) Circulating IAPP may play an important physiological and/or pathophysiological role in control of food intake. 2) The differences in amino acid sequence between rat and human IAPP (6 of 37 amino acids) may be sufficient to impair binding of human IAPP to the receptor involved in the feeding response.

597.14

C-FOS IMMUNOHISTOCHEMICAL LOCALIZATION OF CENTRAL SITES RESPONSIVE TO PERIPHERAL ENTEROSTATIN. Q. Tian, L. Lin, D.A. York* and G.A. Bray. Pennington Biomedical Research Center, Baton Rouge, LA 70808.

Enterostatin is the aminoterminal pentapeptide of pancreatic procolipase which is released by tryptic cleavage. We have shown that enterostatin is a potent inhibitor of food intake and that this anorectic effect is specific to fat when rats are given a choice of macronutrients. The peptide is effective after peripheral or central administration. To gain insight into the possible sites of action in the CNS, we have compared the effects of peripherally administered enterostatin and the lipoprivic feeding agent, 2-mercaptoacetate (2MA) on induction of the immediate-early gene c-fos. Rats were adapted to a high fat diet for 10 days and then injected with either 80 μg enterostatin, 2MA (800 μMol/kg) or saline vehicle immediately prior to providing access to food. After two hours, the rats were perfused intracardially with 4% paraformaldehyde, the brain removed, fixed and subsequently 50 μM sections were cut. Sections were incubated with c-fos antisera and antibody-antigen complexes detected by a biotinylated IgG-Steptavidin/peroxidase procedure. The distribution of c-fos immunoreactivity induced by enterostatin and 2MA was different. Enterostatin induced c-fos appearance in the Supraoptic, Arcuate and Pons nuclei, nucleus tractus solitarius, periaqueductal grey and the hippocampus. In contrast, the 2MA induced c-fos immunoreactivity predominated in the Area Postrema, Dorsomotor Nucleus, Parabrachial nucleus, Central nucleus of amygdala and the locus coruleus. The data suggest enterostatin and 2MA may act at different sites to regulate fat feeding.

597.15

CHRONIC RESTRAINT STRESS ENHANCES THE PROPHAGIC EFFECT OF THE κ-AGONIST U-50,488H . A. Badiani * and J. Stewart. Center for Studies in Behavioral Neurobiology, Psychology, Concordia University, Montréal, Québec, Canada, H3G 1M8.

Chronic treatment with amphetamine enhances the prophagic effect of the kappa-opioid agonist U-50,488H (U50). Since, cross-sensitization between stress and amphetamine has been reported, we studied the effect of chronic stress on the feeding response to U50.

An automatized apparatus was used for the continuous monitoring of feeding, drinking, and locomotor activity. Eight male Wistar rats underwent ten daily (6 h after the onset of the light phase) stress sessions (20 min of restraint) whereas 8 control animals were only briefly handled. All rats had food and water *ad libitum*. There was a slight increase in food intake in stressed animals in the 30 min following stress. After the last stress session the rats were left undisturbed for 120 hours and then tested for two consecutive days in a counterbalanced order, each animal being injected i.p. with saline on one day and with 3 mg/kg of U50 on the other day. U50 increased feeding and reduced drinking in the following two hours; more rats ate (100% vs. 62.5% after saline) and ate a larger first meal. The prophagic effect of U50 was greater in stressed rats. Food intake in the first hour was 2.81±0.37 g in group Stress-U50 vs. 1.77±0.41 g in group Control-U50. The size of the first meal (intermeal interval ≥ 30 min) was 3.19±0.46 g in group Stress-U50 vs. 1.79±0.35 g in group Control-U50. The antidipsic effects of U50 was unchanged.

These results demonstrate that chronic exposure to a relatively mild and non-anorectic stress can sensitize the rat to the prophagic effect of the highly selective kappa-opioid agonist U-50,488H.

597.16

DECREASED FOOD-INTAKE AND MORPHINE ANALGESIA FOLLOWING CENTRAL GYLBENCLAMIDE. D.S. Roane*, and N.E. Boyd. Div. Pharmacology and Toxicology, School of Pharmacy, NE La. Univ., Monroe, LA 71209-0470

Previous research has indicated the presence of a reciprocal relationship between food-intake and opioid mediated analgesia. We believe the most likely cellular candidate common to both ingestive and nociceptive behaviors is the ATP-sensitive K^+ channel (K_{ATP}). This ion channel appears to be opened by mu and delta-1 opioid receptor agonists in the service of analgesia and closed as cellular ATP rises -- potentially providing an explanation for why some feeding paradigms and alterations in glucose metabolism affect morphine analgesia.

To further examine the role of the K_{ATP} in the relationship between feeding and opioid function we administered 80 nmol of glybenclamide (an K_{ATP} antagonist) into male S.D. rats via the lateral ventricle. Chow consumption in the treated animals was significantly reduced over the following 48 hours ($F=2.62$, $p<0.013$) with the peak effect (78% of control) occurring at 6 h. In the tail-flick test, 4 mg/kg morphine sulfate provided analgesia of 42.38 ± 8.4% and 18.89 ± 7.67% in vehicle and treated animals, respectively, ($p<0.05$, n=8/group, one-tailed t-test). These results support the hypothesis that food-intake and analgesia are reciprocally modulated through activity at the K_{ATP}.

597.17

GRF-INDUCED FEEDING IS AN OPIOID-DEPENDENT AND PROTEIN SELECTIVE EFFECT. P.R.Dickson*[1] and F. J. Vaccarino[2]. 1. Dept Psychology, Northeastern University, Boston, Ma. 02115 2. Dept Psychology, University of Toronto, Toronto, Canada, M5S 1A1

Work in our lab has shown that growth hormone-releasing factor (GRF) acts centrally (in the suprachiasmatic nucleus/medial preoptic area, SCN/MPOA) to stimulate food intake in rats, independent of its endocrine effects. Injection of GRF into the SCN/MPOA during the middle of the light cycle stimulates protein (P) intake, while carbohydrate (C) and fat (F) intake is unaffected. This feeding effect is dependent on opiate actions in the paraventricular nucleus of the hypothalamus (PVN), since opiate blockade in this site blocks expression of GRF-induced feeding. The present experiments further explored the effects of GRF on macronutrient selection, and the role of opiates in expression of this behavior.

In all experiments, animals were given two macronutrient choices (P/10% F; C/10% F). The first study determined the effect of PVN morphine (MOR) on macronutrient selection. Animals were injected in counterbalanced order with 0, 1, 10, 17 ug MOR, and intake measured at 1, 2, and 4 hours. MOR selectively stimulated P intake up to 4 hours post-injection; the effects on C intake were inconsistent. The next study examined the ability of selective deprivation to alter the feeding effects of either SCN/MPOA GRF (1 pmol) or 2 mg/kg MOR. Rats were tested in ad lib feeding conditions, and when they had been 24 h deprived of either P or C (indep. groups). GRF increased P intake in ad lib and P deprivation conditions. C deprivation was not able to switch the GRF effect from augmentation of P intake. Systemic MOR showed variable effects, increasing P intake following P deprivation, and C intake following C deprivation. A final study examined involvement of GRF in expression of circadian patterns of macronutrient selection. Injection of GRF antibodies into the SCN/MPOA resulted in a selective, dose-dependent suppression of nomally elevated P intake at dark onset. Conclusions of these studies are that SCN/MPOA GRF is involved in feeding behavior and nutrient regulation mediated by PVN opioids, and this effect is P selective. NSERC grant #35036 to FJV.

597.18

MORPHINE-INDUCED FEEDING: A COMPARISON OF THE LEWIS AND FISCHER 344 INBRED RAT STRAINS. D.D. Krahn and B.A. Gosnell, Dept. of Psychiatry, University of Michigan, Ann Arbor, MI 48109.

Rats of the Lewis inbred strain have been shown to self-administer more morphine than rats of the inbred Fischer 344 (F344) strain (Suzuki et al., *Japan. J. Pharmacol.* 47:425, 1988). We therefore measured whether these stains also differ in their feeding response to morphine. In Exp. 1, rats were maintained on powdered rat chow and given s.c. injections of morphine sulfate (1, 3 or 10 mg/kg) or saline. Food intake was measured 2, 4 and 6 hrs after injection. This procedure was repeated 3 additional times (2 days apart), such that all rats were tested with all doses of morphine and with saline. In Exp. 2, rats were given a choice of 2 diets: a fat/protein diet and a carbohydrate/protein diet. Feeding responses to morphine were measured in a manner identical to that in Exp. 1. In both experiments, the feeding response to morphine was greater in Lewis rats than in F344 rats. To determine whether these responses might be explained by differences in the levels of morphine achieved in blood or brain, rats of each strain were given s.c. injections of morphine sulfate (3 mg/kg) and sacrificed either 30 min (n=6/strain) or 3 hrs (n=6/strain) after injection. Serum and brain morphine levels were determined by RIA. Lewis rats had significantly less brain morphine than F344 rats at 30 min (61±2 vs. 42±1 ng/g, $p<.05$); they did not differ in morphine content at 3 hrs. Serum levels were similar at 30 min and at 3 hrs. Thus, differences in tissue levels cannot readily explain the differences in feeding responses to morphine. These results indicate a strain difference in the feeding response to morphine which complements previously observed differences between Lewis and Fischer 344 rats in the self-administration of morphine. Supported by NIDA Grants DA05471 and DA06827.

597.19

THE EFFECTS OF CONTINUOUS MORPHINE INFUSIONS ON DIET SELECTION AND FOOD INTAKE. B.A. Gosnell and D.D. Krahn. Dept. of Psychiatry, University of Michigan, Ann Arbor, MI 48109.

The administration of morphine causes a short-term increase in food intake, and repeated administration of morphine has been shown to cause progressively larger increases in intake and/or the relative intake of dietary fat. In this experiment, we measured the effects of continuous morphine infusions on diet choice and total intake. Male rats were given ad lib access to two diets: a high-carbohydrate diet (CHO; 80% carb., 20% protein) and a high-fat diet (FAT; 80% fat, 20% protein). Diet intakes were measured daily for 21 days. Via the implantation of osmotic minipumps, one group (n=11) received continuous infusions of morphine sulfate (approx. 2.8 mg/kg/hr) for days 1-7 and of saline for days 8-14. A second group (n=11) was infused with saline for days 1-7 and with morphine for days 8-14. A third group (n=12) received sham surgery but no minipumps. Regardless of whether morphine was infused when the diets were introduced or after 7 days of adaptation to the diets, morphine caused a significant decrease in CHO intake for the first 5 days of the 7 day infusion period (compared to the sham group). Intake of the FAT diet was generally elevated at the beginning of the drug infusion period and depressed toward the end of this period. Total caloric intake was significantly decreased on final 6 days of morphine infusions. Percentage of total caloric intake consumed from the FAT diet was significantly increased for only the first 2 days of the infusion periods. Termination of morphine infusions caused a decrease in the intake of both diets, with no change in relative diet preference; intakes generally returned to control (sham) levels within 1-3 days. These effects (a sustained decrease in total intake with only an initial increase in fat preference) differ from those reported after repeated intermittent administration of morphine in short-term trials. Supported by NIDA Grants DA05471 and DA06827.

597.20

DIFFERENTIAL INVOLVEMENT OF CENTRAL OPIOID RECEPTOR SUBTYPES IN SACCHARIN INTAKE IN RATS. I.W. Beczkowska*, J.E. Koch and R.J. Bodnar. Dept. of Psychology, Queens Col., CUNY, Flushing, NY 11367.

Saccharin intake in rats is decreased following naltrexone (NTX) and increased following mu and delta agonists. Our laboratory recently evaluated the central roles of general (NTX), mu (beta-funaltrexamine, B-FNA), kappa (nor-binaltorphamine, Nor-BNI), mu_1 (naloxonazine), $delta_1$ (DALCE) and $delta_2$ (naltrindole, NTI) opioid antagonists upon intake of sucrose which possesses palatable and nutritive qualities. Sucrose intake was reduced by NTX, B-FNA and Nor-BNI, implicating mu_2 and kappa receptors. The present study evaluated over 1 h central antagonist effects upon intake of saccharin which possesses palatable qualities without postingestive consequences. NTX (20-50 ug) significantly reduced saccharin intake (25-60 min, 60-67%) confirming opioid involvement. In contrast, neither B-FNA nor Nor-BNI affected saccharin intake, suggesting that mu and kappa effects upon sucrose intake altered postingestive factors. Saccharin intake was significantly reduced by DALCE (5-15 min, 51-60%) and NTI (5-60 min, 65-80%), implicating delta receptor mediation. Since sucrose and saccharin differ in their postingestive consequences, any opioid involvement in palatability should consider post-ingestive factors in assessing receptor mediation. (Supported by DA04194).

597.21

DIFFERENTIAL INVOLVEMENT OF CENTRAL OPIOID RECEPTOR SUBTYPES IN TAIL-PINCH FEEDING IN RATS. J.E. Koch* and R.J. Bodnar. Dept. of Psychology, Queens Col., CUNY, Flushing, NY 11367.

Hyperphagia during tail-pinch stress is decreased following general (e.g., Science 209: 1259-1261, 1980; Life Sci. 26: 2113-2118, 1980) and mu (CTOP, Soc. Neurosci. Abstr. 17: 491, 1991), but not by kappa (nor-binaltorphamine, Nor-BNI) or delta (naltrindole, NTI) opioid antagonists. Since there are multiple mu (mu_1 & mu_2) and delta ($delta_1$ & $delta_2$) opioid receptor subtypes, the present study evaluated whether tail-pinch feeding was differentially altered following central administration of mu (beta-funaltrexamine, B-FNA), mu_1 (naloxonazine, NAZ), kappa (Nor-BNI), $delta_1$ (DALCE) and $delta_2$ (NTI) antagonists. Like CTOP, B-FNA significantly and dose-dependently (1-20 ug, icv) reduced tail-pinch eating by 30%. In contrast, NAZ (50 ug) failed to alter tail-pinch feeding, implicating the mu_2 opioid receptor subtype. Neither Nor-BNI (1-20 ug), DALCE (40 ug) nor NTI (20 ug) altered tail-pinch eating, indicating that kappa, $delta_1$ and $delta_2$ binding sites are involved. Since no antagonist altered the latency or duration of food contact during tail-pinch, opioids appear to modulate ingestive rather than activational mechanisms. The selective influence of the mu_2 binding site in tail-pinch feeding is similar to its effects on glucoprivic, high-fat and sucrose intake. (Supported by DA04194 and DA07135).

DRUGS OF ABUSE: ETHANOL AND MONOAMINES

598.1

SEROTONIN POTENTIATES ETHANOL-INDUCED EXCITATION OF VTA NEURONS *IN VITRO*, M.S. Brodie* and S.A. Shefner, Dept. Physiology and Biophysics, University of Illinois, College of Medicine, Chicago, IL 60680.

Dopamine neurons of the ventral tegmental area (VTA) are important components of brain pathways which mediate drug-induced reward. Ethanol (EtOH) increases the firing rate of VTA neurons *in vivo* (Gessa, et al., 1985), and *in vitro* (Brodie, et al., 1990). The EtOH-induced increase in firing rate of VTA neurons may underlie the rewarding effects of ethanol. It has been shown that serotonin reuptake blockers reduce alcohol intake in human alcoholics and in animal studies. We have begun studies to assess the effects of serotonin (5-HT) on ethanol-induced excitation of VTA neurons.

Coronal brain slices containing the VTA were prepared from young adult rats (100 to 200 gm), and the slice was superfused in a recording chamber maintained at 35-36° C. Twenty-one neurons from 17 rats were studied. All drugs were administered in the superfusate. Concentrations of ethanol (40 - 160 mM) were tested in the absence and presence of 5-HT (1 - 50 μM). All neurons studied were excited by EtOH, and had electrophysiological characteristics typical of putative dopamine-containing neurons. In most cases, serotonin alone had a small, transient excitatory effect on these neurons; this excitation subsided within 15 minutes. In 15 of 21 neurons (71%), the excitatory effect of ethanol was increased in the presence of 5-HT by more than 10%. Increases in EtOH-induced excitation of 100% over pre-5-HT controls were seen typically, while the largest increase in EtOH excitation observed was 821%.

These studies suggest that serotonergic agents may be useful in altering the potency of EtOH in reward areas of the brain and may provide an opportunity for the development of pharmacotherapeutic agents useful in the treatment of alcoholism. Supported by P.H.S. grant #AA-05846-09.

598.2

EVALUATION OF m-CHLOROPHENYLPIPERAZINE (mCPP) EFFECTS ON ETHANOL (E) INTAKE AND BEHAVIOUR IN WISTAR RATS. D.M. Tomkins, Y. Buczek and E.M. Sellers*. Departments of Pharmacology, Medicine and Psychiatry, University of Toronto and Clinical Research and Treatment Institute, Addiction Research Foundation, Toronto, Ontario M5S 2S1, Canada.

The 5-HT_1 agonist, mCPP, has been reported to produce subjective effects similar to those following alcohol intake and increase desire for alcohol in chronic alcoholics. The aim of the present study was to assess the effects of mCPP on E intake and the behavioural profile exhibited by the rats prior to and during an E limited access procedure. Following an acquisition phase, rats were placed in individual drinking cages at 1630 h each day and 15 mins later were given 40 mins access to 12% E solution and water. The rats' behaviour was scored every 15 secs as: drinking, active, grooming or resting. In E consuming rats (0.55 ± 0.06 g/kg), mCPP (0.1-1 mg/kg s.c.) significantly reduced E intake by 33% to 85% ($p < 0.05$) compared to vehicle treatment days. Water intake was unaffected. On vehicle-treated days, rats exhibited a consistent behavioural profile. Following 1 mg/kg mCPP, prior to E access, rats rested more and exhibited fewer tube directed behaviours ($p < 0.05$). Rats with no E preference (0.07 ± 0.01 g/kg) also exhibited a similar shift in their behavioural profile. Thus, mCPP caused a marked attenuation in E intake in E-preferring rats. At the lower doses investigated this reduction was selective and not due to behavioural disruption. The effect on preparatory behaviour at 1 mg/kg may reflect a reduced anticipation for E. Alternatively, the anxiogenic properties of mCPP may underlie this profile as is supported by our observations in the low drinking rats.

598.3

ETHANOL PRODUCES NALOXONE-BLOCKABLE ENHANCEMENT OF EXTRACELLULAR DOPAMINE IN NUCLEUS ACCUMBENS OF LEWIS RATS. E.L. Gardner* and J. Chen, Departments of Psychiatry and Neuroscience, Albert Einstein College of Medicine, New York, NY 10461

Activation of brain reward mechanisms is hypothesized to constitute the essential reinforcement produced by abusable substances (Wise, *Pharmacol. Biochem. Behav.* 13[suppl.1]:213-223, 1980), and brain dopamine (DA) systems projecting to the nucleus accumbens (Acc) are hypothesized to constitute a crucial substrate for brain reward (Wise & Bozarth, *Brain Res. Bull.* 12:203-208, 1984). However, the action of ethanol on these brain systems has been less clear than for other abusable drugs. We now report that acute ethanol challenge (0.5-2.5 g/kg, i.p.) induces a dose-dependent increase in basal extracellular levels of DA and its main metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in Acc of freely moving Lewis rats as measured by *in vivo* brain microdialysis. Naloxone at 0.1 mg/kg i.p. produced 50% blockade of this effect, and naloxone at 0.5 mg/kg i.p. produced 100% blockade. These data confirm previous microdialysis findings of ethanol-induced activation of forebrain DA systems (Di Chiara & Imperato, *Proc. Nat. Acad. Sci. U.S.A.* 85:5274-5278, 1988), but additionally implicate endogenous opioid peptide mechanisms in ethanol's activation of brain reward substrates. Thus, the present data may help to explain previous findings that naloxone blocks ethanol's enhancing effects on electrical brain-stimulation reward (Lorens & Sainati, *Life Sci.* 23:1359-1364, 1978). (Supported by NIH grant RR 05397, NIDA grant DA 03622, and a research grant from the Aaron Diamond Foundation).

598.4

QUINPIROLE MICROINJECTED INTO THE VENTRAL TEGMENTAL AREA, DECREASES ETHANOL REINFORCED RESPONDING IN THE RAT. H.H. Samson*, C.W. Hodge, M. Haraguchi, R.S. Lewis and H.L. Erickson. Alcohol and Drug Abuse Institute, University of Washington, Seattle, WA 98195.

The role of the mesolimbic dopamine system in drug reinforcement processes has received wide attention over the last several years. However, the role this system may play in ethanol reinforcement remains to be elucidated. In this study, rats were trained to lever press for oral ethanol (10% v/v) reinforcement using a sucrose-substitution procedure. They were neither food nor water restricted during the experiment. When stable responding was achieved, bilateral cannula guides were implanted 1mm above the ventral tegmental area (VTA). Following recovery, quinpirole at doses of 0.001, 0.01, 0.1 and 1.0 ug/brain injected into the VTA were tested at weekly intervals.

At doses of 0.1 and 1.0, responding was significantly decreased compared to both saline and sham control sessions. The decrease in responding was primarily a result of an early termination of the normal response pattern. This effect was identical to that observed in prior research on the response pattern following microinjection of the D2 DA antagonist raclopride into the n. accumbens. The results suggest that a blockage of DA transmission in the mesolimbic dopamine system at either the VTA or the n. accumbens reduces ethanol reinforced behavior. This supports the hypothesis that ethanol reinforcement involves this mesolimbic DA pathway as implicated for reinforcement with other drugs of abuse.

598.5

SENSITIZATION TO THE DOPAMINE RELEASE-ENHANCING EFFECTS OF ETHANOL DEMONSTRATED IN MALE LONG-EVANS RATS. D. Benjamin*, E.R. Grant, K.R. Goldstein, and L.A. Pohorecky. Center of Alcohol Studies, Rutgers University, Piscataway, NJ 08855.

In contrast to Wistar and Sprague-Dawley rats, dopamine (DA) release in the n. accumbens (NA) of male Long Evans rats does not increase in response to an initial exposure to ethanol (ET). The present study characterized the effect of repeated exposures to ET on the release of DA, serotonin (5-HT), and their metabolites using intracerebral microdialysis coupled with HPLC-EC. ET-naive male Long-Evans rats were implanted with guide cannulae over the NA and the ventral tegmental area (VTA). One week after surgery, microdialysis probes with active membrane lengths of 2.5 mm in the NA and 2 mm in the VTA were lowered. The probes were perfused with Ringer's solution at a rate of 1 μl/min. On the first day of dialysis, baseline amounts of neurochemicals were determined over 3-5 samples taken at 20 min intervals, after which saline was injected IP. Following one or more post-saline samples, ET (1.0 g/kg, IP) was injected and 4 samples were collected, then a second 1 g/kg injection was given. This procedure was then repeated 24, 48, or 72 h later. We have found that, in Long-Evans rats, DA release in the NA does not increase in response to the initial exposure to ET. Subsequent exposure to 1 g/kg caused 303$\pm$11% ($p < 0.05$, n=6) increments in DA release in the NA 24 h later. Substantial increments were found at the later timepoints. These results demonstrate increases in the response of the mesolimbic DA system to ET with repeated exposures, and suggest that increases in DA release in the NA might underlie sensitization to the reinforcing/rewarding effects of ET in Long-Evans rats (see Goldstein *et al.*, this volume). Further characterization of the mesolimbic DA sensitization to ET is in progress. (Supported by Smithers Prevention and NIAAA grants AA05306 and AA08499).

598.6

EFFECT OF ETHANOL ON THE RELEASE OF MONOAMINES IN THE NUCLEUS ACCUMBENS OF THE ALCOHOL PREFERRING AA AND ALCOHOL AVOIDING ANA RATS. K. Kiianmaa*, J. Kokkonen, M. Nurmi and I. Nykänen. Biomedical Research Center, Alko Ltd, Helsinki, Finland.

The importance of central monoamines, with the emphasis on the dopaminergic neurons, in the control of voluntary ethanol consumption was examined by studying the effect of ethanol on the extracellular levels of monoamines in the nucleus accumbens of the alcohol preferring AA (Alko Alcohol) and alcohol avoiding ANA (Alko Nonalcohol) rats with *in vivo* microdialysis. Samples were collected from freely moving animals every 15 minutes, and concentrations of the monoamines and their metabolites were determined in the dialysate with smallbore HPLC. Ethanol (0.5, 1, or 2 g/kg, IP) significantly increased the extracellular levels of DOPAC and HVA in a dose dependent manner suggesting stimulation of dopamine release by ethanol. A similar trend was found with dopamine itself although the results from the present material did not reach significance. The extracellular levels of 5-HT, 5-HIAA or noradrenaline were not affected.

598.7

ETHANOL AND SEROTONIN (5-HT)-MEDIATED MEMBRANE RESPONSES IN RAT HIPPOCAMPAL CA1 NEURONS. Alex. H.L.Lau* and Gerald.D. Frye. Dept. of Med. Pharmacol. & Toxicol., Texas A&M Univ., Coll. of Med., College Station, TX 77843-1114.

Increasing evidence suggests that 5-HT plays an important role in ethanol tolerance and reinforcement. Intracellular recordings in hippocampal brain slices were used to evaluate the effects of ethanol on 5-HT mediated membrane hyperpolarization and spike afterhyperpolarizations (AHPs) block in CA1 pyramidal neurons. Neither acute ethanol (30 mM) treatment in vitro nor in vivo ethanol dependence affected 5HT-mediated (1-100μM) membrane hyperpolarization. Acute ethanol (30mM) increased AHPs during the 40 min superfusion making its apparent biphasic effect on 5-HT-mediated (1-100μM) inhibition of AHPs difficult to interpret. Most striking was the complete block of ethanol-induced enhancement of the AHP by 5-HT (100μM). In vivo ethanol dependence did not alter 5-HT inhibition of the AHP. These results suggest that ethanol may interact with presumed 5-HT_4 receptors thought to mediate AHP block in the hippocampus, while 5-HT_{1A} receptors appear relatively resistant to ethanol. Supported in part by AA06322 & RSDA, AA00101 to GDF.

598.8

BEHAVIORAL EVIDENCE FOR DOWN-REGULATION OF 5HT3 RECEPTORS PRODUCED BY CHRONIC ETHANOL. C.J. Wallis, S.M. Rezazadeh , and H. Lal* Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX, 76107.

Ethanol treatment increases the efficacy of ligand gated ion channels; thus it may result in down regulation of such receptors. In the 5HT receptor family, only 5HT3 receptors are located on ligand gated ion channels. MDL72222 (MDL, a 5HT3 receptor antagonist, 10 mg/Kg,1h) produced no effect on the time spent in the open arms of an elevated plus maze (EPM, Lal et al., Alcohol 8:467-471, 1991) in naive rats, but significantly reduced the total arm entries. In these rats, MDL (10 mg/Kg) given 1h prior to an injection of ETOH (1.5 g/Kg, ip) resulted in a reduction of ETOH-induced incoordination (rotorod) without changing the rate of recovery (additive with ETOH tolerance). After chronic ETOH treatment (7 d in liquid diet, 4.5%), open arm and total arm activity was reduced in animals tested 12 h after a final dose of ETOH, 3 g/Kg. Animals given MDL (5,10, or 20 mg/Kg, 1h) showed a further reduction in open arm and total arm activity, demonstrating exacerbation of these symptoms of ETOH withdrawal. MDL (10mg/Kg) given to naive animals trained to discriminate pentylenetetrazol (PTZ) from saline (Lal et al., JPET 247:508-518, 1988) resulted in a reduction of the threshold dose for PTZ discrimination. ETOH withdrawal also reduced the threshold dose for PTZ discrimination and this effect was additive with that of MDL. It is hypothesized that ETOH treatment results in down regulation of 5HT3 receptors as part of the development of tolerance and/or dependence. Supported by NIAAA Grant AA06890

598.9

EXTRACELLULAR CONCENTRATIONS OF DOPAC, HVA AND 5HIAA IN NUCLEUS ACCUMBENS ARE CORRELATED WITH MEASURES OF ETHANOL-SEEKING BEHAVIOR IN RATS. B.A. Blanchard*, S. Wang, S. Steindorf & S.D. Glick, Dept. of Pharmacology & Toxicology, Albany Medical College, Albany, NY 12208

The mesolimbic dopamine (DA) system has been proposed as a neurochemical substrate for the reinforcing effects of drugs of abuse such as stimulants, opiates and ethanol (EtOH). Rats with high EtOH preference, as a group, have been reported to have lower levels of DA and 5HT in nucleus accumbens (NAC) relative to non-preferring rats. We examined the role of individual differences in DA, DOPAC, HVA and 5HIAA in NAC and striatum (STR) in individual differences in EtOH consumption. Levels of these compounds were assessed in freely moving adult male and female Long-Evans rats by in vivo microdialysis and HPLC. Rats were then trained to bar press for oral EtOH reinforcement. Intake of and preference for 10% (v/v) EtOH were significantly correlated with several neurochemical measures in NAC: DOPAC, HVA and 5HIAA, but not DA, were inversely correlated to measures of EtOH-seeking behavior. There were no significant correlations of STR metabolites with EtOH intake. These findings indicate that individual differences in EtOH intake are related to differences in neurotransmitter function in a terminal region of the mesolimbic system, providing further support for its role in the reinforcing properties of EtOH.

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598.10

EFFECTS OF CHRONIC ETHANOL IN NMDA-EVOKED RELEASE OF ^{3}H-NORADRENALINE IN THE RAT HIPPOCAMPUS. R.Labarca[1], C.Sepúlveda[1], M.Seguel[1], K.Gysling[2], G.Bustos*.[1]Faculties of Medicine and [2]Biological Sciences, Catholic University, Santiago, Chile.

Recent findings support the notion that ethanol-induced behavior is mediated, at least in part, by the NMDA/ionophore complex. For this reason, we decided to study the effects of chronic ethanol administration on the function of NMDA receptor using NMDA-evoked release of ^{3}H-Noradrenaline from hippocampal slices as a functional model.

In CA1-CA3, ethanol in vitro, at contrations of 10, 20,50 and 100 mM, evoked the release of aspartate (ASP), the highest concentrations (50 and 100 mM) being less potent; omission of Ca++ ions from the superfusion media completely abolished the ethanol-induced effect. The evoked release of GLU was much weaker, and only observed at higher concentrations of ethanol (20,50 and 100 mM). At 20 mM, ethanol enhanced K+-evoked release of ASP but not that of GLU.

Twenty four hours after a chronic liquid ethanol diet for 3 months, NMDA-evoked release of ^{3}H-Noradrenaline was marginally augmented in the dentate girus (DG) but not in CA1-CA3. After 30 days of withdrawal, however, the NMDA-evoked release of ^{3}H-Noradrenaline in the DG was Markedly inhibited, whereas in CA1-CA3 a trend of this phenomenon was observed, without reaching statistical significance.

These results suggest that chronic ethanol consumption produces changes in NMDA/ionophore complex that may be related, at least in part, to the pharmacological effects of ethanol. (Supported by Fondecyt Nº 0739/91).

DRUGS OF ABUSE: COCAINE AND BIOCHEMISTRY

599.1

ACUTE AND CHRONIC COCAINE ADMINISTRATION: DOSE RESPONSE EFFECTS ON ZIF268, C-FOS, PREPROENKEPHALIN, AND PREPRODYNORPHIN mRNAs IN RAT BRAIN. J.B. Daunais*, W.T. Bohler, and J.F. McGinty Dept. of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, N.C. 27858.

Wistar rats were administered saline or cocaine HCl (NIDA) i.p. at 10, 20, or 30 mg/kg once daily for 1 or 10 days. One hour following the last injection, the rats were anesthetized and decapitated. Brains were removed and frozen until 12µm sections were cut on a cryostat. Sections were fixed, defatted, pretreated, and hybridized with a 48mer oligonucleotide probe to preprodynorphin (PPD) or preproenkephalin (PPE), or a 40mer oligo probe to c-fos or zif268 at 37°C for 20hr. After stringent washing, the slides were dried and apposed to Kodak X-OMAT film for 2 wk (PPD) or 1 wk (PPE, c-fos, zif).

Zif268 mRNA increased in a dose-dependent fashion after acute and chronic cocaine in layers IV and VI of frontal and parietal cortex, layer II of piriform cortex, olfactory tubercle, dorsal striatum including the fundus striati, islands of Calleja, and tenia tecta. Acute cocaine induced a far more robust signal than chronic cocaine in these areas. Minimal induction of Zif268 hybridization signal was present in the nucleus accumbens, particularly in the core of cocaine-treated animals after acute or chronic administration.

In the acutely treated rats, c-fos mRNA increased dose dependently in piriform cortex, olfactory tubercle, and dorsal striatum, whereas signal was lacking in the chronic cocaine groups, except in the piriform cortex, indicating a downregulation of c-fos.

PPE and PPD signal hybridization were robust in the dorsal and ventral striatum of all groups. Digital image analysis is currently under way to determine if there are any appreciable differences in signal between the cocaine-treated animals and their respective controls after acute or chronic administration.. These data suggest that widespread changes occurring at the molecular level after acute cocaine may differ from those following chronic cocaine. Supported by DA 03982.

599.2

AP-1 AND CRE-BINDING ACTIVITY ARE REGULATED IN THE RAT LOCUS COERULEUS AND NUCLEUS ACCUMBENS FOLLOWING CHRONIC MORPHINE OR COCAINE. B.T. Hope*, H. Nye, and E.J. Nestler, Lab. of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale School of Medicine, New Haven, CT 06508.

We have studied changes associated with transcription factors, which control gene expression, in two model systems of drug addiction in the rat: chronic morphine in the locus coeruleus (LC) and chronic cocaine in the nucleus accumbens (NAc). Correlating with increases in c-fos and c-jun in the LC during morphine withdrawal (*Brain Res.*, 525:256), AP-1 binding in gel shift assays has now also been shown to increase. A decrease in CREB phosphorylation with acute morphine, and an increase during morphine withdrawal has been shown recently in the LC (*J. Neurochem.*, 58:1168). We now report that although there is no change in CRE-binding during acute morphine, there is an increase following chronic morphine. This suggests the possibility that chronic morphine increases the total amount of some CRE-binding protein(s) in this brain region. In the NAc, in addition to the previously shown desensitization in c-fos and c-jun expression and persistent increase in AP-1 binding following chronic cocaine (Hope et al., *PNAS*, 1992), there is also an increase in CRE-binding, which increases even further following 1 week of withdrawal. There are also striking regional differences in CRE-binding patterns in gel shift assays, further indicating regional heterogeneity of transcription factors in the brain. The results indicate that levels of transcription factors are altered in the brain following chronic drug treatment, and that such alterations may underlie functional changes associated with addiction.

599.3

ACTIVATION OF TRANSCRIPTION FACTOR GENES IN STRIATUM BY COCAINE IS MEDIATED BY BLOCKADE OF BOTH 5-HT AND DA UPTAKE. **R.V. BHAT* AND J.M. BARABAN. Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.**

Cocaine elicits robust increases in the expression of several transcription factors in striatum. As this response is abolished by D_1 receptor blockade, it has been assumed that blockade of DA uptake mediates the striatal activation of transcription factors. However, we have found that the selective 5-HT uptake inhibitors, fluoxetine (5-10 mg/kg) and citalopram (2 mg/kg) which do not induce c-Fos or *zif268* by themselves, markedly potentiate the ability of mazindol, an inhibitor of DA uptake, to activate c-*fos* or *zif268* in striatum. These findings suggest that cocaine's blockade of 5-HT uptake contributes to its activation of these genes. To assess the role of the 5-HT system, rats were treated with p-chloroamphetamine to lesion the 5-HT system selectively. This treatment markedly reduces activation of *zif268* and c-*fos* by cocaine in the striatum. In contrast, lesioning of the NE system with DSP-4 does not. These results indicate that, along with the DA system, the 5-HT system plays a key role in mediating cocaine's activation of transcription factor genes in the striatum. The involvement of both these systems may help explain several anomalous features of the pharmacology of this response.

599.4

REGULATION OF PREPROTRH mRNA LEVELS IN RAT BRAIN BY CHRONIC COCAINE. K.A. Sevarino* and R.J. Primus, Div. of Molecular Psych., Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06508.

Short-term and long-term consequences of chronic cocaine exposure on levels of preproTRH (ppTRH) mRNA were examined in adult male rats. Rats received i.p. injections of cocaine hydrochloride (15 mg/kg, b.i.d.) for fourteen days, and were then sacrificed 45 min, 18 hr, 72 hr, or one week following the last cocaine injection. RNA was extracted from the amygdala (AMY), hippocampus (HIPPO), hypothalamus (HYPO), and nucleus accumbens (NAc), and levels of ppTRH mRNA were measured by solution hybridization assay. PpTRH mRNA was decreased by 30-40% in HYPO and NAc at 45 min and 18 hr following the last chronic cocaine injection. While levels of ppTRH mRNA returned to control levels by 72 hr post-injection in the NAc, levels remained significantly decreased in the HYPO after one week of cocaine withdrawal. In the AMY, ppTRH mRNA was decreased by 45% at 45 min post-injection. PpTRH mRNA levels in the AMY returned to control levels by 18 hr post-injection and then increased significantly both 72 hr and one week following cocaine withdrawal. PpTRH mRNA in the HIPPO increased slightly at 45 min post-injection, increased by 40-50% at both 18 hr and 72 hr post-injection, and remained increased, by 20%, one week following cocaine withdrawal.

This study shows that levels of ppTRH mRNA are regulated by chronic cocaine exposure in a time-dependent and region-specific manner. The long-term changes in ppTRH mRNA may suggest a role for this peptide in drug craving and addiction.

599.5

THE USE OF SUBTRACTION HYBRIDIZATION TO DETECT COCAINE UP-REGULATED MESSENGER RNAs IN RAT NUCLEUS ACCUMBENS. J.R. Walker*, E.J. Nestler, and K.A. Sevarino. Division of Molecular Psychiatry, Departments of Psychiatry and Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06508.

Cocaine is one of the most reinforcing substances known. Further, chronic use results in sensitization to the drug, a phenomenon which may be related to its powerful addictive effects. Behavioral, electrophysiological, and biochemical evidence indicates that the nucleus accumbens (NAc) is part of the neural pathway mediating the reinforcing and sensitizing properties of cocaine. To identify protein alterations in the NAc that underlie cocaine's effects, we have developed a subtraction hybridization procedure to detect cocaine-regulated mRNAs. Subtraction enrichment was performed by hybridizing cDNA synthesized from cocaine-treated NAc mRNA with mRNA from untreated rat NAc. The subtracted cDNA was used as a probe to screen a rat NAc cDNA library, and sequences representing mRNAs up-regulated by chronic cocaine were isolated. The mRNAs detected to date fall into three groups: several novel proteins, mitochondrially-encoded proteins, and artifactually-isolated ribosomal RNAs. We are currently characterizing the cocaine-regulated proteins, both known and novel, for their pharmacological specificity to cocaine, the time course of their regulation, and the anatomical pattern of their regulation. We are also improving the subtraction hybridization protocol to eliminate the artifactual detection of ribosomal RNAs.

599.6

DOPAMINE TRANSPORTER: SPECIES DIFFERENCES IN MOLECULAR WEIGHT. A. Patel, L. Markham+, R. Lew*, G. Uhl+ and M.J. Kuhar. Labs. of Mol. Pharmacology & +Mol. Neurobiology, ARC/NIDA, Box 5180, Baltimore, MD 21224.

The dopamine transporter (DAT) is glycosylated in a brain-region-species fashion, but species differences in these post-translational modifications have not been described. Four potential N-linked glycosylation sites are found in the rat DAT 2nd extracellular loop and only 3 sites in the human DAT cDNA although it encodes an almost-identical number of amino acids (see abstract by D. Vandenbergh et.al.). We now report use of the DAT photoaffinity label ^{125}I-DEEP {[^{125}I]1-[2-(diphenyl methoxy) ethyl]-4-[2-(4-azido-3-iodophenyl) piperazine} and SDS-polyacrylamide gel electrophoresis to estimate the sizes of the mature DAT protein in striatal membranes from the dog, rat, and human and its size in primate kidney COS cells expressing the rat DAT cDNA. Prestained standards were used to estimate molecular mass and 30 μM (-) cocaine was used to define nonspecific labelling. Canine, rat, human and COS cell membranes displayed transporter sizes ranging from 76 to 110 kDa. Initial results using endo- and exo-glycosidases indicated that in the striatum and nucleus accumbens, the differences in apparent transporter size largely resulted from differential glycosylation.

599.7

CHARGE ISOFORMS OF DOPAMINE TRANSPORTERS. R.A. Vaughan, M.T. McCoy and M.J. Kuhar*. NIDA Addiction Research Center, P.O. Box 1580, Baltimore, MD 21224

Dopamine transporters from rat striatum and nucleus accumbens membranes were photoaffinity labeled with either ^{125}I-DEEP, (a GBR analog) or ^{125}I-RTI-82 (a cocaine analog) (Brain Res. 1992, 596:173). Radiolabel-pure preparations were subjected to isoelectric focusing in one dimensional urea-polyacrylamide slab gels in order to investigate sample heterogeneity. Following electrofocusing, radioactivity corresponding to dopamine transporters was distributed into several sharp bands of approximate pI 5.6-6.6. Very similar patterns were obtained using tissue from either region and either photoaffinity probe. Since the predicted isoelectric point of the protein based on cDNA sequence is 7.05, the multiple acidic forms observed may be a consequence of heterogenous post-translational modifications. Enzymatic deglycosylation and dephosphorylation of dopamine transporter samples are being used to test this hypothesis.

599.8

[^{3}H]GBR12935 AND [^{3}H]BTCP LABEL MULTIPLE BINDING SITES IN RAT STRIATAL MEMBRANES. H.C. Akunne[1]*, C. Dersch[1], G.U. Char[1], J.S. Partilla[1], B.R. de Costa[2], K.C. Rice[2] and R.B. Rothman[1]. [1]LCP, NIDA Addiction Research Center, Baltimore, MD 21224 and [2]LMC, NIDDK, NIH, Bethesda, MD 20892. *Neurosciences Section, Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

The present study addressed the hypothesis that there exist multiple sites/states associated with the dopamine (DA) transporter. We used [^{3}H]GBR12935 AND [^{3}H]BTCP to label the DA transporter present in striatal membranes, and conducted the assays under identical assay conditions : 18-24 hr incubations at 4^0 C in 55.2 mM sodium phosphate buffer, pH 7.4, with a protease inhibitor cocktail. In order to obtain data suitable for quantitative curve fitting, it was necessary to periodically repurify the [^{3}H]ligands by HPLC. Under these conditions, we observed greater than 90% specific binding. The method of binding surface analysis was used to characterize the interaction of GBR12935, BTCP, mazindol, and CFT with binding sites labeled by the [^{3}H]ligands. Fitting of the data to one and two site binding models, using MLAB-PC, demonstrated that for both [^{3}H]ligands, the two site model fit the data far better than did the one site model. Structure-activity studies suggested that while each radioligand labeled a site common to both, each also labeled a distinct site. These results support the hypothesis of multiple binding sites/states associated with the DA transporter. Identification of selective agents for these sites may be valuable tools for further studies of the DA transporter.

599.9

COCAINE RECEPTORS SOLUBILIZED FROM RHESUS STRIATUM ARE HETEROGENEOUS ON THE BASIS OF CHARGE. L.M. Gracz* and B.K. Madras. Harvard Medical School, New England Regional Primate Research Center, Southborough, MA 01772.

High- and low-affinity binding components for [^{3}H]cocaine and [^{3}H]CFT (2β-carbomethoxy-3β-(4-fluorophenyl)tropane) associated with the dopamine transporter have been identified in monkey caudate-putamen. The heterogeneity of binding sites for [^{3}H]CFT was not an artifact produced by the freezing and thawing of tissue as both sites were observed in freshly prepared and in frozen striatal homogenates from squirrel monkey. Futhermore, both components of [^{3}H]CFT binding were preserved in solubilized striatal membranes (1% digitonin in Tris-HCl buffer; 25% yield) from rhesus monkey. Size-exclusion gel chromatography of the solubilized preparation failed to resolve two distinct binding components and instead yielded a single, broad peak of [^{3}H]CFT binding centered about an apparent molecular weight of 200,000. In contrast, DEAE chromatography yielded three distinct peaks of [^{3}H]CFT binding. The pharmacological profiles of the peaks were determined in competition experiments with [^{3}H]CFT and other drugs (n = 2). [^{3}H]CFT binding was fully inhibited by cocaine cogeners, monoamine uptake inhibitors, and dopamine in each peak. Additionally, the peaks exhibited different high- and low-affinity binding profiles for cocaine, GBR 12909, and dopamine. However, the individual DEAE peaks could not be resolved by size-exclusion gel chromatography. The results suggest that cocaine binding site heterogeneity may be a function of the charge state of the dopamine transporter. Supported by USPHS grants DA00499, DA06303, MH14275, and RR00168.

599.10

CATIONIC AND ANIONIC REQUIREMENTS FOR THE BINDING OF [^{3}H]CFT TO THE DOPAMINE UPTAKE CARRIER. M.E.A. Reith* and L.L. Coffey. Dept. of Basic Sci., University of Illinois College of Medicine, Peoria, IL 61656.

[^{3}H]CFT binds to the dopamine transporter with a higher affinity than its parent molecule [^{3}H]cocaine. In the present study with freshly prepared Sprague-Dawley striatal P_2 membranes, [^{3}H]CFT binding occurred to a single site. When Na^+ was the only cation present at 1-200 mM and phosphate the only anion, optimal binding was observed at 30-50 mM Na^+ with a profound reduction in binding at higher [Na^+]. Similar binding values were observed when [Na^+] was varied with Cl^-, NO_3^-, or $S_2O_4^{2-}$ as the co-varying anion in the presence of a fixed concentration of 10 mM sodium phosphate as the buffer; much lower binding, over the entire sodium concentration range, occurred when the fixed sodium phosphate concentration was reduced to 1 mM. Curves describing binding at varying [NaI] with 1 or 10 mM sodium phosphate were above those for varying [NaCl], [$NaNO_3$], or [Na_2SO_4] at the respective [sodium phosphate]; again phosphate anion had a stimulatory effect. With I^-, maximal binding occurred at a higher [Na^+] (150 mM or more) than with any other anion tested. With 1 mM sodium phosphate, substitution of 10 mM KCl or LiCl for NaCl virtually abolished binding. At 10, 30, and 200 mM sodium phosphate the K_d of binding was 22, 9, and 32 nM, respectively; with 50 mM sodium phosphate, substitution of 49 mM NaCl increased the K_d from 9 to 30 nM; with 130 mM sodium phosphate, substitution of 120 mM NaI reduced the K_d value by one-half; there was little or no change in the B_{max} (214 pmol/g fresh tissue). These results are compatible with the notion that [^{3}H]CFT binding requires low concentrations of Na^+, is stimulated by phosphate anion or I^-, but is unaffected by Cl^-, NO_3^-, or SO_4^{2-}. Concentrations of Na^+ above 50 mM become inhibitory, an effect counteracted by I^-.
Supported by NIDA 03025.

599.11

MERCURIC CHLORIDE HAS A BIPHASIC EFFECT ON [^{3}H]METHYLPHENIDATE BINDING TO THE DOPAMINE TRANSPORTER. M.M.Schweri* Mercer Univ.Sch. of Med., Macon,GA 30207.

In previous work, we have shown that one or more Cys residues are present in or near the cocaine-sensitive domain on the dopamine transport complex recognized by the radiolabeled stimulant, [^{3}H]methylphenidate ([^{3}H]MP) [*Neuropharmacology* 29:901, 1990]. Because Hg^{+2} is known to form a strong complex with sulfhydryl groups, the present work was undertaken to determine the effect of $HgCl_2$ on binding to this site in striatal tissue preparations of male Sprague-Dawley rats. In the presence of 0.05-1000 μM $HgCl_2$, a biphasic dose-response curve was obtained. Binding was enhanced below 5 μM $HgCl_2$ (maximum = 160% of control at 2.5 μM), but was completely inhibited at higher concentrations (IC_{50} = 8.5 μM). Scatchard analyses of [^{3}H]MP binding in the presence of 1 and 10 μM $HgCl_2$ gave the following results (3 experiments were conducted in each series):

SERIES	[$HgCl_2$]	K_D (M)	B_{max} (pmols/mg protein)
I	0	84 ± 4	7.5 ± 0.2
	1 μM	58 ± 4*	8.5 ± 0.5
II	0	93 ± 10	9.8 ± 0.5
	10 μM	220 ± 41**	6.6 ± 0.1*

*p<.01, **p<.05 compared to paired value at 0 $HgCl_2$, one-tailed paired t-test. $HgCl_2$ may differentially affect stimulant binding via interaction with specific Cys residues in the transporter macromolecule.

599.12

CHARACTERIZATION OF FETAL RAT BRAIN COCAINE RECEPTORS LABELED WITH [^{3}H]COCAINE OR THE POTENT COCAINE ANALOG [^{125}I]RTI-55. L.P. Shearman*, R.L. Maguire, and J.S. Meyer. Dept. of Psychology, Neuroscience and Behavior Program, Univ. of Massachusetts, Amherst, MA 01003.

[^{3}H]cocaine binding sites are present in the fetal rat brain (Meyer and Collins, Soc. Neurosci. Abs. 15:255, 1989). To characterize these sites, we assessed the potency of various monoamine uptake blockers to inhibit [^{3}H]cocaine (10 nM) binding to gestational day (GD) 20 whole-brain membranes. The following rank order of potency (IC_{50}) was obtained: cocaine > GBR 12909 ~ zimelidine ≥ desipramine ~ mazindol ≥ clomipramine ~ nisoxetine (all drugs yielded pseudo-Hill coefficients less than 0.7). Thus, cocaine appears to bind primarily to the dopamine and serotonin transporters in the fetal brain. In subsequent experiments, we studied the binding of the potent cocaine analog [^{125}I]RTI-55. GD20 whole-brain membranes were incubated with 10 pM [^{125}I]RTI-55 along with increasing concentrations of unlabeled RTI (0.1 pM to 100 nM). 50 μM cocaine was used to define nonspecific binding. A 2-site model was preferred statistically over a 1-site model by non-linear computerized curve fitting. Kinetic constants were as follows: K_D=0.13 nM, B_{Max}=0.18 pmol/mg protein for the high-affinity site, and K_D=11.56 nM, B_{Max}=0.59 pmol/mg protein for the low-affinity site. The K_D of the high-affinity site closely parallels that reported for adult rat striatum (Boja et al., Eur. J. Pharmacol. 194:133, 1991), whereas the K_D of the low-affinity site is somewhat higher. [^{125}I]RTI should be a useful ligand for studying the effects of prenatal cocaine on the subsequent development of cocaine binding sites. Supported by DA-06495.

599.13

EFFECTS OF INJECTION OF KINASE INHIBITORS INTO THE A10 DOPAMINE REGION ON COCAINE-INDUCED MOTOR ACTIVITY. J.D. Steketee*. Department of Pharmacology and Therapeutics, Louisiana State University Medical Center, Shreveport, LA 71130-3932.

Acute peripheral injection of cocaine stimulates motor activity in parallel with an increase of extracellular dopamine in the nucleus accumbens, as measured by in vivo microdialysis. The augmented behavioral and neurochemical responses which occur with repeated, intermittent treatment are termed sensitization. In other neuronal models, an increase in protein kinase C (PKC) activity has been associated with sensitization. Before examining the role of PKC in sensitization, it was necessary to examine the effects of different kinase inhibitors on the acute motor-stimulant response to cocaine. Cannulae were bilaterally implanted above the ventral tegmental area (A10 region), a region proposed to be critically involved the sensitized behavioral response to cocaine, for intra-cranial injections. One week after surgery animals received intra-A10 injections of saline or a kinase inhibitor 5 min before receiving peripheral injections of saline or cocaine (15 mg/kg). Animals received each of the 4 possible treatment combinations with a minimum 72 hr inter-trial interval. The kinase inhibitors used in these studies included polymyxin B (PMB), a calmodulin kinase and PKC inhibitor, H7 hydrochloride, a PKC and protein kinase A (PKA) inhibitor and H8 hydrochloride, a PKA and cGMP-dependent protein kinase inhibitor. Preliminary data demonstrated that H7 dose-dependently inhibits, while PMB does not alter cocaine-stimulated motor activity. The most effective dose of H7 (30 nmol/side) did not significantly alter baseline motor activity. These data suggest that PKA may play a role in the acute motor-stimulant response to cocaine and will be verified with intra-A10 injections of H8. In vivo microdialysis studies are currently being conducted to determine whether the H7-induced inhibition of cocaine-stimulated motor activity, is associated with an inhibition of cocaine-induced dopamine release in the nucleus accumbens.

599.14

EFFECTS OF CHOLERA TOXIN INFUSION INTO THE NUCLEUS ACCUMBENS ON LOCOMOTOR BEHAVIOR. S. T. Cunningham[1]* and A. E. Kelley[2]. [1]Dept. of Psychology, Harvard University, Cambridge, MA 02138, [2]Dept. of Psychology, Northeastern Univ., Boston, MA 02115.

Intracellular signal transduction mechanisms are currently the focus of much research. Although manipulation of second messenger systems is widespread in cell biology, there are very few experiments examining the consequences of such manipulation on behavior. In three separate experiments, we investigated the effects of microinfusion of cholera toxin (CTX, a bacterial toxin that stimulates production of cyclic AMP) into the nucleus accumbens (N. Acc.) on locomotor activity in rats (N=30). For Experiment I, three groups of rats received either saline or CTX (50 or 500 ng/µl) into the N. Acc. Locomotor activity (horizontal and vertical activity) was measured for 4 h following a single CTX infusion and subsequently for 4 h on 6 consecutive days. No acute effects on motor activity were observed. However, the 500-ng dose of CTX induced long-lasting hyperactivity that was apparent 24 h later and that lasted 4 days. A smaller but significant hypermotility occurred on days 4 and 5 following infusion of the 50-ng dose. Site-specificity of this behavioral phenomenon was investigated in Experiment II by infusion of CTX (250 ng/µl) into either the N. Acc. or the posterior dorsal striatum (PDS). CTX treatment of the PDS had no behavioral effects, while the long-lasting hyperactivity following treatment of the N. Acc. was replicated. In Experiment III, the effect of intra-accumbens pretreatment with saline or CTX (10 ng/µl) on *d*-amphetamine (0.5 mg/kg)-induced motor activity was investigated. This low dose of CTX did not increase baseline motor activity one day later; however CTX did induce a sensitized locomotor response to amphetamine. These data suggest that CTX induces long-lasting upregulation of the cyclic AMP system which is reflected by enhanced motor responses normally mediated by the N. Acc. Further, the results may have important implications for mechanisms underlying drug-induced sensitization.

AGING AND BEHAVIOR II

600.1

SEROTONIN UPTAKE IN THE BRAINS OF AGING RATS: PAROXETINE BINDING AND THE EFFECTS OF IMIPRAMINE AND TETRAHYDROACRIDINE. R.C.Arora,* J.W.Crayton, and A. Gulati. Section on Biol. Psychiatry, Hines VA Hospital, Dept. of Pharmacology and Psychiatry, Loyola Stritch Sch. of Med., and Dept. of Pharmacodynamics, Univ. of IL., Chicago, IL.

To examine the relationship between aging and serotonin (5-HT) metabolism, we studied 5-HT uptake in the frontal cortex (FC) and hippocampus (H) of young (4mos.), adult (15 mos.) and old (24 mos.) Fischer 344 rats. 5-HT uptake was studied by measuring binding of the specific 5-HT uptake site ligand, ^{3}H-paroxetine (PA). Bmax of PA binding was significantly higher in the H of adult rats compared with either young or old rats. The difference in Bmax between young and old rats was not significant. Kd of PA binding did not change significantly with age. In contrast to the findings in H, FC showed no difference in Kd or Bmax in PA binding. Imipramine inhibited PA binding in FC and H but there was no difference in IC_{50} values between the three groups. Tetrahydroacridine (THA) inhibited PA binding in FC and H but was 2000 times less potent than imipramine. IC_{50} values for THA did not change with age in either brain region. These findings suggest that the number of 5-HT transporter sites in the hippocampus increase with brain maturity but then drops significantly during old age, and may have implications for the normal and pathological age-related decrements in learning and memory thought to be mediated by hippocampal structures.

600.2

C-FOS mRNA RESPONSE TO ACUTE HALOPERIDOL IS BLUNTED IN THE STRIATUM OF AGED RATS. D.J. Doble*, K.M.Merchant and D.M. Dorsa. Dept. of Psychiatry, Univ. of WA, Seattle WA 98195 and GRECC, VAMC, Seatttle WA 98108

Previous studies demonstrated that administration of the dopamine D2 receptor antagonist haloperidol (H) induces transcription of the neurotensin/neuromedin N (NT/N) gene in neurons of the dorsolateral striatum (DLSt). This effect may be mediated in part by the proto-oncogene c-fos. The administration of H results in a rapid increase in transcription of the c-fos gene in the DLSt; this precedes the induction of the NT/N mRNA response to H in this region.

We have observed a blunting of the striatal NT/N mRNA response to H in aged animals. To further characterize the subcellular mechanisms underlying this blunted NT/N response, 3 month old and 24 month old Fischer 344 rats were treated acutely with saline or H (1 mg/kg i.p.). Animals were sacrificed after 1/2 or 1 hour and blood was collected for H levels. In situ hybridization for c-fos was performed using a ^{35}S labelled oligonucleotide probe. Densitometric analysis of film autoradiograms revealed a significant increase in striatal c-fos mRNA in both of the H treated groups compared with controls ($p<0.001$). However, the c-fos mRNA response was decreased in the old H-treated animals when compared with the young H-treated animals ($p<0.01$). These data suggest that the age-related blunting of the NT/N mRNA response to H may result in part from decreased transcription of the c-fos gene. We will explore the possibility that this alteration in post-synaptic response reflects the senescent decline in D2 receptors. The effect of diminished c-fos transcription on the expression of primary transcripts of the NT/N gene in these animals will also be discussed.

600.3

LOCALIZATION OF AGING-RELATED CHANGES IN α_2-ADRENERGIC RECEPTORS TO SPECIFIC BRAIN REGIONS OF FISCHER 344 RATS D.R. Wallace* and N.R. Zahniser. Dept. of Pharmacology, Univ. of Colorado Hlth. Sci. Ctr., Denver, CO 80262.

Aging-related regulation of central α_2-adrenergic receptors was examined in specific brain regions of 8- and 27-month-old Fischer 344 rats. For these studies, saturation isotherms were generated with the partial α_2-adrenergic receptor agonist p-^{125}I-iodoclonidine (^{125}I-PIC) and quantitative autoradiographic analysis. Assays were carried out with ^{125}I-PIC (0.05 - 3 nM) and 50 mM Tris-HCl buffer (pH 7.4) containing 10 mM $MgCl_2$ and 1 mM EGTA for 90 min at 21°C. In the three brain regions investigated -- cerebral cortex, hypothalamus and locus coeruleus -- ^{125}I-PIC bound to a single class of noninteracting sites. The affinity of the receptor for ^{125}I-PIC did not differ among the three brain regions or between the two age-groups; the Kd values ranged from 0.4 - 1.5 nM. The rank order of the density of α_2-adrenergic receptors was hypothalamus > locus coeruleus > cerebral cortex. The density of receptors in the hypothalamus of the aged rats was reduced by approximately 60% (adult: 1350 ± 162 fmol/mg; aged: 561 ± 155). In contrast, no significant aging-related reductions were observed in either locus coeruleus (adult: 482 ± 144; aged: 350 ± 59) or cerebral cortex (adult: 246 ± 17; aged: 212 ± 21). These results demonstrate that there is a marked loss of α_2-adrenergic receptors in the hypothalamus of aged Fischer 344 rats, which is not observed in either locus coeruleus or cerebral cortex. The receptor down-regulation in the hypothalamus may contribute to altered behaviors in aged animals. Further, in locus coeruleus and cerebral cortex, functional changes, rather than changes in receptor affinity or density, would be expected to mediate aging-related changes which involve α_2-adrenergic receptors. (Supported by USPHS AG04418)

600.4

P CHANNEL AND CALBINDIN ARE DIFFERENTIALLY AFFECTED FOLLOWING CORTICOSTERONE TREATMENT IN AGING. D. Hillman*, S. Chen, T.T. Aung, B. Cherksey, M. Sugimori, and R. Llinas, Department of Physiology and Biophysics, New York University Medical Center, New York, NY 10016

A polyclonal antibody (Cherksey et al. 1991 Soc. Neurosci.) against P type calcium channels (Llinas et al. 1989 *Proc. Natl. Acad. Sci. USA* **86**:1689-1693) was used to localize these channels in the mammalian CNS (Hillman et al. 1991 *Proc. Natl. Acad. Sci. USA* **88**:7076-7080). Electron microscopic examination revealed intensely labeled patches on the plasma membrane and ER of Purkinje cell dendrites and spines. Immunoreactions for calbindin protein produced the highest levels of reaction in the same types of neurons as the P channel. In the dentate gyrus of Fischer 344 aged rats (23 mo.), densitometric analysis showed a marked reduction in calbindin (as compared to 3 mo. of age); where as, P channel protein was moderately reduced. Corticosterone treatment of young rats, for one week, increased calbindin by 40% above normal, but P channel levels were unaffected. Aged rats treated with corticosterone, however, had about a 50% recovery of the calbindin aging-effect, while P channels were unaffected by the treatment. This study shows that steroid levels in aging may be a critical factor in controlling the level of calcium sequestration through calcium binding proteins. Supported by NIH-NINCDS: NS-13742 and NIH-NIA: AG-09480

600.5

BEHAVIORAL ANALYSIS OF CHRONIC NIMODIPINE TREATMENT IN AGED RATS. J. Hengemihle, E.L. Spangler, J.A. Joseph, D. Roberts, P. Garofalo, E. Bresnahan*, and D.K. Ingram, Gerontology Research Center, Natl. Inst. Aging, NIH, Baltimore, MD 21224.

Nimodipine is a calcium channel antagonist reported to have beneficial effects on treatment of ischemic damage as well as the potential for retarding aspects of brain and behavioral aging when provided chronically to rats (A. Scriabine *et al., FASEB J.* 3:1799, 1989). We treated aged male F-344 rats (22-24 mo) with nimodipine in subcutaneous pellets in the following doses: 0 (controls), 20 mg (low-dose) or 40 mg (high-dose) replenished after 6 wk. After 3 mo of treatment, surviving rats and a group of young controls (6 mo) were tested in a behavioral battery involving exploratory activity in an open field and in a runwheel cage as well as motor abilities required for remaining on an inclined screen, suspended from a wire, and balanced on a rotorod. Rats were also pretrained for one-way active avoidance in a straight runway before being trained in a 14-unit T-maze. During 20 trials rats were required to negotiate each of 5 maze segments within 10 sec to avoid footshock (0.8 mA). According to analysis of variance (ANOVA), nimodipine treatment produced no significant effects ($ps>0.05$) on body weight, food intake, or survival of aged rats. ANOVA of behavioral results indicated significant ($ps< 0.05$) age-related decline in performance of all tasks except in open-field behavior. Nimodipine treatment had no significant effects on performance of aged rats except in maze learning. Rats on the high-dose regimen performed significantly ($p<0.05$) better than aged controls in the maze. The results indicate that chronic nimodipine treatment of aged rats had no toxic effects and might be beneficial for preventing age-related decline in learning performance.

600.6

[^{3}H]CGS 19755 BINDING TO NMDA RECEPTORS IN RAT HIPPOCAMPUS: EFFECTS OF AGE AND CHRONIC NIMODIPINE. R.D. Kusztos[1], D.K. Ingram[2], J.A. Joseph[2], E.L. Spangler[2] and E.D. London[1]*. Addiction Res. Ctr., NIDA[1]; Gerontology Res. Ctr. NIA[2], Balto., MD 21224.

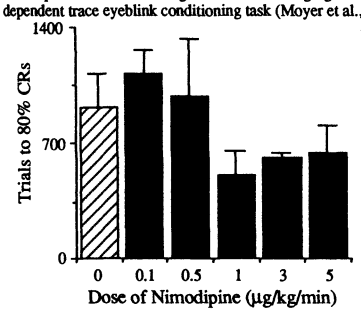
Previous reports have indicated marked age-related declines in hippocampal NMDA receptor concentrations in rats (e.g. M Tamaru et al., *Brain Res.*, **542**: 83, 1991). Nimodipine is a Ca^{2+} channel antagonist, reported to offer protection against cerebral ischemia, and to retard behavioral and brain aging in rats (A Scriabine et al., *FASEB J.*, **3**:1799, 1989). We have examined binding of [^{3}H]CGS 19755, a radioligand for NMDA receptors (DE Murphy et al., *Br. J. Pharmacol.*, **95**:932, 1988), in hippocampi of naive male F-344 rats of different ages, and of aged male F-344 rats treated chronically (3 mo) with nimodipine and provided with extensive behavioral testing (3 wk). Nimodipine was delivered via s.c. pellets of 20 mg (low dose) or 40 mg (high dose), which were replaced once after 6 wk. Assays were performed on well-washed, Triton-treated crude membranes.

In naive and untreated rats, marked age-related declines (42%) in [^{3}H]CGS 19755 binding were observed (23-25 mo vs. 7 mo). There was no significant age difference in protein content of the hippocampi. In contrast, hippocampi of rats receiving extensive training did not show an age-related decline in [^{3}H]CGS 19755 binding. Furthermore, compared to corresponding control rats, aged rats treated with low (but not high) doses of nimodipine revealed significantly higher hippocampal [^{3}H]CGS 19755 binding ($p < 0.05$). Thus, our findings suggest that age-related decline in hippocampal NMDA receptor binding appears only in naive rats, while chronic treatment with nimodipine may up-regulate NMDA receptor binding in highly trained, aged rats.

600.7

DOSE DEPENDENT EFFECT OF NIMODIPINE ON LEARNING RATE IN AGING RABBITS. M. Kowalska and J.F. Disterhoft*. Department of CMS Biology, Northwestern University Medical School, Chicago, IL 6061

Nimodipine, a dihydropyridine calcium entry blocker, facilitates learning in aging rabbits (Deyo *et al.*1989). The present study is determining the relation between i.v. nimodipine dose and learning enhancement of aging rabbits in the hippocampally - dependent trace eyeblink conditioning task (Moyer et al.,1990).



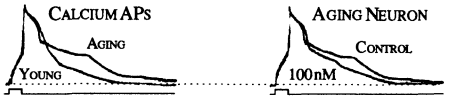
20 aging female rabbits (mean age=38.5 mo) have been trained using a 500 msec trace eyeblink (nictitating membrane) task to a behavioral criterion of 80% conditioned responses (CRs) or for a maximum of 25 days (2000 trials). Groups of animals were given either various doses of nimodipine or vehicle.

There were no differences in latencies or amplitudes of CRs or UCRs between the groups. The results suggest that a 1ug/kg/min nimodipine is the most effective in enhancing learning rate. It facilitates aquisition approximatelly two-fold in comparison to vehicle. Determining the optimal dose of nimodipine is important for further system and cellular studies on nimodipine actions in aging brain. (Supported by NIH 1 ROI AG08796 and The Miles Institute).

600.8

NIMODIPINE DECREASES CALCIUM ACTION POTENTIALS IN AGING AND YOUNG RABBIT CA1 NEURONS. J.R. Moyer, Jr.* and J.F. Disterhoft. CMS Biology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

The dihydropyridine calcium antagonist, nimodipine, facilitates learning in aging rabbits (Deyo *et al.*, 1989) and increases spontaneous firing of CA1 neurons *in vivo* (Thompson *et al.*, 1990). We previously reported that 100 nM nimodipine reduced the post-burst afterhyperpolarization (AHP) of aging but not young CA1 neurons and that aging neurons had larger AHPs (Moyer *et al.*, 1991). Since the AHP is primarily a Ca^{2+}–activated K^+ current, we studied the effects of nimodipine on calcium action potentials (APs) in aging and young CA1 neurons.



Intracellular recordings were made from 18 CA1 neurons (11 young, 7 aging) using current-clamp. 2M CsCl electrodes were used to block voltage-dependent K^+ currents and 4 µM TTX was added to the aCSF to block Na^+ currents. Cells were exposed to bath applied vehicle followed by increasing concentrations of nimodipine (100 nM to 10 µM). Aging neurons had prolonged calcium APs. 100 nM nimodipine decreased the calcium AP in aging neurons; 1 µM had little additional effect. In young neurons, 1 µM nimodipine had little or no effect on the calcium action potential, but 10 µM reduced it slightly. Nimodipine acted on the plateau phase of the calcium AP, which had a time course similar to the AHP and may underlie the enhanced AHP seen in those neurons. These data support the hypothesis that aging neurons have increased calcium influx that may contribute to their larger AHPs. Nimodipine reduced the calcium AP in an age- and concentration-dependent manner and may underlie it's learning enhancement in aging animals. (Supported by 1 RO1 AG08796 & The Miles Institute).

600.9

REDUCED CONTROL OF MOTOR OUTPUT IN A HUMAN HAND MUSCLE OF OLDER SUBJECTS DURING SUBMAXIMAL CONTRACTIONS.

M. Galganski, A.J. Fuglevand, and R.M. Enoka*. Depts. Exercise & Sport Sciences and Physiology, Univ. Arizona, Tucson, AZ 85721.

Aging is associated with a reduction in the number of motor units innervating a muscle but with an increase in the innervation ratio of surviving motor units. The purpose of the study was to determine the effect of these age-related changes on the capability of subjects to control force during isometric contractions at submaximal targets. Twenty-three healthy, neurologically normal human subjects (young: ages 20-37 years; old: ages 60-75 years) participated in the study. Motor unit activity was recorded from first dorsal interosseous muscle while the left index finger exerted an abduction force. The maximum voluntary contraction force (MVC) was not different among subjects (29 vs 24 N for the young and old subjects, respectively). Subjects were required to hold the isometric force at four different levels (5%, 20%, 35% and 50% MVC) for 20 seconds. Elderly subjects displayed greater force fluctuations about each force level. The coefficient of variation about each force level (6.6%, 3.7%, 2.9%, 2.9% vs 11.0%, 5.0%, 4.2%, 3.9% for the young and old subjects, respectively) was statistically greater for the older subjects. Moreover, the force exerted by single motor units (young: n=102; old: n=88) was found to be statistically greater in elderly subjects (29.1 vs 16.2 mN). However, the motor unit discharge characteristics were similar for the two groups of subjects. The presence of motor units with larger force amplitudes in elderly subjects may account for their decreased ability to control force.

Supported by NIH grants AG 09000, GM 08400, NS 07309 and NS 08634.

600.10

AGE DIFFERENCES IN VISUOMOTOR CONTROL.

E.A. Roy[1]*, T. Winchester[1] and S. Black[2]. [1]Department of Kinesiology, University of Waterloo, Waterloo and [2]Sunnybrook Health Sciences Centre, Toronto, CANADA.

The movement characteristics of a simple aiming task were examined in two groups of right-handed subjects involving either younger (20-35 years) or older (60-75 years) adults. A WATSMART system was used to record positional coordinates of a marker placed on a stylus which was grasped in the right hand. Discrete pointing movements were made to a target of two different widths (12 mm or 57 mm) over two different amplitudes (150 mm or 300 mm) giving rise to indices of difficulty ranging from 2.4 to 5.65 bits. Subjects were instructed to move as quickly and accurately as possible. The results revealed that the overall movement time was greater for the older subjects for all conditions and reflected the relative difficulty of the task condition. Peak velocities achieved were considerably larger for the longer movements for both groups, with the younger subjects reaching higher peak velocities in all conditions. While th2e time to peak velocity was similar between the groups, time after peak velocity differed significantly with the older subjects spending more time decelerating toward the target. The significance of these findings for understanding the effects of age on reaching is discussed with reference to current theories of motor control.

600.11

AGE-RELATED SHRINKAGE OF THE MAMILLARY BODIES: *IN VIVO* **MRI EVIDENCE.**

I. Torres, N. Raz, J. Acker#, C.J. Long*. Dept. Psychology, Memphis State Univ., Memphis, TN 38152 and Baptist Memorial Hospital, Memphis, TN 38119.

Mamillary bodies (MB) are hypothesized to play a critical role in declarative memory - a cognitive function that declines with age. In this study, age-related differences in the size of MB were examined using magnetic resonance (MR) imaging. The cross-sectional area of the MB was estimated from MR images of the brain in healthy volunteers and neurologically intact patients (N=82, age 18-78). The cross-sectional area of the tectum was used as a control region of interest (ROI). The area of the MB declined with age ($r = -.51$, $p < .001$); the area of the tectum did not ($r = -.08$, ns). Covarying skull size and sex did not alter the results. Supported by MSU Center for Applied Psychological Research.

600.12

SELECTIVE AGING OF HUMAN CEREBRAL CORTEX: EVIDENCE FROM IN VIVO MRI MORPHOMETRY.

N. Raz*, I. Torres, W. Spencer, J. Acker. Dept. Psychology, Memphis State Univ., Memphis, TN 38152 and Baptist Memorial Hospital, Memphis, TN 38119.

The effects of aging on the size of selected cortical regions in 29 healthy volunteers and 54 patients with negative radiological findings were examined using magnetic resonance imaging (MRI) scans. In both samples, similar patterns of cortical aging emerged. The size of sampled regions of association cortices (dorsolateral prefrontal and inferior parietal) correlated negatively with age, whereas no significant correlations between the size of primary somatosensory and visual cortices and age were found. In the first but not in the second sample, some of the correlations were attenuated after controlling statistically for skull size and sex. Overall, there were small but consistent trends for leftward asymmetry of the white matter, and rightward asymmetry of the grey matter. The results support the notion of pathoclysis (selective aging) of association areas accompanied by relative sparing of sensory cortices. Supported by MSU Center for Applied Psychological Research.

600.13

AGE-RELATED COGNITIVE DECLINE AND EEG SLOWING IN DOWN'S SYNDROME AS A MODEL OF ALZHEIMER'S DISEASE.

H. Soininen*, J. Partanen, V. Jousmäki, E.-L. Helkala, M. Vanhanen and P.J. Riekkinen Sr. Dept. of Neurology and Clinical Neurophysiology, University of Kuopio, 70211 Kuopio, Finland.

The patients with Down's syndrome (DS) invariably develop Alzheimer-type neuropathological changes at 40 years of age and older and display deficits in the ascending cholinergic and monoaminergic projections as do patients with Alzheimer's disease (AD). To address whether studying DS patients of different ages could serve as a model for progression of AD, we studied quantitative EEG and neurophysiological performance in an aging series of 31 DS patients, 36 patients with probable AD and age-matched controls. We found an age-related decline of cortical functions and slowing of the EEG in DS patients aged from 20 to 60 years. EEG slowing, decrease of the peak frequency, was significantly related to Mini-Mental status scores, and visual, praxic and speech functions, and memory in the DS patients similarly as in the AD patients. Such correlations were not demonstrated for young or elderly controls. This study provides neuropsychological and electrophysiological data to suggest that studying DS patients of different ages can serve as a model for progression of AD.

600.14

ENHANCED CORTISOL AND ACTH RESPONSES TO HYPERTONIC SALINE INFUSION IN OLDER HUMANS.

E.R. Peskind*, M.A. Raskind, D. Wingerson, M. Pascualy, D.J. Dobie, R.C. Veith, D.M. Dorsa, and C.W. Wilkinson. Dept. of Psychiatry, Univ. of Washington School of Medicine, and Seattle and American Lake VA GRECC, Seattle, WA 98195

Hypertonic saline administration is a physiologic stimulus of the hypothalamic-pituitary-adrenal (HPA) axis. It increases plasma ACTH and cortisol in young normal humans and elevates CRH mRNA in the paraventricular nucleus of rats. This study addressed the hypothesis that the enhanced HPA axis responsivity demonstrated in old rats also occurs in normal older humans and, if so, whether it is altered in Alzheimer's disease (AD). We administered a 90 minute hypertonic saline infusion (5% sodium chloride at 0.06 ml/kg/min) and a 90 minute placebo infusion (0.9% sodium chloride at 0.06 ml/kg/min) to normal young men (n=11, age=29 ± 2 yrs), normal older men (n=7, age=63 ± 3 yrs), and otherwise healthy older men with AD (n=17, age=67 ± 2 yrs). Hypertonic saline produced substantial and equivalent increases in serum osmolality, serum sodium, plasma vasopressin, and plasma norepinephrine among groups. In contrast, the responses of plasma ACTH and plasma cortisol to hypertonic saline infusion as compared to placebo infusion were significantly greater in older normal and AD subjects than in young subjects. Cortisol and ACTH responses did not differ between older normal and AD subjects. These results suggest increased responsivity of the HPA axis in human aging. Supported by AGO5136, AGO8419, and the Dept. of Veterans Affairs.

601.1

ADHESIVE PROPERTIES OF CELLS OVEREXPRESSING ß/A4 AMYLOID DNA. G.E. Maestre*, B. A. Tate, R.E. Majocha, C.A. Marotta. Massachusetts General Hospital and Harvard Medical School, Boston 02114 USA and University of Zulia Apdo 526 Maracaibo, Venezuela.

Several clones of PC12 cell overexpressing ß/A4-C terminal region of amyloid precursor protein (APP) have been examined for alterations in adhesive properties. The rate of attachment to substrate of transfected and control cells was assessed. Following plating in uncoated plastic petri dishes, unattached cells were collected and counted at several time intervals for up 24 to hours. At 30 minutes, approximately half of control cells while none of the transfected cells adhered to the dish. By six hours both transfected and control showed 100% attachment. The strength of adhesion to substrate was also assessed. After 48 hours of incubation, under similar disruptive forces, about 65% of transfected cells and only 25% of the control cells remain attached. Transfected cells at the light microscopy level showed increased cell clumping. These data suggest that overexpression of the ß/A4-C terminal region of APP may alter both cell-substrate and cell-cell adhesion.

601.2

Evidence for Altered APP Processing Following Acute Head Trauma in The Rodent. D. Games, K. M. Khan, F.G. Soriano, J. Lieberburg* and S. Sinha. Athena Neurosciences, South San Francisco, CA. 94080.

The epidemiological association between traumatic head injury and Alzheimer's Disease, the detection of beta amyloid (βAP) deposition in the brains of professional boxers and reports of βAP deposits following acute head injury in the human prompted us to ask if altered amyloid precursor protein (APP) localization and expression resulted after head injury in the rodent. The injury was delivered by a 10 gram weight dropped on the exposed dural surface overlying the parietal cortex from a height of 10 cm. After 24 hours dystrophic cells, neurites and fiber tracks confined to the injured area were intensely stained with N- and C- terminal APP antibodies, as well as with an antibody raised to a synthetic peptide containing the amino terminal portion of βAP. Specific staining was evident 4 hours after injury and persisted for up to one week. This aberrant APP staining correlated with the appearance of amino terminally truncated APP fragments on Western blots, clustered at ~22 Kd and ~70-80 Kd, also confined to the injured and penumbral regions. Gross alterations in total APP levels were not evident. The coincidence of increased APP staining in dystrophic cells and axons and the appearance of aberrant carboxyl terminal fragments confined to the injured regions suggest that altered APP processing, perhaps as a consequence of localized up regulation, occurs as an acute response to head trauma.

601.3

A 17-MER PEPTIDE SEGMENT OF THE AMYLOID β/A4-PROTEIN (APP) REDUCES NEUROLOGIC DAMAGE IN A RABBIT SPINAL CORD ISCHEMIA MODEL. M.P. Bowes, T. Saitoh, J.A. Zivin, J.-M. Roch, and K. Ueda*, Univ. California, San Diego, Dept. of Neurosciences, La Jolla, CA 92093-0624, USA.

A synthetic 17-mer peptide corresponding to Ala319-Met335 of β/A4-protein precursor (APP) has a neurotrophic effect on rat neuroblastoma cells (L.W. Jin et al., reported at this meeting). We evaluated the ability of this peptide to reduce CNS damage in a rabbit spinal cord reversible ischemia model (Zivin et al., Arch. Neurol. 39:408-412, 1982). Ischemia of the caudal lumbar cord is produced by temporary occulsion of the abdominal aorta. Saline or peptide (200, 500 or 1000 nM) was administered i.t. 20 minutes prior to ischemia and once daily for three days thereafter. Neurologic outcome was evaluated after four days. Durations of ischemia encompassing all grades of neurologic function were included. The 500 nM dose significantly reduced neurologic damage. The average ischemia duration necessary to produce permanent neurologic damage increased from 30.1±1.77 min in controls to 41.4±4.7 min in the 500 nM group. The 200 nM dose produced a nonsignificant trend toward reduced neurologic damage. Our results demonstrate that this 17-mer peptide is capable of reducing neurologic damage in vivo in a model of CNS ischemia, a finding consistent with a neurotrophic effect of APP.

601.4

LOCALIZATION OF ß-AMYLOID PRECURSOR PROTEIN (ß-APP) IN THE RAT CENTRAL NERVOUS SYSTEM (CNS). A.C.Y. Lo*, D.L. Price and S.S. Sisodia. Neuropathology Lab., The Johns Hopkins University Sch. of Medicine, Balto., MD 21205.

Previous studies have documented that ß-APP undergoes fast anterograde axonal transport in the rat peripheral nervous system and is present at nerve terminals in the CNS. Using sucrose gradient fractionation of cortical membranes and immunoisolation with antisynaptophysin beads, we showed that a small fraction of APP colocalizes with synaptophysin, a small synaptic vesicle (SSV) marker. However, the vast majority of APP fractionates with vesicle populations that are immunopositive for transferrin receptor, an endosomal marker, suggesting colocalization in endosomes. Current studies are directed towards further characterization of APP localization in nerve terminals at the ultrastructural level by subcellular fractionation and immunoelectron microscopy techniques using synaptosomes as an experimental model.

601.5

MICE TRANSGENIC FOR HUMAN β-AMYLOID PROTEIN ARE IMPAIRED IN SPATIAL, BUT NOT CUED LEARNING. G. Tirado-Santiago*[1], J. Nalbantoglu[2], J.P. Julien[2], & M.L. Shapiro[1], Depts. of [1]Psychology and [2]Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada.

Brain deposition of β-amyloid is an early marker of Alzheimer's disease. Mice (B6C3, 8 months old) transgenic for a human β-amyloid fragment were compared to normal litter mates in spatial and nonspatial learning tasks in the Morris water maze. A cDNA fragment including amino acids 591-695, spanning the amyloid-forming and C-terminus portion of the β-amyloid precursor protein, was cloned into the first exon of the human neurofilament NF-L gene, under the transcriptional control of the NF-L promoter. Northern analysis showed 3 of 7 transgenic lines expressed human β-amyloid transcripts in brain tissue. Spatial and non-spatial learning were tested using standard procedures in the Morris water maze task (n: transgenic = 4, control=7). Behavior was tested blind to the genetic history of the mice. Three probe tests were given: (1) the water temperature was reduced from 25° to 20° C, (2) the platform was moved to the opposite quadrant to distinguish spatial from procedural learning, and (3) the platform was made visible. Two measures of learning and performance have been analyzed statistically thus far: escape latency and the number of trials correct [reaching the platform before the end of a trial]. Transgenic mice were impaired relative to their litter mates in spatial learning in both measures and each probe test (e.g. number correct: $F(1,9) = 9.2$, $p < 0.02$) except when the platform was visible, when the two groups of mice performed similarly ($F(1,9) = 0.01$, $p = 0.9$). Eighteen month old mice are now being tested. The results suggest that expression of human β-amyloid protein may produce a selective learning deficit in 8 month old mice.

601.6

HISTOLOGICAL DEMONSTRATION of AMYLOID-IMMUNOREACTIVE DEPOSITS IN THE BRAINS OF APP-TRANSGENIC MICE. Rudy Krause II, Robert W. Manning, J. Patrick Card, Darlise DiMatteo, Leonard G. Davis*. CNS Research, The Dupont Merck Pharmaceutical Co., Wilmington DE 19880.

The creation of transgenic mice expressing the human form of the amyloid precursor protein (APP, 751 amino acid form) allows an investigation of the relationship between APP expression and AD. Immunohistological analysis of such transgenic mice (founders) at 15-26 months of age with antibodies (previously characterized and used to identify plaques in AD tissue) allowed us to observe an increase of antibody reactive elements in the brains of transgenic founder animals (n=13) when compared to non-transgenic littermates (n=10), and an older (ca. 30 months of age) control group (n=4). Three major brain regions of differential staining were evident: cortex, hippocampus, and cerebellum. The antibody reactive bodies were demonstrated in several forms: a group of localized "laminar-like dispersed spots" in the hippocampus similar to that described [M. Jucker et al., Science 255, p. 1443, 1992], these were also present in some controls; vascular staining similar to cerebro-vascular plaques; and intense cellular and some extracellular staining. The differences between the transgenic and control groups demonstrate that expression in mice of the human form of APP751 results in an increase in the amyloid-immunoreactivity. It is worth noting that although the mouse APP is >90% homologous to the human APP, potentially significant differences occur in the first fifteen amino acids in the β-amyloid sequence which could contribute to the inability of the mice to appropriately process the transgene (human APP-751). Further evaluation of these animals will be necessary before their usefulness as an AD model can be determined.

601.7

CREATION OF TRANSGENIC MICE POSSESSING NORMAL AND MUTATED HUMAN APP. Robert W. Manning*, Christian Reid, Rudy Krause II, Geneva Barkley, Leslie Smith, Leonard G. Davis. CNS Disease Research, The Dupont Merck Pharmaceutical Co., Wilmington DE 19880-0328.

One of the characteristic features of Alzheimer's Disease (AD) is the deposition of β-amyloid into neuritic- and cerebrovascular-plaques. This pathology has been implicated in the manifestation of the clinical symptoms. β-amyloid is a 4200 dalton peptide which is derived from a larger precursor protein (APP). Alternate exon usage results in the generation of at least three APP forms (695, 751, and 770 amino acids) that contain the sequence for β-amyloid. It has been suggested that either overexpression of APP, disruption of the tissue specific balance of the APP forms, or abnormal processing of the precursor protein may result in the deposition. More recently, single nucleotide mutations in APP have been reported in some Familial AD pedigrees and in Hereditary (Dutch) Cerebral Hemorrhage with Amyloidosis (HCHWA-D). In order to test the causal relationship of the above hypotheses on plaque formation as part of the etiology in AD, we have used transgene based expression of the human APP751 and APP695 cDNAs in transgenic mice under the control of the mouse thy-1.2 promoter. The thy-1.2 promoter has been shown to direct neuronal expression in transgenic mice. Additionally, site specific mutagenesis was used to generate single nucleotide changes that result in the amino acid substitutions previously reported in the inherited disorders. Transgenic mice possessing the two mutated forms in APP751 and in APP695 have also been created. The construction of the vectors and preliminary molecular biological analysis will be presented.

601.8

SECRETED FORMS OF AMYLOID PRECURSOR PROTEIN (APP^S) REGULATE INTRANEURONAL CALCIUM LEVELS: ROLES FOR APP^S IN NEURONAL PLASTICITY. M. P. Mattson[1]*, I. Lieberburg[2] and R. E. Rydel[2]. [1]Sanders-Brown Center on Aging and Dept. of Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536; [2]Athena Neurosciences, South SanFrancisco, CA 94080.

Amyloid precursor proteins (APP) are membrane-associated proteins produced in neurons and axonally transported. Normal enzymatic processing of APP results in the release of secreted forms of APP (APP^S), whereas in Alzheimer's disease (AD) aberrant processing results in the liberation of intact ß-amyloid peptide which forms aggregates that can destabilize calcium homeostasis and increase neuronal vulnerability to excitotoxicity (*J. Neurosci.*, 12:376, 1992). The normal function(s) of APP^S are unknown. We now report that APP^S695 and APP^S751 cause a potent dose-dependent (10 pM - 1 nM) and rapid (sec to min) reduction in intraneuronal free calcium levels (measured using fura-2) in cultured human cortical and rat hippocampal neurons. The reductions in calcium levels were reversible and were blocked by antibodies that recognize a region common to both APP^S695 and APP^S751 (aas. 444-592 of APP695). APP^S-induced reductions in calcium levels occurred in cell bodies, neurites and growth cones; preliminary data indicate that APP^S may influence growth cone motility. We are currently exploring the mechanism whereby APP^S lower calcium levels. We propose that by influencing neuronal calcium homeostasis APP^S play roles in regulating neurite outgrowth and modulating synaptic plasticity (LTP). Abnormal processing of APP in AD may result in a loss of calcium homeostasis and neurofibrillary degeneration as the result of disruption of normal APP^S functions, and the accumulation of ß-amyloid peptide. (supported by: NIH, Alzheimer's Association, Eli Lilly & Co., and Athena Neurosciences).

601.9

EVIDENCE THAT BRAIN SYNAPTIC MEMBRANES CONTAIN A RECEPTOR FOR THE ALZHEIMER AMYLOID βA4 PEPTIDE. B.A. Bahr*, B. Abai, S. Shahrestani, S. Viteri, and G. Lynch. Center for the Neurobiology of Learning and Memory, University of California, Irvine, California 92717-3800

Alzheimer's disease neurodegeneration is associated with deposition of the 39- to 43-amino acid amyloid βA4 polypeptide in neuritic plaques and the cerebral vasculature. The precursor protein for βA4 (APP) has been suggested to be a specialized adhesion molecule necessary for synapse formation and function (Breen *et al., J. Neurosci. Res.* 28:90, 1991; Schubert *et al., Brain Res.* 563:184, 1991); other such proteins have been identified (Bahr & Lynch, *Biochem. J.* 281:137, 1992). Furthermore, symptoms of Alzheimers have been shown to be strongly linked to the development of synapse pathology and synaptic loss (Masliah *et al., Neurosci. Lett.* 103:234, 1989). Accordingly, rat synaptosomal membranes (SPMs) were seeded onto microtitre wells coated with portions of APP or extracellular matrix proteins in order to test for homotypic and/or heterotypic receptors that recognize synaptic matrices. Subsequent assays using antibodies to the nerve terminal marker synaptophysin determined that neocortical SPMs bound to merosin, fibronectin, entactin, and to a lesser extent laminin, fibrinogen, and collagen. In comparison, it appears that the greatest amount of SPM attachment was directed to neurotoxic carboxyterminal fragments of APP including *i)* βAPP-C104 which spans the βA4 and cytoplasmic domains of APP, *ii)* amino acids 25 to 35 of βA4 (βA25-35), and to a degree *iii)* βA1-40. No SPMs bound to βA1-16, BSA, or substance P which has a similar amino acid sequence as βA25-35. The βA25-35 binding had the following regional selectivity: hippocampus ≈ neocortex » striatum > cerebellum ≥ thalamus ≈ brain stem > olfactory bulb. The binding was inhibited by 100 μM βA1-40 and by the adhesion blocker GRGDSP (EC_{50}=2.8 ± 0.1 mM), but not by 1 mM βA1-16, 100 μM substance P, 100 μM physalaemin, 10 mM GRADSP, or 3 mM REDV. Merosin-SPM attachment, however, was blocked by REDV (EC_{50}=230 ± 10 μM) but not by βA1-40 or βA25-35. This data suggest that at least two receptor types are involved in synaptic matrix recognition, perhaps one of which plays an active role in synaptic pathogenesis (Supported by the HFSP).

601.10

DISTRIBUTION OF BINDING SITES FOR THE NEUROTOXIC PEPTIDE, βAPP-C104, IN RAT BRAIN. M.R. Kozlowski*, E. Hall, R.L. Neve[2]. Department of Screening and Biochemical Research, Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492-7660, and [2]Molecular Neurogenetics Laboratory, McClean Hospital, Belmont, MA 02178.

We have reported the existence of a binding site for a naturally occurring, neurotoxic fragment of the amyloid precursor protein (APP): βAPP-C104 (Kozlowski et al., J. Neurosci., in press). The binding characteristics of this site suggest that it may be involved in the production of βAPP-C104 neurotoxicity. In the present study, we report the regional distribution of this binding site in rat brain, determined autoradiographically. Structures showing the greatest amount of binding included cerebellum and related areas, superior and inferior colliculi, and hypothalamus. Moderately high amounts of binding were observed in cortex, hippocampus, caudate nucleus, and most thalamic nuclei. Only small amounts of binding were found in globus pallidus, ventroposteromedial thalamic nucleus, and in white matter.

The finding of moderately high levels of βAPP-C104 binding in cortex and hippocampus, two structures that are particularly affected in Alzheimer's disease, supports the idea that βAPP-C104-induced toxicity may be an etiological factor in this disorder. It is curious, however, that the greatest amount of binding was found in the cerebellum, a structure not damaged in Alzheimer's disease. The apparent mismatch between binding site density and putative toxic response to the peptide may be due to a higher concentration of the toxic peptide in susceptible regions or particular sensitivity of certain areas to the events that follow binding.

601.11

A DIRECT ROLE FOR PROTEIN KINASE C AND THE TRANSCRIPTION FACTOR JUN/AP-1 IN THE REGULATION OF THE β-AMYLOID PRECURSOR PROTEIN GENE. J. Trejo, N.N. Dewji, R.M. Bayney, and J.H. Brown*, Department of Pharmacology, U.C. San Diego, La Jolla, CA 92093.

The mechanisms involved in β-amyloid deposition in Alzheimer's disease are largely unknown but might in part involve overexpression of the β-amyloid precursor protein (β-APP) gene. The promoter region of this gene contains two upstream sequences homologous to the transcription factor activator protein-1 (AP-1) recognition site. Activation of protein kinase C (PKC) leads to the induction Fos and Jun (major constituents of AP-1), increased AP-1 DNA binding activity, and transcriptional activation of AP-1 responsive genes. To determine whether PKC activation also regulates β-APP gene expression we measured β-APP mRNA levels and transcriptional activation of a 593 bp β-APP promoter-luciferase reporter gene construct (β-APP-LUC) in a human glial (1321N1) cell line. Activation of PKC with the phorbol ester PMA results in a 4-fold increase in β-APP mRNA levels. In transient transfection experiments the β-APP-LUC is transcriptionally activated by PMA as well as by co-expression of a constitutively activated PKC. We used gel mobility shift assays to determine if PMA induces protein DNA binding to either of two oligonucleotides corresponding to the putative AP-1 sites. The upstream AP-1 site effectively binds factors in nuclear extracts from PMA stimulated cells while the downstream AP-1 site fails to exhibit binding activity. To directly establish a role for Fos and Jun in transcriptional regulation of the β-APP gene, cells were co-transfected with β-APP-LUC reporter together with Fos and Jun expression plasmids. Surprisingly the β-APP promoter is transactivated by co-expression of Jun while Fos appears to repress transcriptional activation. We conclude that β-APP gene transcription is regulated through the activation of PKC and induction of Jun/AP-1 which binds to the upstream AP-1 site to activate transcription.

601.12

NICOTINE EFFECTS ON REGULATION OF APP SPLICING AND NEUROTROPHIN LEVELS IN SH-SY5Y CELLS. L. Monteggia*, S. P. Arneric, and T. Giordano. Neuroscience Research, Abbott Laboratories, Abbott Park, IL 60064-3500.

It has been reported that an inverse relationship exists between nicotine intake and incidence of Alzheimer's Disease (AD). Altered splicing patterns of APP and changes in neurotrophin expression have been implicated in AD and behavioral deficits in rats. In this study we sought to determine if the beneficial nicotinic effects observed in AD are related to changes in APP splicing and/or neurotrophin levels by studying differentiated SH-SY5Y cells. Cultured SH-SY5Y cells were differentiated with 10μM retinoic acid (RA) for eight days; nicotine (100nM or 1 μM) was added on the eighth day, 24hrs prior to RNA isolation. We used rtPCR to measure the splicing ratio of APP, along with the levels of NGF, BDNF and NT3 transcripts relative to cyclophilin RNA. Although there were no differences in the APP splicing ratio (APP 695 transcript represented 65% of the total APP RNA at each condition) there was a statistically significant increase (1.6X; $p<0.05$) in total APP RNA relative to cyclophilin RNA with 1 μM nicotine. Changes in the levels of NGF, NT3 and BDNF transcripts relative to cyclophilin RNA following nicotine administration were also observed although not statistically significant. Thus, in this in vitro model, the effects of nicotine can not be attributed to altered APP splicing or induction of neurotrophins.

601.13

RELEASE OF THE AMYLOID PRECURSOR PROTEIN IN FIBROBLASTS TRANSFECTED WITH PROTEIN KINASE C-α. B.E. Slack*[1], R.M. Nitsch[1], G.M. Kunz Jr.[1], E. Livneh[2], AND R.J. Wurtman[1], [1]Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge MA 02139, USA and [2]Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Protein kinase C (PKC) has been implicated in the proteolytic processing of the Alzheimer amyloid precursor protein (APP) (Buxbaum et al., PNAS 87: 6003, 1990) and concomitant release of an N-terminal fragment (Caporaso et al., PNAS 89: 3055, 1992). The effect of the PKC activator phorbol 12-myristate 13-acetate (PMA) on APP release was compared in Swiss 3T3 fibroblasts transfected with PKC-α and the gene for neomycin (neo) resistance, and in control (neo resistant) cells. Basal APP release from cells transfected with PKC-α was significantly increased (to 177 ± 24% of control, n=11), while levels of cell-associated APP were unchanged. PMA (100 nM) increased APP release from control fibroblasts by approximately 2.4-fold within 1 hr. The effect was dose-dependent (from 1 nM to 1 μM) and was inhibited by the PKC antagonist H-7 (100 μM). In fibroblasts transfected with PKC-α, the maximum response to PMA (expressed as fold increase over basal release) was reduced by more than 50% relative to controls. This reflected an increase in basal APP release in the transfected cells rather than a reduction in the absolute amount of APP released by PMA treatment. The EC50 for PMA was reduced from 60 nM (in controls) to 8 nM (in PKC-α transfected cells). These results show that APP release is regulated by PKC-α in Swiss 3T3 fibroblasts. Possibly, altered function of one or more subspecies of PKC may contribute to abnormal APP processing and subsequent amyloid deposition in Alzheimer's disease brain. (Supported by NIMH)

601.14

Neuronal activity driven expression of amyloid precursor protein (beta-APP) in glial cells

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There is conflicting evidence concerning the pathophysiologically relevant source of the amyloid precursor protein (beta-APP) and the mechanisms that lead to its deposition in the amyloid plaques in Alzheimer's disease. The *in vivo* expression of the amyloid precursor protein was investigated in lesions models of the the rat central nervous system. By means of immunocytochemistry, Western and Northern blot analysis we found the amyloid precursor protein constitutively expressed in glial and other non-neuronal cell types. Under normal conditions amyloid precursor protein expression showed regional differences in different brain regions. In the experimental lesion models the expression of amyloid precursor protein was not only near the site of lesion but also in projection areas remote to the sites of primary neuronal de/regeneration. An increase in the expression of the amyloid precursor protein occurred in distinct glial elements within hours after the experimental lesions. The swiftness by which the amyloid precursor protein is expressed suggests a fast signal from neuron to glia.

601.15

NEURODEGENERATION INDUCED BY β-AMYLOID PEPTIDES *IN VITRO* MAY FOLLOW AN APOPTOTIC PATHWAY. A. Copani*, D.T. Loo, C.J. Pike, A.J. Walencewicz, and C.W. Cotman, Irvine Research Unit in Brain Aging, *University of California, Irvine, CA 92717 USA.*

In the nervous system cell death may occur through two distinct processes: necrosis and apoptosis. Necrotic cell death, resulting from acute injury, is characterized by cell swelling and lysis; conversely, apoptotic cell death is characterized by cell shrinkage and release of membrane-bound bodies. The factors that trigger apoptosis in the nervous system have been proposed to activate an endogenous program of cellular self-destruction, yet they remain poorly defined.

In Alzheimer's disease, β-amyloid is hypothesized to contribute to neuronal loss. We have reported that aggregated β-amyloid peptides (βAPs) can be neurotoxic *in vitro*. Here, we analyze morphological and biochemical events associated with βAP-induced neurodegeneration to understand the mechanism by which treated neurons die. Degenerating neurons, observed under phase-contrast microscopy during a 24h exposure to βAPs, exhibited condensed cell bodies with dystrophic neurites. The degenerative events proceeded asynchronously and affected a significant portion of the neuronal population. At 24h a disparity between the number of degenerating neurons and the release of lactate dehydrogenase was observed, suggesting that cell lysis is not the initial event in the degenerative process. A breakdown of cellular DNA into oligonucleosome-length fragments is often associated with apoptosis. Using agarose gel electrophoresis, we found that βAP-treated cultures exhibited a regularly spaced "ladder" of DNA fragments. Aurintricarboxylic acid, an inhibitor of nucleases *in vitro*, suppressed the DNA fragmentation and delayed neuronal lysis when added to the cultures simultaneously with the peptide. Untreated cultures exhibited measurable DNA fragmentation, likely resulting from normally occurring cell death *in vitro*, but it was significantly less than in βAP- treated cultures.

Our study suggests that neurons exposed to βAPs degenerate in a manner consistent with morphological and biochemical changes characteristic of apoptosis, perhaps as a result of accelerated normal cell death *in vitro*.

601.16

NEUROTOXICITY INDUCED BY CHRONIC APPLICATION OF ß AMYLOID FRAGMENT 25-35: INVOLVEMENT OF APOPTOSIS. G.Forloni*, R. Chiesa, N. Angeretti and S. Smiroldo. Istituto di Ricerche Farmacologiche "Mario Negri", 20157 Milano, Italy.

The ß amyloid protein (ß-AP) is the major component of the senile plaques and cerebral vascular deposits characteristic of Alzheimer's Disease (AD). Recently, it has been shown a neurotoxic activity of ß-AP and its fragments. Other authors have reported that ß-AP itself is not neurotoxic, but it renders neurons more sensitive to excitotoxic damage. In this study, we investigated the neurotoxic effect of chronic exposure of rat cultured hippocampal neurons to ß amyloid fragment 25-35 (ß-25-35). Moreover, we tested, in the cells exposed to ß-25-35, the presence of the cascade of events characteristic of programmed cell death, defined apoptosis. Primary hippocampal neurons were derived from fetal rat brain at embryonic day 17,plated at a concentration of $5x10^5$/ml and cultured in the presence of fetal calf serum (10%). The neuronal death was determined visually and quantified by spectrophotometric method. The cells were exposed to micromolar concentrations of ß-25-35. A single application of ß-25-35 did not induce any neuronal alteration even at the highest concentration (100 μM). When the exposure to ß-25-35 (25-100μM) was repeated every two days for 10 days, a significant reduction of cell survival was evident. Furthermore, the morphological analysis and the DNA fragmentation indicated that the neuronal death induced by ß-25-35 involved also the mechanism of apoptosis. These results confirm the potential pathogenetic role played by ß-AP in AD and indicate that ß-AP may induce neuronal death through a specific programmed process.

601.17

Nimodipine-sensitive, β-amyloid(25-35)-induced $[Ca^{2+}]_i$ responses in single cultured rat hippocampal neurons. J.C.Chisholm*, J.N.Davis, and E.J.Hunnicutt, Jr., Miles Inst. Preclin. Pharmacol. West Haven, CT 06516.

Fragments of β-amyloid (βAP), a protein deposited in plaques characteristic of Alzheimer's disease, enhance delayed, Ca^{2+}-dependent excitotoxicity in CNS neurons. Exposure to various fragments of peptide for 1-4 days increases toxicity of glutamate, kainate, and NMDA measured in human and murine neurons in culture (Koh, Brain Res. 533:315;'90; Mattson, J.Neurosci. 12:376;92). Using calibrated fura2 measurements of $[Ca^{2+}]_i$ in single neonatal rat hippocampal neurons, cultured for 8-10 days, we report dose-dependent increases in $[Ca^{2+}]_i$ during acute application (10 - 100 μM) of a toxic βAP(25-35) fragment. Within 1 min of application, βAP increased $[Ca^{2+}]_i$ (n=20 of 20) to well over 3 μM in some cells. Response included transient and sustained components. It persisted for up to 40 min, the longest time tested, in the absence of Mg^{2+} and presence of the glutamate potentiator, glycine (100 nM). Nimodipine (10 - 100 nM) blocked these responses dose-dependently when applied before βAP. In conclusion, finding of an acute, dose-dependent effect of βAP(25-35) suggests the possibility of interaction with a specific binding site. Furthermore, nimodipine block of $[Ca^{2+}]_i$ responses induced by βAP(25-35) suggests a possible role for nimodipine in attenuating effects of βAP mediated by increases in $[Ca^{2+}]_i$.

601.18

MOLECULAR MODELING OF THE FAMILIAL ALZHEIMER'S DISEASE MUTATIONS IN AMYLOID PRECURSOR PROTEIN. R.V. Fishleigh, R.P. Mee, B. Robson and B.A. Yankner* - Proteus Molecular Design Limited, Marple, UK, Harvard Medical School and The Children's Hospital, Boston, MA 02115.

Point mutations in the transmembrane domain of amyloid precursor protein (APP) have been identified in some families with the early-onset form of Alzheimer's disease. Molecular dynamics simulations, which model the motions of a molecule as it changes over time by calculating interatomic forces, have been used to explore the conformational effects of the APP mutations. The Prometheus™ software (Proteus Molecular Design Ltd.) was used in combination with supercomputing capability for this study. The native Val-717 form of APP formed a stable transmembrane α-helix with some unwinding in the region of Gly(708)-Gly(709). The transmembrane α-helix was markedly destabilized in the Gly-717 APP mutation, with helix breakdown at the substitution site and frequent propagation of irregularity to Gly(708)-Gly(709) in the β amyloid domain. Helix instability also occurred in the Ile-717 and Phe-717 APP mutants. The predicted altered conformation of the transmembrane domain of the mutants may play a role in modifying the balance between alternative APP processing pathways to promote the formation of β-amyloid. Further studies on the APP transmembrane domain using explicit molecular models of the lipid bilayer are currently underway.

602.1

SYNTHESIS AND SECRETION OF ACTIVE α1-ANTICHYMOTRYPSIN BY MURINE ASTROCYTES. K. Kanemaru and C. R. Abraham*. Arthritis Ctr. Boston Univ. Sch. of Med., Boston, MA 02118.

α1-antichymotrypsin (ACT), a serine protease inhibitor, was found to be one of the components of senile plaque amyloid (Abraham et al., Cell 52:487, 1988). In situ hybridization studies showed that ACT mRNA was expressed in reactive astrocytes around senile plaques, indicating that reactive astrocytes were the origin of ACT (Pasternack et al., Am. J. Pathol. 135:827, 1989; Koo et al., Neurobiol. Aging 12:495, 1991). To confirm this possibility, we studied the synthesis and secretion of ACT using primary murine astrocyte cultures obtained from one day old neonatal brains. We stained cultured astrocytes with a polyclonal antibody to human ACT (Dako) which revealed cytoplasmic granular labeling consistent with compartmentalization in secretory granules. This staining was completely abolished by preincubation of the antibodies with human ACT. The presence of ACT in the conditioned medium was examined by Western blotting. A 60kDa protein was detected in the culture medium with ACT antibodies. Secretion of the ACT-like protein was stimulated by fetal calf serum. In addition, the ACT-like molecule from the culture medium of murine astrocytes could form SDS-insoluble complexes with iodinated human cathepsin G. A complex of approximately 90kDa was detected by autoradiography. These results suggest that reactive astrocytes synthesize and secrete an active ACT-like protein. ACT, secreted by reactive astrocytes, may disturb the protease-antiprotease balance, resulting in abberant processing of the ß amyloid precursor protein. (Supported by NIH AG-09905).

602.2

BINDING OF SECRETED HUMAN NEUROBLASTOMA PROTEOGLYCANS TO THE ALZHEIMER'S AMYLOID A4 PEPTIDE. Howard Fillit[1,2*], Luc Buee[1,3], Wanhong Ding[1], and Andre Delacourte[3] 1Ritter department of Geriatrics and Adult Development, [2]Fishberg Center for Neurobiology, The Mount Sinai Medical Center, New York, NY, 10029-6574, U.S.A. and [3]INSERM U156, Place de Verdun 59045 Lille Cedex FRANCE. Proteoglycans (PGs) may play a fundamental role in amyloid deposition. PGs were purified from conditioned medium of human neuroblastoma cells (SKNSH-SY 5Y). A heparan sulfate PG (HSPG) and a dermatan sulfate PG were identified by enzyme susceptibility. A monoclonal antibody to the protein core of a vascular HSPG found in senile plaques in Alzheimer's disease also reacted with HSPG protein core secreted by human neuroblastoma cells. High affinity binding between $^{35}SO_4$ labelled neuroblastoma PGs and the Alzheimer amyloid (A4) peptide was demonstrated by affinity chromatography. Specificity studies demonstrated that binding of human neuroblastoma PGs to the amyloid peptide was primarily mediated by a heparan sulfate glycosaminoglycan. No significant binding of neuroblastoma PGs was found to two other basic peptides also containing a putative heparin-binding domain derived from the beta-amyloid precursor. Human neuroblastoma PGs may bind to the Alzheimer amyloid peptide in a region with a heparin binding consensus sequence which also contains the cleavage site of the beta amyloid precusor protein. Neuronal HSPG may either regulate the secretion of the amyloid protein precursor or modify the binding of the amyloid protein precursor to other cellular adhesion molecules. Alterations in HSPG-A4 binding may be related to the pathogenesis of amyloid deposition in Alzheimer's disease.

602.3

BETA AMYLOID DEPOSITION AND NEURONAL DEGENERATION IN CULTURED HUMAN CORTICAL NEURONS. J. Busciglio* and B.A. Yankner. Dept. of Neurology, Harvard Medical School and The Children's Hospital, Boston, MA 02115.

We have established a method for long-term culture of human cortical neurons. Chronic administration of 4 μM of the β amyloid peptide β1-40 resulted in progressive neuronal degeneration. Immunocytochemical analysis showed that β1-40 gradually accumulated specifically on the neuronal soma as the incubation time increased, forming compacted deposits that contain the cell bodies of degenerating neurons and are surrounded by dystrophic neurites. Amyloid deposition was not observed in the absence of cells or after incubation with a control peptide. Immunocytochemical analysis with an antibody to a phosphorylated tau isoform showed marked induction of this antigen which preceded and accompanied neuronal degeneration caused by β1-40. Western blot analysis with this antibody showed the induction of a 57 kD band after treatment with β1-40. Increased immunoreactivity with Alz-50 but not with antibodies to MAP-2 or MAP-5 was also observed after treatment with β1-40. These results suggest that the formation of neuronal β amyloid deposits causes neuronal degeneration and alteration of the neuronal cytoskeleton. Compact amyloid plaques in Alzheimer's disease may form around degenerating neurons.

602.4

SUBSTRATE-BOUND BETA-AMYLOID PEPTIDE: NEUROTROPHIC EFFECT ON PRIMARY CEREBRAL CORTICAL NEURONS IN VITRO. **J.R. Wujek*, M.D. Dority, J. Silver, and R.C.A. Frederickson. Gliatech, Inc., Cleveland, OH 44122 & Case Western Reserve Univ., Cleveland, OH 44106.**

Beta-amyloid peptide (βAP), when dissolved in culture medium, has been reported to be neurotoxic to primary neurons (Yankner et al., 1989, Science 245:417). However, β-Amyloid in Alzheimers diseased brain is found primarily as insoluble plaques. Therefore, we have examined the effect of substrate-bound βAP on primary cerebral cortical neurons in vitro. A small volume of βAP_{1-40}, βAP_{1-28} or βAP_{25-35} was applied to culture dishes coated with a layer of poly-L-lysine over a layer of nitrocellulose. Each βAP solution dried and formed a dot approximately 1mm in diameter. A cell suspension of enriched primary embryonic cortical neurons (E15) was then seeded onto the dish. The supernatant was removed twenty minutes later and replaced with fresh culture medium. The cells adhered preferentially to the βAP_{1-40} dot and the βAP_{25-35}. No preferential adhesion was seen on the βAP_{1-28} dot. Over a period of 30 days, the neurons extended many neurites. Neurons off the dots also extended neurites, but were observed to degenerate at 3 weeks in culture. In contrast, neurons on the βAP dots, survived beyond 4 weeks in culture. These results indicate that βAP, in its insoluble form, exhibits adhesive and neurotrophic properties for primary cortical neurons in vitro.

602.5

SECRETED FORMS OF APP INCREASE NEURONAL SURVIVAL AND PROTECT CULTURED RAT AND HUMAN CNS NEURONS AGAINST HYPOGLYCEMIC DAMAGE. B. Cheng[1]*, V. L. Smith-Swintosky[1], I. Lieberburg[2], R. E. Rydel[2] and M. P. Mattson[1]. [1]Sanders-Brown Center on Aging and Dept. of Anatomy & Neurobiol., Univ. of Kentucky, Lexington, KY 40536; [2]Athena Neurosciences, S. San Francisco, CA 94080.

In the accompanying abstract (Mattson et al.) we reported that secreted forms of amyloid precursor protein (APP^S) exert a potent calcium-lowering effect in cultured rat hippocampal and human cortical neurons. We now report APP^S695 and APP^S751 dose-dependently (100 pM - 100 nM) promote neuronal survival and protect these same neuronal populations (as well as rat septal neurons) from hypoglycemic damage. Neuroprotection was observed when the APP^S were added before or up to 12 hr following the onset of hypoglycemia. APP^S695 and APP^S751 each reduced resting levels of intraneuronal calcium and prevented the hypoglycemia-induced elevation in intraneuronal calcium levels. Antibodies that recognize a region common to both APP^S695 and APP^S751 (aas. 444-592 of APP695) abolished the neuroprotective effect of APP^Ss in glucose deprived cultures, whereas antibodies directed against other regions of the APPs were ineffective. APP^Ss also prevented neurofibrillary tangle-like antigenic changes in tau and ubiquitin normally caused by a hypoglycemic insult. Since hypoglycemic damage involves NMDA receptor activation, these data suggest that APP^S might also modify neuronal responses to glutamate, a possibility now being explored. APP^S may normally play a neurotrophic/neuromodulatory role and aberrant processing of APPs may contribute to a loss of calcium homeostasis in AD by: (1) disrupting a normally trophic/neuroprotective function of APP^S and (2) liberating ß-amyloid peptide which may enhance neuronal vulnerability to ischemic/excitotoxic insults. (supported by: NIH, Alzheimer's Association, ILSI, Athena Neurosciences and Eli Lilly & Co.).

602.6

INTRACELLULAR APPLICATION OF β-AMYLOID PEPTIDE INDUCES EXCITATORY CURRENTS IN RAT HIPPOCAMPAL NEURONS. L.K.Simmons*, M.O. Chaney, S.P. Little, and R.L. Berry. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285.

The accumulation of extracellular deposits of β - amyloid peptide (A4P) in senile plaques is believed to be a primary event in the pathogenesis of Alzheimer's Disease. Several groups have shown that A4P is neurotoxic and potentiates other forms of neurotoxicity *in vitro*. Since there is increasing evidence that A4P can also accumulate inside neurons, we used electrophysiological protocols to investigate the intracellular effects of the peptide.

Pyramidal neurons from primary cultures of rat hippocampal cells were voltage-clamped using the whole-cell clamp configuration. Patch pipettes contained either A4P (1-40) or a control scrambled sequence 1-40 peptide in an "internal" salt solution. A4P (0.5 - 20 nM) elicited large, spontaneous inward currents. Occassionally the currents exhibited seizure-like activity, with periods of high frequency bursting interspersed with periods of low activity. These currents were never observed with the control peptide (up to 1 mM concentrations), had a reversal potential near 0 mV, and were voltage-dependent -- increasing in frequency when the cell membrane was clamped above -40 mV. These inward currents were not blocked by extracellular application of TTX or TEA, but were reversibly blocked with Cd^{2+}.

Mattson et al. (1992; J. Neurosci., 12:376) reported that prolonged exposure to μM concentrations of extracellular A4P potentiates neurotoxicity by disrupting cellular Ca^{2+} homeostasis. We show that a Ca^{2+}-dependendent excitatory current is activated with intracellular dialysis of nM concentrations of A4P, suggesting that at least part of the toxicity observed *in vitro* could be due to small amounts of A4P entering the cell.

602.7

N-TERMINAL CLEAVAGE OF B-AMYLOID BY CATHEPSIN G IS DEPENDENT ON PH AND IONIC CONDITIONS. J.L., Sonnenberg, S.R., Sahasrabudhe, J. Hulmes, A.J.Blume and M.P Vitek* Departof Molec. Pharmacology, Lederle Laboratories, American Cyanamid Company, Pearl River, N.Y., 10965.

BAP formation depends on its extraction from APP. The peptide HSEVKMDAEF spans the precursor site where ami**No-BetA-endoPeptidase (N-BAPase)** should cleave between Met and Asp (M/D cut) to generate the amino-terminus of BAP. When a putative N-BAPase like activity, Cathepsin-G, is incubated with the HSEVKMDAEF substrate, we observe several products that are separated by HPLC and identified by amino-acid sequencing. With the suggestion that APP processing (Golde and Greengard) and Cathepsin G localization occurs in the lysosomal compartment of the cell, we wished to test the effect of pH, with a focus on acidic pH, on our peptide/Cat-G system. We find 2 dominant cleavage patterns where the levels of the product pairs HSEVK plus MDAEF (K/M cut) and HSEVKM plus DAEF (M/D cut) are dependent on the pH and ionic strength of the reaction. At alkaline pH the K/M cut is favored while acidic pH favors the M/D cut which is more sensitive to high ionic strength.

	0 mM NaCl			150mM NaCl			500mM NaCl		
pH	K/M	M/D	Uncut	K/M	M/D	Uncut	K/M	M/D	Uncut
4.8	0%	65	35	0	40	60	0	19	81
5.3	5	70	25	3	39	58	0	27	7
6.2	57	42	0	42	59	5	4	47	48
7.3	70	30	0	79	21	0	75	24	0
8.3	94	5	0	92	8	0	81	19	0
9.2	100	0	0	94	6	0	75	25	0

These results suggest that chymotrypsin-like proteases and compartmental environment may play a role in directly generating the N-terminus of BAP or if we hypothesize an additional amino-exo-peptidase activity, that Cathepsin-G may also play an indirect role in BAP formation.

602.8

"CALPAIN INHIBITORS SIGNIFICANTLY DECREASE THE CATALYTIC ACTIVITIES OF A MAJOR EXTRA-LYSOSOMAL PROTEINASE, THE MULTICATALYTIC PROTEINASE COMPLEX." M.E. Pereira and S. Wilk*, Department of Pharmacology, Mount Sinai School of Medicine, C.U.N.Y., New York, N.Y. 10029

The calcium activated proteinases calpain I and II have been the focus of many recent studies addressing protein degradation in neurodegenerative diseases. In addition to calpains, brain contains another major extra-lysosomal proteinase known as the multicatalytic proteinase complex (MPC) (Wilk, and Orlowski, J. Neurochem. 40: 842:1983). MPC has unique catalytic properties, splitting peptide bonds on the carboxyl side of hydrophobic (chymotrypsinlike activity), basic (trypsinlike activity) and acidic amino acids (peptidyl glutamyl peptide activity; PGP) . A fourth catalytic site appears to be responsible for the initial breakdown of proteins such as ß-casein (caseinolytic activity). MPC has been suggested to be the "catalytic core" of the Ubiquitin/ATP-dependent protein degradation pathway. The neuropathological finding of ubiquitinated proteins in neurofibrillary tangles in Alzheimer's disease and in other neuronal inclusion bodies may implicate MPC-malfunction in neurodegenerative disorders, resulting in abnormal accumulation of ubiquitin-protein conjugates. Two recently synthesized peptide aldehydes, N-acetyl-Leu-Leu-norleucinal or calpain inhibitor I (CPI_1) and N-acetyl-Leu-Leu-methioninal or calpain inhibitor II (CPI_2), have been proposed as selective calpain inhibitors. Since MPC is also inhibited by some peptide aldehydes, we determined the effect of calpain inhibitors I and II on the catalytic activities of an apparently homogeneous preparation of bovine pituitary MPC. All four catalytic activities were inhibited by CPI_1 and CPI_2 with the caseinolytic activity being the most sensitive (IC_{50}'s of 7.2 μM and 11.9 μM respectively). Chymotrypsinlike activity (hydrolysis of Cbz-Gly-Gly-Leu-pNA) was markedly decreased in the presence of CPI_1 and CPI_2 with approximate IC_{50}'s of 9.9 μM and 24.9 μM res, ectively. The trypsinlike (Cbz-D-Ala-Leu-Arg-2NA) and PGP (Cbz-Leu-Leu-Glu-2NA) activities declined moderately with increasing inhibitor concentrations, up to 100 μM. Caution must therefore be exercised when these inhibitors are used to infer calpain function. (Supported by the Alzheimer's Association/New York City Chapter Pilot Research Grant PRG-90-114 to M.E.P.; NIH grant NS-17392 and an ADAMHA Research Scientist Award MH-00350 to S.W.)

602.9

ß-AMYLOID CONTAINING PEPTIDES PRODUCED IN NEWBORN RAT CEREBRAL CORTEX PRIMARY CULTURES. Xue R, LeBlanc AC, LaSala D, Gambetti, P*. Inst. Pathol. Case Western Reserve U. Cleveland, OH 44106

Combined morphological, immunocytochemical and biochemical studies have shown that the changes in cell composition and organization undergone by primary cultures of rat cerebral cortex between 7 and 32 days in vitro (DIV) are associated with changes in expression and processing of the amyloid precursor protein (APP). Cultures at 7 DIV which are composed mostly of evenly distributed astrocytes, microglia and neurons express full-length forms of APP and a series of five smaller C-terminal peptides, the largest of which appears to contain the entire ß-amyloid peptide (ßAP). Between 14-32 DIV, clusters of small cells, closely associated with neurons and immunocytochemically identified as immature oligodendrocytes, increase significantly in the cultures. These cultures simultaneously show an increase in the larger ßAP-containing peptide while the four smaller C-terminal APP peptides decrease significantly . Moreover, an additional ßAP reactive peptide which migrates at a slightly slower rate than synthetic ß-amyloid (1-40) peptide is also detected. Immunocytochemistry has revealed cell surface immunoreactivity to ßAP and APP C-terminal antibodies around these clusters. Preliminary results of pulse-chase experiments indicate active synthesis of APP by oligodendrocytes.

These results suggest that immature cells of the CNS cerebral cortex are producing and extruding relatively large amounts of ßAP-containing peptides.Supported by NIH NIA RO1 AGNS 08155, Britton fund and NIH NIA AGO8992-01.

602.10

EFFECT OF AGGREGATED AMYLOID ON CULTURED HIPPOCAMPAL NEURONS. R.K. Lartius* and E. Uemura. Neuroscience Program, Iowa State University, Ames, IA 50011.

β-amyloid protein (β-AP) is a peptide that abnormally deposits in diseases such as Alzheimer's disease (AD). Recent evidence has suggested that the accumulation of this peptide may result in neuronal death and the formation of neurofibrillary tangles. Toxic effects have been reported when cultured neurons were exposed to synthetic amyloid peptides. Further, it has been suggested that peptide aggregation alters toxicity. In this study, we examined effects of aggregated amyloid fragments on cultured hippocampal neurons. Synthetic peptides homologous to amino acids 1-40 (β 1-40) and 25-35 (β 25-35) of β-AP were dissolved in ultrapure water (10 mg/ml) and allowed to aggregate for at least 2 days. At this time, the aggregated peptide was removed by centrifugation. Small amyloid islands approximately 1 mm in diameter were formed by precipitating the aggregate onto polylysine-treated or untreated 35 mm^2 culture dishes. Control dishes with no amyloid were prepared in an identical manner. Hippocampal neurons isolated from E18 rat pups were then seeded onto the dishes. By 4 h, neurons had attached to both the β 25-35 and the β 1-40 islands. By 24 h, the majority of these cells had extended neurites and were largely multipolar. Attachment and outgrowth to the islands were independent of polylysine treatment. After 7 days, the cultures were fixed and processed for MAP 2 and Tau-1 immunohistochemistry. There were no significant differences in cell survival or somal MAP 2 or Tau-1 staining compared to control following exposure to either amyloid peptides. These finding suggests that, by itself, amyloid in the aggregated form is not neurotoxic. Further, since neurons attached and elaborated processes on these peptides, amyloid has apparent substrate properties. The latter may explain the abnormal neurite sprouting that has been observed in the plaque regions of AD. (Supported by Hoechst-Roussel Pharmaceuticals, Inc.)

602.11

ENHANCED EXPRESSION OF AMYLOID PRECURSOR PROTEIN AFTER MIDDLE CEREBRAL ARTERY OCCLUSION. J. A. Clemens*, D.T. Stephenson and K. Rash. The Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285.

Induction of amyloid precursor protein mRNA after persistent focal ischemia in rat cerebral cortex has recently been reported (Abe et al., *J. Cereb. Blood Flow Met.*, 11: 1991). In order to determine if expression of APP itself is enhanced after cerebral ischemia and its cellular origin, we performed immunocytochemistry using antibodies to various epitopes of APP on tissue from rats subjected to permanent middle cerebral artery occlusion (MCAO). Male spontaneously hypertensive rats were anesthetized with isoflurane and the middle cerebral artery was occluded at the level of the rhinal fissure. This treatment produces an infarct restricted to the cerebral cortex. At 4 and 7 days following MCAO the rats were deeply anesthetized and the brains were perfusion fixed with periodate lysine paraformaldehyde. Ten micron thick frozen coronal sections were made through the infarct and immunostained with different polyclonal antibodies directed to C-terminal epitopes of APP (kindly provided by D. Selkoe). Pathological structures including dystrophic neurites and axonal spheroids were strongly labeled at the border of the infarct zone. In addition, many neuronal cell bodies were stained intracellularly in this region. Modified bielschowsky silver staining of near-adjacent sections demonstrated argyrophilic neurons and axons in this same border zone. This finding complements the recent reports of APP accumulation following stab lesion and kainic acid injection. These data suggest APP accumulates in regions of neurodegeneration/regeneration following focal ischemia and implicates that APP may play a role in the reaction of the CNS to injury.

602.12

β-AMYLOID PEPTIDES INDUCE MORPHOLOGICAL CHANGES IN CULTURED RAT MICROGLIA. A.R. Korotzer*, C.J. Pike, and C.W. Cotman. Irvine Research Unit in Brain Aging, *University of California, Irvine, CA 92717 USA.*

Microglia are often associated with senile plaques, a pathological hallmark of Alzheimer's disease. The insoluble core of plaques consists largely of aggregated β-amyloid protein. Synthetic β-amyloid peptides (βAPs) can induce neurodegeneration in vitro, an effect reported to be influenced by the state of peptide aggregation. We have examined the effects of synthetic βAPs on rat microglia in culture.

Two different βAPs were used: β1-42, a full-length homolog of the β-amyloid protein deposited into senile plaques, and β25-35, an active fragment of β-amyloid protein that exhibits both aggregative and neurodegenerative properties. Cultures treated with both β25-35 and β1-42 contained visible precipitate, indicating the presence of aggregated peptide.

Within hours after addition to purified cultures of ramified microglia, β25-35 had induced severe beading of processes; often processes appeared discontinuous under light microscopy. β1-42 also induced this morphological effect, although often with less intensity than β25-35.

These observations indicate that aggregated βAPs, in addition to their reported neurodegenerative properties, also induce morphological changes in microglia in vitro.

602.13

AMYLOID P COMPONENT AND ALZHEIMER AMYLOID LESIONS. L.S. Perlmutter*, M.A. Myers, E. Barrón, D. Saperia, H.C. Chui. Dept. of Neurology, University of Southern Calif. Sch. Med., Los Angeles, CA 90033.

Amyloid P component (APC) is a globular glycoprotein associated with the amyloid proteins that accumulate in virtually all amyloidoses, including Alzheimer disease (AD). This study continues our analyses of APC's association with the lesions of AD, particularly "preamyloid" and neurofibrillary tangles (NFT). Autopsy samples from AD and non-AD cases were fixed in mixed aldehydes and immunocytochemically labeled with a polyclonal antibody against APC (DAKO-Patts). Reaction product was visualized with the avidin-biotin-peroxidase technique (Vectastain), using diaminobenzidine as chromagen. In all cases, regional foci of labeled neurons, astrocytes, microglia, and/or oligodendrocytes were seen; thus APC accumulation is not specific to AD. In AD, the cytosol of intracellular-NFT-laden neurons was labeled, while the extracellular- (E-) NFT themselves were darkly stained. Some plaques resembling "preamyloid" deposits were dispersed through the neuropil. More classical-appearing accumulations contained immunolabeled knob-like structures that, at the light microscopic level, appeared to be dystrophic neurites . However, ultrastructural analyses revealed that dystrophic neurites were never immunolabeled. Hence, these accumulations may not be classic plaques, but rather diffuse accumulations of APC around E-NFT.

An acute phase reactant in rodents, APC's function in humans is unknown. Recently, APC immunostaining has been shown to parallel that of complement proteins, suggesting an association with complement activation and the initiation of phagocytosis (Akiyama et al, Brain Res, 1991, 548:349). APC's localization to "preamyloid," and its association with biochemically diverse types of amyloids, further suggests a central role in amyloid processing. (CA AD Prog., AG07127, AG05142, AG07624)

602.14

ALZHEIMER AMYLOID PRECURSOR PROTEIN OCCURS AS A CHONDROITIN SULFATE PROTEOGLYCAN IN GLIOMA CELLS, **J.Shioi*, J.P.Anderson, J.A.Ripellino, S.Efthimiopoulos, L.M.Refolo, and N.K.Robakis. Dept. of Psychiatry and Fishberg Res. Ctr. for Neurobiol., Mount Sinai Med. Ctr., New York, N.Y. 10029**

Amyloid ß-protein is a major component of senile plaques found in brains of patients with Alzheimer's disease. It is hypothesized that this small protein is a pathological degradation product of the amyloid precursor (APP). We have found that both full length and truncated, secreted APP exist as the core protein of a chondroitin sulfate proteoglycan (CSPG). The secreted APP CSPG in C6 cell culture medium was partially purified by ion-exchange and gel filtration chromatography. Western blot analysis revealed a diffuse band (ca 140k-250kD). This material was detected by several antibodies which specifically recognize different regions of APP. Chondroitinase AC- or ABC-treatment completely eliminated this high molecular weight band with a concomitant increase in the APP band (120kD). The digested product reacted with an anti-stub monoclonal antibody which recognizes 4-sulfated disaccharide. Heparinase or heparitinase did not have any effect on this PG. The N-terminal sequence of this CSPG core protein matched with that of APP. Densitometric analysis showed that about 90% of the secreted APP can occur in the CSPG form. CSPG form of full length APP was also detected on the cell surface. The close proximity of two consensus CS attachment sites to both the N-terminus of the amyloid ß-protein and the secretase cleavage site, suggests that the CS chains may affect the APP processing and/or production of the amyloid ß-protein.

602.15

PHOSPHORYLATION OF β/A_4 AMYLOID PRECURSOR PROTEIN (APP) *IN VIVO*. I.P. Shapiro*,[1,2], L. McConologue[3], K.E. Eidman, T.R. Soderling[2]. [1]Department of Pathology and [2]Vollum Institute, Oregon Health Sciences University, Portland, OR 97201 and [3]Athena Neurosciences, South San Francisco, CA 94080.

We wanted to determine the protein kinase(s) which phosphorylate APP, the site(s) of APP phosphorylation, and whether changes in the biochemistry of APP might be associated with APP phosphorylation. Cultured Sf9 insect cells, infected for 48h with recombinant APP_{695}-baculovirus, were labeled with ^{32}P-orthophosphate and immunoprecipitated with a polyclonal anti-$APP_{590-695}$ antibody (C-terminal-immunoreactive). Under basal conditions, APP was phosphorylated on Ser only.

PKC(α) stimulated the ^{32}P-phosphorylation of full-length APP (≥2-fold) and of a ~10 kDa C-terminal APP fragment (≥10-fold) in Sf9 cells co-expressing PKC and APP. The same site in the cytoplasmic domain of APP was phosphorylated by PKC in full-length APP and in the *in situ*-generated C-terminal fragment, as indicated by the presence of the same PKC-stimulated tryptic phosphopeptide in both APP species. PKC stimulated ^{32}P incorporation into PSer only. One possibile explanation for the large relative increase in ^{32}P labeling of the C-terminal fragment is that PKC stimulates the accumulation of this fragment. Experiments to characterize the C-terminal fragment, the precise PKC phosphorylation site, and the mechanism of increased ^{32}P incoporation into this APP C-terminal fragment are ongoing.

These studies demonstrate that PKC phosphorylates APP and that APP phosphorylation may regulate the processing of APP *in vivo*.

602.16

Solvent effects on rat and human β-protein toxicity and amyloid formation in vivo. S.A. Frautschy[1]*, Waite, J.[2,3] G.M. Cole[2], A. Baird[1], J. Pitha[4] Thal, L.J.[2,3] [1]Dept. MCGB Whittier Institute, 9894 Genesee, La Jolla, Ca. 92037, [2]Dept. of Neurosciences, UCSD, La Jolla, Ca. 92093 [3]Dept. Neurology, Veterans Admin., San Diego, 92161, [4] Gerontology Res. Cent., Baltimore, MD 21224

Evidence for mutations in and around the β-protein coding region of the amyloid precursor protein (APP) gene in families with early onset Alzheimer's disease (AD) suggests that altered APP metabolism may play a role in AD. Although some studies have reported β-protein toxicity, this subject is controversial. Our purpose was to determine the effects of various solvents on β-protein toxicity and amyloid formation. Fifteen μg of human β(1-40) or rat β(1-42) were dissolved in 35% acetonitrile, water, or a 35% 2-hydroxypropyl-β-cyclodextrin (cyclodextrin) solution in PBS. A 1 μl volume was infused into 16 male Fischer 344 rats into the CA1 region of the hippocampus. Solvent alone or solvent plus reverse sequence peptide was infused on the control side. Rats were sacrificed at 7 d and analysis of necrosis was performed on frozen sections using Nissl stain, β-protein distribution using immunocytochemistry and amyloid formation using Thioflavin-S or Congo Red staining. Results demonstrated that acetonitrile caused gross toxicity which was markedly enhanced by β-protein. β-protein in water was also toxic. However, with cyclodextrin/PBS, the toxic effects were almost negligible. There was intensive staining for β-protein around the tip of β-protein needle injections. On the contralateral side light staining of glial cells was associated with the needle track. The CA3 pyramidal neurons, distal to injury stained for β-protein on both sides. Intriguingly, staining for amyloid patterns was similar to anti-β-protein except that the cyclodextrin/PBS treatment showed no Congo Red birefringence and weak Thioflavin-S fluorescence. We conclude that solvent effects play a major role in acute β-protein neurotoxicity, and that cyclodextrin may inhibit amyloid formation and amyloid toxicity.

602.17

β-AMYLOID-INDUCED NEURODEGENERATION IN VITRO: RELATIONSHIP TO PEPTIDE AGGREGATION. C.J. Pike[1]*, D. Burdick[2], A.J. Walencewicz[1], C.G. Glabe[2] and C.W. Cotman[1]. [1]Irvine Research Unit in Brain Aging and [2]Department of Molecular Biology and Biochemistry, *University of California, Irvine, CA 92717 USA.*

The β-amyloid protein is present as an insoluble aggregate within senile plaques, the definitive lesions of Alzheimer's disease (AD). We have demonstrated previously that β1-42, a full-length synthetic β-amyloid peptide (βAP), readily forms aggregates in vitro and is neurotoxic to cultured hippocampal neurons when in an aggregated state. Here we have examined an overlapping series of βAPs to investigate further the structure-function relationship between the neurodegenerative and aggregative properties of β-amyloid protein. βAPs were studied both after initial solubilization in water and after a 7-day incubation of these solutions. Aggregation of βAP solutions was assessed by light microscopy, electrophoresis, and ultracentrifugation studies. We found that only βAPs containing a substantial portion of the hydrophobic transmembrane region formed aggregates that were stable under tissue culture conditions. Following addition to cultures of E-18 rat hippocampal neurons, only those βAPs that exhibited aggregation were observed to cause significant neurodegeneration. Partial reversal of βAP aggregation by solvent treatment resulted in a corresponding attenuation in toxicity. These data suggest not only that hydrophobic sequences may affect the stabilization of βAP aggregation, but also that the state of aggregation of βAPs may be relevant to their biological activities. Together these observations support the theory that β-amyloid protein contributes to AD pathology either directly as a neurotoxin and/or indirectly by increasing neuronal vulnerability to other forms of injury.

603.1

CULTURE OF POSTNATAL MESENCEPHALIC DOPAMINE-CONTAINING NEURONS FROM WILD-TYPE AND WEAVER MICE. B.B. Ligon and S. Roffler-Tarlov*, Program in Neuroscience, Tufts Univ. Sch. Med., Boston, MA

Weaver is an autosomal recessive mutation in mice that affects the differentiation and vitality of several classes of neurons. In the midbrain, many of the dopamine-containing cells of the substantia nigra fail to differentiate normally and die after the first postnatal week. This is a report of our preliminary results of the fate of these neurons placed in culture.

For these experiments, the ventral mesencephalon and the striatum were removed from the brains of 7 day-old pups that were identified as being either homozygous weaver or homozygous normal mice by the appearance of the cerebellum. Disassociated cells from midbrain and striatum of the same mouse were plated together at high density on cover slips coated with polylysine, laminin and fibronectin. The coverslips were placed in 24-well plates with modified DMEM and serum.

After 4 days in culture, the cells were fixed and stained for the presence of tyrosine hydroxylase (TH) to identify the dopamine-containing populations. Both weaver and wild-type TH-positive neurons displayed both fusiform and polygonal morphology. In cultures of weaver, the TH-positive fusiform cells outnumbered the polygonal types by a ratio of 4 to 1; whereas polygonal forms outnumbered fusiform by a ratio of approximately 3 to 1 in the wild-type. Pyknotic nuclei were abundant in the weaver cultures and scarce in the wild-type indicating that cell death took place in the weaver cultures during this period at a greater rate than in the wild-type cultures.

This culture system appears to reflect the early cell death of the weaver's dopamine-containing neurons that takes place *in vivo*. These cultures may serve as a useful assay for conditions that influence the development and long-term survival of dopamine-containing neurons. NIH NS20181

603.2

MECHANISMS OF CELL DEATH PRODUCED BY CALCIUM INTOXICATION IN DIFFERENTIATED PC12 CELLS. P.P. Michel, S. Vyas, M. Ruberg, P. Anglade, F. Javoy-Agid* and Y. Agid, INSERM U289, Hôpital de la Salpêtrière, 75013 Paris, France.

Normal neuronal cell death either in vivo, during embryonic development or in vitro in cell culture models has been reported to be an active process, dependent on gene expression and protein synthesis (Ellis et al., Annu. Rev. Cell Biol., 7, 663, 1991, Martin et al., J. Neurosci., 10, 184, 1990). The question is whether there is a comparable activation of a cell death program in Parkinson's disease, a neurodegenerative state that is characterized by a selective and progressive loss of dopaminergic neurons. Since disruption of calcium homeostasis is a feature common to most models of cell degeneration, we studied the effects on gene expression of the ionophore A23187 in differentiated PC12 cells, i.e., a type of cells which exhibits properties similar to dopaminergic neurons.

In PC12 cells grown for 8-12 days in presence of NGF2.5S (25ng/ml) and serum proteins, treatment with low concentrations of the calcium ionophore A23187 (3-10μM) for 24hrs produced selective neurite disruption with limited loss of the number of cell bodies as characterized by the trypan blue exclusion criterion. Accordingly, [3H]-dopamine uptake, an index of neurite integrity was impaired in this range of concentrations. Neuronal differentiation could be resumed upon withdrawal of A23187 in the presence of NGF. Widespread cell degeneration occured at higher concentrations only (>30μM). Electron microscopical studies revealed morphological changes in the cytoplasm (formation of lipid droplets; mitochondrial shrinkage; reticulum vacuolation) but no obvious nuclear alterations such as chromatin segregation. In addition, DNA fragmentation could not be detected on agarose gels suggesting that in this culture model, nuclease activation is not a prerequisite for cell death. Other factors such as Ca^{2+} activated proteases or deregulation of certain genes that lead to irreversible damage are being studied.

603.3

NMDA-MEDIATED GLUTAMATE NEUROTOXICITY IN MESENCEPHALIC DOPAMINE NEURONS IN CULTURE. S. Kikuchi and S.U. Kim* Dept. of Neurology, Univ. of British Columbia, Vancouver, Canada.

Neurotoxic action of excitatory amino acids has been considered as one of the causes for the neuronal loss in neurodegenerative diseases that include Parkinson disease. In this study, we investigated the neurotoxic effect of glutamate in dopaminergic neurons grown in culture. Dissociated cell cultures were prepared from 13 day fetal mouse mesencephalon, while 10-14 day old cultures were exposed to glutamate for 10 minutes, and 24 hours later cultures were evaluated for glutamate neurotoxicity by tyrosine hydroxylase (TH) and MAP2 immunostaining and by dopamine uptake assay. In the glutamate-exposed cultures, the number of TH-positive and MAP2-positive neurons and the level of dopamine uptake were decreased to 30-50% of the control. Glutamate neurotoxicity was completely blocked by MK801, an NMDA receptor antagonist, and was reduced by magnesium ion. Phorbol ester and gangliosdies (GM1 and GT1b) were also found to block glutamte neurotoxicity, suggesting that protein kinase C translocation and activation might be important steps in the pathomechanism of glutamate neurotoxicity.

603.4

MK-801 DOES NOT PREVENT DOPAMINERGIC CELL DEATH INDUCED BY MPP+ IN RAT MESENCEPHALIC CULTURES. Y. Agid, B. Zalc*‡ and P.P. Michel, INSERM U289 et ‡U134, Hôpital de la Salpêtrière, 75013 Paris, France.

Neuroprotective effects of MK-801 were tested in a culture model reproducing the selective degeneration of dopaminergic neurons in parkinsonian brains (Michel et al., J. Neurochem., 54, 1102, 1990). Dissociated mesencephalic cells derived from embryonic rat brains were exposed to the 1-methyl-4-phenylpyridinium ion (MPP+), the active metabolite of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPP+ at low concentrations (3 and 10μM) produced selective and dose-dependent toxic effects towards dopaminergic neurons as quantified by the loss of tyrosine hydroxylase positive (TH+) neurons and the loss of [3H]-DA uptake whereas non dopaminergic cell types remained unaffected. MK-801 at 3 and 10μM did not rescue degenerative neurons. At 50μM, i.e., the highest concentration that is not toxic by itself in this culture system, MK-801 was also ineffective. Additionally, degree of dopaminergic damage was not reduced when repeated additions of the glutamate antagonist were performed during exposure to MPP+ or when mesencephalic cultures were left after intoxication for several days in a culture medium still supplemented with MK-801 but lacking the toxin. Furthermore, in control cultures, MK-801 did not affect the uptake of [3H]-DA significantly, thereby suggesting that this compound does not interfere with the accumulation of MPP+ into dopaminergic nerve terminals. At higher concentrations tested (100μM), MPP+ produced a non selective destruction of all cultured cells as characterized by the loss of the number of trypan blue excluding cells and the loss of [3H]-GABA uptake. In these conditions, MK-801 was also found ineffective. Altogether these results indicate that MPP+ neurotoxic effects are not related to an excitotoxic process and/or that MK-801 does not interfere with MPP+ toxic scenario. Therefore antiglutamates may prove not to be effective in preventing dopaminergic cell degeneration in Parkinson's disease.

603.5

DEPRENYL AND CLORGYLINE SUPPRESS HYDROXYL RADICAL GENERATION DURING DOPAMINE OVERFLOW ELICITED BY 2'-METHYL-MPTP. S.-J. Huang, C.C. Chiueh*, and D.L. Murphy, Lab. of Clinical Science, NIMH, Bethesda, MD 20892.

We have recently reported the use of *in vivo* intracranial microdialysis perfusion of salicylate to assess hydroxyl radicals (•OH) formation in the brain. The •OH adducts of salicylate were measured by HPLC-EC. Intrastriatal infusion of 2'-methyl analog of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (2'-methyl-MPTP, 1 nmol/min, for 60 min) but not MPTP caused a sustained increase in dopamine (DA) efflux lasting for more than 4 hours. In addition, increased •OH generation was also detected during the sustained DA overflow.

Pretreatment with deprenyl and clorgyline (5 mg/kg, i.p.) suppressed not only the DA overflow but also the •OH generation elicited by 2'-methyl-MPTP. Our ongoing *in vitro* results indicate that 2'-methyl-MPTP can potentiate iron-catalyzed DA autoxidation which leads to formation of •OH and neuromelanin. Thus enhanced •OH formation may be due to autoxidation of the released DA in the basal ganglia where high levels of iron are present. •OH may also be formed during the deamination of released DA and the oxidation of 2'-methyl-MPTP by A and B type monoamine oxidase. This data leads to a new working hypothesis that monoamine oxidase inhibitors may protect against MPTP-induced selective DA neurotoxicity by suppressing the generation of cytotoxic •OH radicals in the iron-enriched basal ganglia.

603.6

A METHOD FOR MEASURING DOPAMINE-PROTEIN CONJUGATES AS AN INDEX OF DOPAMINE OXIDATION. M.J. Zigmond* and T.G. Hastings, Dept. of Cellular and Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Dopamine (DA) and free radicals are implicated in the induction of striatal damage associated with certain neurotoxic events. In the presence of oxygen, DA oxidizes to free radical species and DA quinones. The quinones may attack and bind to nucleophilic groups on proteins resulting in potentially cytotoxic alterations in cellular function. To establish a method for measuring DA-protein conjugates as an index of DA oxidation, we incubated striatal slices with ^{3}H-DA under various buffer conditions and determined the amount of radioactivity bound to acid-precipitated protein. The amount of tritium bound to protein was greatly influenced by the concentration of reducing agent (ascorbate or glutathione) present in the incubation buffer. After a 1 h incubation with 60 nM ^{3}H-DA, there was a 3.4-fold increase in the amount of tritium bound to protein in a Krebs-bicarbonate buffer without ascorbate, as compared with the same buffer containing 0.85 mM ascorbate (2.52 ± 0.43 pmol/mg prot and 0.74 ± 0.04 pmol/mg prot, respectively). The difference increased to 6.6-fold after 120 min. The amount of tritium bound to protein was reduced by 90% in the presence of 10 µM glutathione. To characterize the DA-protein conjugate, striatal slices were incubated with 100 µM ^{3}H-DA and the labeled protein was hydrolyzed in 6 N HCl. Two major electroactive peaks containing radioactivity and corresponding to the elution positions of cysteinyl-DOPAC and cysteinyl-DA were isolated by HPLC in a 1 to 2.5 ratio. These findings suggest that: 1) the oxidation products of DA and DA metabolites are binding to protein; 2) binding occurs by covalent interactions with protein sulfhydryl groups; and 3) DA oxidation associated with cytotoxicity would be strongly influenced by cellular maintenance of antioxidant capacities. (Supported in part by USPHS grants NS19608, MH00058, and NS09076.)

603.7

PROSTAGLANDIN SYNTHASE-CATALYZED OXIDATION OF DOPAMINE. T.G. Hastings* and M.J. Zigmond. Dept. of Cellular and Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.

The oxidation of dopamine (DA) is implicated in neuromelanin formation and cytotoxicity, and is a likely contributor to the selective vulnerability of dopaminergic neurons. Although the oxidation of DA will occur nonenzymatically, examples of enzymatic activity mediating the toxicity of DA and related compounds have been reported. Numerous peroxidase-type enzymes are capable of catalyzing the oxidation of DA to reactive quinones, however, these enzymes are not found in brain. Prostaglandin (PG) synthase, an arachidonic acid metabolizing enzyme, is prominent in brain and has peroxidase activity that requires reducing cosubstrates such as catechol-containing compounds. As a result of this reaction, DA may be co-oxidized to DA quinones and free radical semiquinones. To investigate this possibility, we examined whether purified PG synthase would catalyze the oxidation of DA in vitro. Utilizing spectrophotometric analysis, we measured the formation of the DA oxidation product, dopachrome. We observed that PG synthase oxidized DA, using either arachidonic acid or hydrogen peroxide as a substrate, at an initial rate of 12.0 nmol/min, 44-fold faster than DA autoxidation. The rate of the catalyzed reaction began to plateau within 2 min, indicative of the enzyme's self-inactivation. In the presence of bovine serum albumin, the PG synthase-catalyzed oxidation of DA resulted in the binding of DA to protein which was identified as cysteinyl-DA conjugates. The amount of cysteinyl-DA isolated from protein was increased 2-4 fold in the presence of PG synthase, using either substrate. The identification of cysteinyl-DA indicates that the enzyme catalyzes the co-oxidation of DA to quinones which are capable of reacting with nucleophilic thiol groups on proteins. DA oxidation catalyzed by PG synthase represents potential mechanisms for neuromelanin formation and cytotoxicity. (Supported in part by USPHS grants NS19608, MH00058, and NS09076.)

603.8

USE OF SALICYLATE TO TRAP HYDROXYL RADICALS IN RAT BRAIN: A METHODOLOGICAL STUDY L.P. Liang*, T.G. Hastings, M.J. Zigmond and A. Giovanni. Dept. Cellular and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Formation of free radicals in brain has been implicated in many neuro-degenerative processes, including those induced by ischemia, excitatory amino acids, and amphetamines. It has been reported that salicylate can react with hydroxyl radicals to form the stable adducts 2,3- and 2,5-dihydroxybenzonic acid (DHBA) (Floyd et al., J. Free Rad. Biol. Med., 1986). Tissue levels of these adducts can be determined by HPLC with electrochemical detection. We assessed the practicality of this method and determined a paradigm for estimating the content of hydroxyl radicals in rat brain. Following its systemic administration (100 mg/kg, i.p.), salicylate levels peaked at approximately 150 nmol/g tissue at 2 hrs and then declined with a t½ of 4 hr in several brain areas, including striatum, frontal cortex, and hippocampus. The major adduct of salicylate formed in vivo was 2,5-DHBA, which reached a peak of approximately 250 pmol/g tissue (2 hr) in the different areas. DHBA levels were not affected by the addition of the reducing agent ascorbate (0.5 mM) and/or the iron chelator DETAPAC (1 mM) during tissue homogenization (sonication at 100 mg/ml in 0.2 M perchlorate). Freezing the tissue (-70°C) also had no effect. We are using this method to examine formation of free radicals in vivo under several conditions associated with an increase in DA availability, such as methamphetamine exposure (see Giovanni, Soc. Neurosci. Abs., 1992) and L-DOPA-treatment. Our data demonstrate the usefulness of this technique to trap free radicals in vivo to quantify these highly reactive and short lived oxygen species. (Supported in part by NS19608, MH18273, and MH00058.)

603.9

METHAMPHETAMINE INCREASES HYDROXYL RADICALS IN RAT STRIATUM: ROLE OF DOPAMINE. A. Giovanni*, T.G. Hastings, L.P. Liang, and M.J. Zigmond. Department of Cellular and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

The role of dopamine (DA) in the induction of dopaminergic neurotoxicity resulting from methamphetamine (METH) treatment supports the hypothesis that DA may be involved in the etiology of Parkinson's disease. DA can autoxidize to form a variety of potentially neurotoxic compounds, including free radicals such as the hydroxyl radical (OH•). To assess the role of free radicals in the toxic effects of METH, we used salicylate to trap OH• in vivo. We then used HPLC with electrochemical detection to measure the striatal content of 2,3-dihydroxybenzonic acid (2,3-DHBA) and 2,5-DHBA, stable adducts resulting from OH• attack upon salicylate (see Liang et al., Soc. Neurosci. Abs., 1992). Adult male rats (325-375 g) were given a neurotoxic regimen of METH (12.5 mg/kg, s.c., 4x, at 2 hr intervals) and neurotoxicity was assessed by examining striatal DA content for long-term depletion. Some rats received salicylate 1 hr after the last dose of METH and the animals sacrificed 2 hr later. Under these conditions, METH caused an increase in the level of 2,5-DHBA in striatum, from 107 ± 13 to 237 ± 34 pmol/g tissue (mean ± SEM, n=8 and 9, respectively). This increase, as well as the decrements in striatal DA, was blocked by pre-treatment with α-methyl-*p*-tyrosine (2 x 50 mg/kg, i.p.), an inhibitor of catecholamine biosynthesis. These results indicate that: 1) the concentration of free radicals in striatum increases in response to neurotoxic doses of METH; and 2) DA plays an essential role in this METH-induced increase in free radicals. These findings also suggest that under other conditions of increased availability, endogenous DA may play a role in neurodegeneration through the formation of free radicals. (Supported in part by NS19608, MH18273, and MH00058.)

603.10

THE EFFECT OF FREE RADICALS ON DOPAMINE METABOLISM, IMPLICATIONS FOR PARKINSON'S DISEASE.

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The mechanism for the accelerated loss of dopaminergic neurons in Parkinson's Disease is unknown. One hypotheses focuses on the oxidative stress associated with the natural metabolism of dopamine (DA). We have used ESR spin trapping to study the kinetics of free radical production from DA metabolism by serum or mitochondrial MAO. The spin adduct observed is the hydroxyl (OH) adduct of the spin trap 5,5-dimethyl-1-pyrroline N-oxide. A quantitative comparison of the ESR with simultaneous enzyme assays shows that the number of OH radicals trapped is a substantial fraction (>10%) of the number of DA metabolized. Surprisingly, kinetic analysis of the ESR data shows that adventitious copper is responsible for the OH production in the serum MAO system. When Gamma irradiation was used as an alternative source for free radical production we detected altered mitochondrial MAO ability to metabolise DA. The role of protective agents and transition metals is under study. These results support the oxidative stress hypothesis associated with DA which plays a role in Parkinson's Disease. (Supported in part by a grant to F.F.Oldfield from the Parkinson's Disease Foundation.)

603.11

MPP$^+$ INDUCED OXIDATIVE STRESS - NEW INSIGHTS INTO THE HYDRIDE TRANSFER MECHANISM. J. D. Adams* and L. K. Klaidman. Dept. Molecular Pharmacology and Toxicology, Univ. Southern Calif., Los Angeles, CA 90033.

We recently proposed a new mechanism for MPP$^+$ induced oxidative stress, involving a two electron transfer, called hydride transfer. This process is mediated by a number of enzymes, especially flavin enzymes. Hydride tranfer to MPP$^+$ results in the formation of 1-methyl-4-phenyl-1,4-dihydropyridine (DHP) which is quickly oxidized by $O_2^{\cdot-}$ in one electron steps. The first product is MPP$^{\cdot}$ which can reduce oxygen to form $O_2^{\cdot-}$ or cleave hydrogen peroxide to form OH$^{\cdot}$, and regenerate MPP$^+$. We now report the quantitation of $O_2^{\cdot-}$, H_2O_2 and OH$^{\cdot}$ during incubations of MPP$^+$ or MPDP$^+$ with enzymes including monoamine oxidase, lipoamide dehydrogenase, aldehyde dehydrogenase and xanthine oxidase. These experiments in addition to oxygen uptake experiments are demonstrating the ability of MPP$^+$ to redox cycle and form radicals that could be damaging to cells. Most of the enzymes examined are found in mitochondria, and perhaps the cytosol, which may indicate the importance of oxygen radical formation by MPP$^+$ in mitochondria. This redox cycling can complement the formation of $O_2^{\cdot-}$ during inhibition of NADH dehydrogenase by MPP$^+$. Mitochondrial and cytosolic generation of oxygen radicals are critical components of the toxicity induced by MPP$^+$.

603.12

SHORT TERM ALTERATIONS OF NIGRAL MALONDIALDEHYDE (MDA) LEVELS AND STRIATAL DOPAMINERGIC MARKERS INDUCED BY INTRANIGRAL IRON INFUSION. G.J. Sengstock[1]*, N. Zawia[3], C.W. Olanow[2], A.J. Dunn[4], G.W. Arendash[1]. Depts. of Biology[1], Neurology[2], Univ. of South Florida, Tampa, FL 33620; NIEHS[3], Research Triangle Park, NC 27709; Dept. of Pharmacology[4], LSU Medical Ctr., Shreveport, LA 71130.

In Parkinson's disease (PD), iron content and lipid peroxidation are increased within the substantia nigra (SN). We recently reported that iron infusions into the rat SN induce localized neurodegeneration as well as dose-dependent reductions in striatal dopaminergic markers (*Brain Res Bull* 28:645, 1992). To test whether iron-catalyzed lipid peroxidation may be associated with the resultant degenerative changes, 0.63nmol of iron or vehicle was infused unilaterally into the SN of adult male rats. Animals were decapitated at several time points through 1 month. Striatal tissue was assayed for biogenic amines by HPLC. To record only **basal** MDA levels in the SN and prevent further MDA formation beyond decapitation, desferrioxamine and BHT were incorporated in our spectrophotometric MDA microassay. Vehicle infusion had no effect on MDA or striatal dopaminergic markers. In contrast, MDA values from iron-infused animals were significantly elevated (72%, $p<0.05$) 1 hour post-infusion compared to nonoperated control animals and returned to control levels by 1 day. Successive to the SN alterations, striatal DOPAC and HVA (catabolites of dopamine metabolism) were significantly increased ($p<0.05$) at 1 day, as was calculated dopamine turnover ($p<0.001$). By one week, all neurochemical values returned to control levels. These data are supportive of the hypothesis that lipid peroxidation, as determined by MDA levels, is acutely increased following iron infusion into the SN This suggests that iron-induced lipid peroxidation may be involved in the degeneration of nigrostriatal dopaminergic neurons in PD.

604.1

SEASONAL VARIATION IN THE INCIDENCE OF NEURODEVELOPMENTAL DISORDERS. J. Liederman * and K. Flannery, Psych. Dept., Boston Univ., Boston, MA 02215.

Neurodevelopmental disorders (NDs) such as learning disabilities, articulation disturbances, attention deficit disorder, cerebral palsy, and mental retardation may sometimes be triggered by events that occur during gestation. One important intrauterine factor may be endocrinological; it has been suggested that imbalances of sex hormones during gestation may be associated with neurodevelopmental disorder. Since hormone levels are known to vary seasonally, the relationship between hormonal variation in utero and ND was indirectly assessed by measuring the incidence of NDs for children conceived during different seasons (defined in terms of length of daylight). This was was examined in a subsample of The National Collaborative Perinatal Project (N = 21,833). There were seasonal variations in the rate of NDs with the highest prevalence for winter/fall conceptions as compared to spring or summer. These findings were robust since they remained unchanged when considering seasonal variations in other risk factors such as maternal weight gain, maternal anemia, and maternal infections.

This work was supported by NIMH Grant # R03 MH47049 to J.L.

604.2

SELECTIVE COGNITIVE DEFICITS LINKED TO GENE SIZE AND PARENTAL INHERITANCE IN NONRETARDED CARRIERS OF FRAGILE X SYNDROME. V.J.Hinton*, J.M.Halperin, C.S.Dobkin, X.H.Ding, W.T.Brown and C.M.Miezejeski. CSI/IBR Center for Developmental Neuroscience, Staten Island, NY 10314.

The relationship between inheritance, the size of the FMR-1 gene, and cognitive function was evaluated in nonretarded adult female carriers of the Fragile X Syndrome. The goals were to determine whether 1) parental inheritance of FMR-1 affects cognitive and genomic expression; 2) mild cognitive impairments are associated with expansion of an unstable CGG region in the FMR-1 gene; and 3) specific cognitive domains are affected by this expansion. Sixteen women with paternally inherited FMR-1 (PI), 11 women with maternally inherited FMR-1 (MI) and 16 controls were compared with regard to DNA fragment size probed with StB 12.3 and neuropsychological measures. The PI group did not differ from controls on IQ, with all being within 1 SD of the mean, and none showed large CGG expansion in the FMR-1 gene. In contrast, five (45%) subjects in the MI group had IQ scores more than 1 SD below the mean and large (>500 bp) CGG expansion in the FMR-1 gene. All other MI subjects were indistinguishable from the PI subjects on both cognitive and molecular measures. The MI group was then subdivided into two groups based on size of CGG expansion. After controlling for IQ, MI subjects with large expansions were found to be selectively impaired ($p < .05$) on measures of perceptual and attentional function, but not verbal skills. Thus, molecular status of FMR-1 appears to be linked to parental inheritance and may selectively affect specific cognitive skills in nonretarded women.

604.3

SPECT EVIDENCE OF DELAYED FRONTAL CORTEX MATURATION IN CHILDHOOD AUTISM M. Zilbovicius*, B. Garreau, Y. Samson, Ph. Remy, B. Bruck, C. Barthélémy, A. Syrota, G. Lelord. SHFJ, CEA, Orsay and INSERM U316, Tours, France.

Neurobiological causes of childhood autism, a severe developmental disorder, are still unknown. Clinical and cognitive data have suggested that the maturation of the cerebral cortex may be delayed in this disorder, particularly involving the frontal lobes. Maturational changes in cerebral function can currently be assessed by measures of regional metabolism (Chugani and Phelps, 1986) or cerebral blood flow (Tzourio et al., 1988). We used SPECT to compare the regional distribution of cerebral blood flow (rCBF) in five young children with primary autism (age: 41 ± 7.8 months) and in five age-matched non-autistic children (33.4 ± 6.8 months). All studies were performed under sedation with the Xenon 133 iv injection method. The normalized frontal rCBF was markedly lower in the autistic group (0.86 ± 0.07) than in controls (0.96 ± 0.08, $p < .0008$). The normalized posterior cortical rCBF was slightly higher in autistic than in control children (1.1 ± 0.1 vs 1.07 ± 0.25, $p < .04$). The mean cerebral cortical blood flow was similar in both groups (autistic: 73.2 ± 10.1 ml/min/100g; control: 72.4 ± 8.5 ml/min/100g, ns). We believe that the marked frontal hypoperfusion found in these young autistic children (2-4 years) may indicate a delayed frontal lobe maturation rather than a permanent frontal abnormality since: 1) such frontal hypoperfusion is found in normal but younger children (1-2 years old), and 2) frontal perfusion is normal in older (5-11 years old) autistic children (Zilbovicius et al., 1992).

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604.4

CHRONIC ABERRANT BEHAVIOR IN THE MENTALLY RETARDED: ANIMAL MODELS, RISK FACTORS, AND ADJUNCTIVE BEHAVIOR. P. S. Loupe, C. J. Stodgell, & R. E. Tessel.* Dept of Pharmacol. & Toxicol., Univ. of Kansas, Lawrence, KS 66045

Spontaneously hypertensive (SHR), Sprague-Dawley (SD) rats neonatally depleted of brain catecholamines by 6-hydroxydopamine injection (6HD; 100 ug/rat) or made microencephalic by prenatal methylazoxymethanol (MAM; 25mg/kg) injection to pregnant dams, and post-weaning social isolation (SI) and prior environmental stress may respectively model genetic,organic and environmental risk factors for self-injurious behavior (SIB), stereotypies, aggression and hyperactivity (Chronic Aberrant Behaviors; CAB) seen in the mentally retarded. It may be that CAB are induced and maintained at high frequencies in the mentally retarded because the risk factors increase susceptibility to the adjunctive (schedule-induced) consequences of intermittent reinforcement. In an attempt to evaluate this hypothesis, group-housed and SI MAM, SHR, 6HD and SD rats with prior histories of repeated exposure to electric foot-shock were magazine trained under a VI 45" schedule of food-pellet presentation to a 5-sec latency criterion for removal of food from a hopper; MAM rats required significantly more sessions to reach criterion than any other group ($p < .01$). The schedule was then changed to an FT 60" and the proportion of inter-pellet intervals in which specified behaviors occurred (behavioral frequency) were determined. Locomotor activity and rearing frequencies during FT session 1 were similar among each of the non-environmental risk factor groups except those in MAM which were significantly greater ($p < .05$). These frequencies increased significantly ($p < .05$) across subsequent sessions in SHR but not in 6HD, MAM or SD. Grooming frequency increased significantly across sessions ($p < .01$) to essentially to the same extent in all groups. No biting (an index of aggression), SIB or effect of housing was observed during training. The relationship of these findings to CAB will be discussed. (Supported by NICHHD grant 1 PO 1HD26927)

604.5

EFFECTS OF METHYLAZOXYMETHANOL (MAM) TREATMENT ON GESTATIONAL DAY 14 (GD14) ON NEUROCHEMISTRY, REGIONAL BRAIN WEIGHT, AND BEHAVIOR. S. A. Ferguson*, R. R. Holson, M. G. Paule and J. F. Bowyer. Divisions of Neurotoxicology and Reproductive & Developmental Toxicology, National Center for Toxicological Research, Jefferson, AR 72079.

Sprague-Dawley rats were injected sc with either MAM (20 mg/kg) or saline on GD14 (plug date=GD 0). Beginning prior to weaning and continuing through adulthood, offspring were assessed on a variety of behavioral tests, including activity measures. Regional brain weights were obtained and DA and 5-HT concentrations in caudate nucleus (CN) were measured. Contrary to what is typically reported, MAM-treated rats exhibited either hypoactivity or normal activity levels; no consistent indications of hyperactivity were noted. Frontal cortex, hippocampus, and CN of MAM-treated rats were most reduced in weight (cortex weight was 50% of control) while brainstem, olfactory bulbs, thalamus, cerebellum, and body weight were relatively unaffected. MAM treatment increased 5-HT concentration in CN two-fold, with little effect on DA concentration. Regional brain weight reductions and increased CN 5-HT concentrations in MAM-treated rats were consistent with previous findings; however, neither activity nor DA levels were consistent with literature reports. The possibility that these data may reflect a strain difference cannot be excluded.

604.6

AN INBRED EPILEPSY-PRONE SUBSTRAIN OF BALB/c MICE SHOWS AN ABSENCE OR REDUCTION OF CORPUS CALLOSUM AND AN ABNORMAL PROJECTION TO BASAL FOREBRAIN. S. Dolina*, C. Morin, C.E. Ribak and R.T. Robertson. Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Epilepsy-prone (EP) and epilepsy-resistant (ER) substrains of mice derived from the BALB/c strain have been developed through 19 generations of close breeding and subsequent inbreeding. The BALB/c strain shows the acallosal phenotype in about 15% of the population. In this study, we determined the presence or absence of a corpus callosum and patterns of efferent projections from motor cortex in EP and ER mice. Data were collected from juvenile (postnatal age 13-14 days) and adult mice. Animals from the 8th and 9th inbred generations were used. Aldehyde fixed tissue was prepared for Nissl stains and DiI for the tracing of cortical efferents.

ER animals had normal corpora callosa (101 of 101 cases). Twenty of 26 EP animals were acallosal and the remaining 6 EP animals displayed a corpus callosum markedly reduced in size. Placements of small crystals of DiI in motor cortex of ER animals revealed the presence of a normal corpus callosum, with anterograde labeling of terminals and retrograde labeling of pyramidal neurons in the contralateral cortex. In acallosal EP animals, DiI labeled axons extended toward the midline, but turned ventrally to course through the medial septum. These fibers appeared to terminate in the basal forebrain in the region of the nucleus of the diagonal band. EP animals with a rudimentary corpus callosum showed some labeled fibers crossing the midline to terminate in the contralateral cortex, but many fibers also coursed ventrally to the basal forebrain.

These results show that aberrant cortical projections to the basal forebrain occur in mice that lack a complete corpus callosum. Further, in this model a strong linkage between acallosal brains and inherited epilepsy is demonstrated.

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604.7

DISTRIBUTION AND MORPHOLOGY OF THE CORTICOSPINAL TRACT NEURONS IN PRENATALY X-RAY IRRADIATED RATS. RETROGRADE TRACING AND INTRACELLULAR LUCIFER YELLOW STAINING STUDIES.

H. Ochiai, S. Miyahara, S. Wakisaka, and M. Inoue*. Dept. of Neurosurgery, Miyazaki Med. College Miyazaki 889-16 and Dept. of Physiology, Fukuoka Univ., Fukuoka 812, Japan.

X ray irradiation to foetus is a potent teratogenic procedure, and such it is the prime cause of mental retardation and behavioral abnormality. In order to clearify the effect of X ray irradiation on development of the corticospinal (CS) tract neurons, fetal rats were irradiated with X ray (1.5 Gy, 140 kV, 4 mA) at pregnant day 12. At the day 30 of postnatal, FAST BLUE or HRP was injected into cervical cord at C3-4 level to identify CS neurons. Three days after the injection, the animals were sacrificed, brain was removed and sectioned at a thickness of 100 micrometer, and the CS neurons were stained intracellulary with Lucifer Yellow, photo-oxydated and analyzed. Most of retrogradely labeled neurons distributed layer V, their row formation was poor and vertical disposition was remarkable. Clustering of ectopic neurons were sometimes seen at layer VI. Most of the CS neurons distributed in layer V was typical pyramidal cell, but the neurons distributed in the deep of layer V had relatively round somata and the apical dendrites were thinning, shortened and sometimes wavy, indicating that the development and differentiation were poor. The ectopic neurons had small and round somata, and it had slender and spokewise dendrites, which had rich dendritic trees. The heterogeneity of the population and variety of shape of the CS neurons in X ray irradiated rats results from the effects of irradiation on early events in neuronal development such as neuronal generation and migration.

604.8

THE HISTOPATHOLOGICAL RESPONSE OF THE FETAL SHEEP BRAIN TO MID-GESTATIONAL HYPOXIA.

D.H. Penning[1], M.R. Grafe[2]*, Y. Matsuda[3], R. Hammond[3], B. Richardson[3]. Queen's Univ., Kingston, Ontario[1]; Univ. of Calif. San Diego[2]; Univ. of Western Ontario[3].

Intrauterine hypoxic/ischemic injury is the cause of significant morbidity and mortality in newborns and children and may produce injuries ranging from learning disorders to severe neurologic impairment. We examined the physiologic and neuropathologic effects of midgestation hypoxia in chronically instrumented fetal sheep. The results of the physiologic studies have been reported (Matsuda et al, Am J Obstet Gynecol, in press). Fetal sheep were catheterized in utero 5 days prior to hypoxia. At 87-99 days gestational age (term=147 days) the ewes inspired a hypoxic gas mixture (9% O2) for 8 hours; maternal and fetal blood gases were monitored throughout the experiment. Fetuses became hypoxic (PaO2=14.8 mmHg vs. controls=29.0), but only mildly acidotic (pH=7.23 vs. controls=7.36). Three days after hypoxia the ewes were euthanized and fetuses removed and immediately perfused with formalin. The brains were routinely prepared for histology and sections were taken to include cerebral cortex with white matter, hippocampus, thalamus, basal ganglia, cerebellum, and brain stem. Control animals included animals subjected to the same surgical procedures, but without hypoxia as well as normal, uninstrumented animals. One hypoxic animal was excluded from further analysis due to acute meningitis. Three of the 4 remaining hypoxic animals had moderate to severe white matter necrosis in the cerebral hemispheres. In the most severely injured regions adjacent cortex was also infarcted, but selective neuronal injury was not seen. One hypoxic animal showed no significant changes. Mild, focal white matter injury was seen in one of the two instrumented controls. Uninstrumented controls (n=3) had no significant pathologic changes. The results of these studies correlate well with the white matter necrosis seen in some premature infants. Similar studies in near-term (>129 days) fetal sheep are underway.

604.9

EARLY POSTNATAL OXYGEN DEFICIENCY AND NEURAL-BEHAVIORAL DEVELOPMENT IN RATS.

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Perinatal oxygen deficiency decreases motor activity and alters its cyclicity immediately after the treatment. In the present study, behavior, neuropathology and neurochemistry of pups deprived of oxygen were studied until postnatal day 21.

Pups at P0 or P1 were deprived of oxygen (placement in airtight chambers until a gasping criterion; 3 identical treatments + rest periods). Pups were housed with the dam. Behavior (open field activity, sensory-motor reflex testing), HPLC biochemistry (dopamine, serotonin of accumbens, striatum, and hippocampus), and histopathology were studied at P1, P7, P14 and P21.

Oxygen deprived and control pups showed no differences in body weights at any age. Preliminary analyses indicate relationships between behavioral, neurochemical and histopathological measures. (Conducted under NIH Guide for Care and Use of Laboratory Animals).

604.10

BIRTHDATES OF NEURONS IN NEOCORTICAL ECTOPIAS OF NEW ZEALAND BLACK MICE.

G.F. Sherman*, L.V. Stone, N.R. Walthour, G.W. Boehm, V.H. Denenberg, G.D. Rosen, and A.M. Galaburda. Department of Neurology, Harvard Medical School, and Beth Israel Hospital, Boston, MA 02215 and Biobehavioral Sciences Program, University of Connecticut, Storrs, CT 06269.

Previous studies have shown that 40-50% of New Zealand Black (NZB/B1NJ) mice, a strain which spontaneously develops severe autoimmune disease, have ectopic collections of neurons in layer I of the cortex (Sherman et al., Acta Neuropathol., 74:239-242, 1985). Usually one ectopia is seen in affected brains, although multiple ectopias are sometimes present. Ectopias contain as few as 10 or as many as 100 or more neurons, and have been seen as early as E13-14. The birthdates of neurons in the ectopias has not been determined and therefore in the present study we examined ectopias in adulthood after BrdU (an S-phase marker) injections of pregnant NZB mice.

NZB mice were time-mated (E0 was the day a vaginal plug was found) and the pregnant females were given an injection of BrdU (Becton-Dickinson; 50 µg per gram of body weight) on one day from the range covering the birth of most neurons in the cerebral cortex (E10-E18). Over 100 offspring were anesthetized in adulthood and perfused with 0.9% saline followed by 4% paraformaldehyde. The brains were removed form the crania and placed into 10% buffered sucrose overnight, followed by 30% buffered sucrose for 24 hours, after which they were frozen and cut coronally at 30 µm on a sliding microtome. Every fifth section was stained with thionin to reveal neocortical ectopias and BrdU immunohistochemistry was performed on adjacent sections.

BrdU staining of neurons was similar to that reported by other authors: after early injections BrdU-positive neurons were present mostly in infragranular layers of the cortex, whereas the supragranular layers were positively-stained after later prenatal injections. However, the layer I ectopias showed a different pattern. Cells born as early as E12-13 and as late as E15 were present in the adult ectopias. The morphology of these cells was neuronal in appearance, however, double-labeling experiments are necessary to make any definitive statements. The older ages (E16-18) are presently being examined. We conclude that the ectopias originate early during the prenatal period and may consist of neurons generated over a wide range of prenatal days. (Supported in part by NIH grant HD 20806.)

604.11

BIRTHDATES OF NEOCORTICAL NEURONS IN INDUCED MICROGYRIA IN THE RAT.

G.D. Rosen*, J.M. Richman, G.F. Sherman, and A.M.Galaburda. Beth Israel Hospital and Harvard Med. School, Boston, MA 02215.

A freezing probe placed on the skulls of newborn rats acutely damages the cortical plate underlying the probe. Within one week of the lesion, the cortex reorganizes forming an area resembling focal microgyria. This four-layered structure consists of an outer molecular layer (i); a second layer (ii) which is contiguous with layer II and III of the adjacent, intact cortex; a *lamina dissecans* (iii); and a fourth layer (iv) which is contiguous with layer VIb. The current experiment was designed to determine the birthdates of neurons comprising this induced neocortical malformation.

Time-mated pregnant Wistar rats were injected with either [^{3}H]thymidine or BrdU on embryonic day (E) 15, E17, E19, or E21. At postnatal day (P) 0 or P1, the pups from these litters were anesthetized by hypothermia and a freezing probe (≈ -70°C) placed on the skull overlying the cortical plate. Animals were sacrificed on either P1, P3, P4, P5, P7, P10, and P60 by transcardial perfusion with saline followed by 4% paraformaldehyde. The brains were post-fixed for 28-96 hours, sectioned coronally, and either immunohistochemically stained for BrdU or prepared for autoradiography.

Neurons born on E15, which are seen in the infragranular layers of the undamaged cortex, were rarely found in the microgyric cortex. When present they could be seen only in microgyric layer iv. Neurons generated on E17 and E19 were found scattered throughout microgyric layer ii, whereas in the undamaged cortex E17 neurons were generally found in inferior laminar locations to those born on E19. A few E21 neurons were seen in the most superficial portions of microgyric layer ii. These results suggest that the majority of neurons comprising the microgyric cortex are generated later in neuronogenesis.

(This work was supported, in part, by NIH Grant 20806).

604.12

SELECTIVE LAMINAR AND AREAL DELETION OF CORTICAL NEURONS BY PRENATAL X-IRRADIATION IN THE MACAQUE MONKEY.

O. Algan*, P.S. Goldman-Rakic, P. Rakic Section of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510

Ionizing irradiation was used to separately diminish or delete cells in infragranular, granular and supragranular layers of the neocortex in fetal monkeys. X-irradiation was performed on specific embryonic (E) days to disturb mitotic division and migration of neurons destined to form particular layers (Rakic, '74-'88). Macaque fetuses were irradiated at E33-40, E39, E70-73, E70-80, E80-90 with individual doses among 40-264 rads (R) and cumulative doses of 100-600R. Pregnant monkeys were anesthetized, and an X-Ray tube was focused on the fetal head, localized with ultrasound. Offspring were tested behaviorally in a separate study and/or sacrificed between P60 and 2 yrs. The brains were cut at 35µm from celloidin blocks and stained with cresyl violet. The density per unit volume, the size and the distribution of neurons in each layer of areas (A) 17 and 24 were examined using an unbiased 3D cell counting method (Williams & Rakic, '88). The effects of doses and schedules of irradiation were evaluated by ANOVA. We found first, that the density of cortical cells was lower in irradiated offspring, who also had larger cerebral ventricles than age matched controls. Second, our initial results indicate that irradiation had different effects at different ages: e.g., at E70-80, irradiation with >400R decreased cell density substantially in layers II-V of A17, while irradiation with similar doses (250-300R) at E80-90, depleted more superficial layers preferentially. Irradiation at E70-80 with 100-400R depleted predominantly middle layers IV and V. Third, different dose response effects were obtained in different areas of the same specimens in accord with the timing of cell origin of these areas. For example, irradiation at E80-90 did not alter cell density in A24, while irradiation at E70-80 affected only layer II which is generated at that time. The preferential depletion of specific layers of primate neocortex by X-irradiation provides an opportunity to analyze the effect of individual laminar loss in selected areas on the local synaptic organization and the pattern of extrinsic connectivity of the cerebral cortex as well as the consequent effects on behavior. Supported by MH44866.

604.13

Pyramidal Cells in the Sensory-motor Cortex of the H-Tx rat with infantile hydrocephalus. H.C. Jones* and N.G. Harris#. Dept of Pharmacology, Univ. of Florida, Gainesville, Fl 32610 and # Dept of Physiology, King's College, London W8 7AH, UK.

The H-Tx rat has congenital hydrocephalus caused by obstruction of the aqueduct at 18-days gestation. The lateral ventricles expand rapidly after birth and the cortical grey matter becomes severely attenuated, particularly in the posterior cortex where there is disruption of the laminar structure in the deeper layers. Layer V pyramidal cells have been studied at 21 days after birth by quantitative analysis of Golgi-stained sections from the sensory-motor (anterior) cortex. The basal dendrites of cells in the most severely affected rats were significantly reduced by 40 -60 % in total length and also in vertical and horizontal extent when compared to control rats. This was confirmed by concentric circle analysis which showed a reduction in the number of dendrite-ring intersections with increasing distance from the soma. The angle of the apical dendrite to the pial surface was between 0 and 17° in control rats but around 40% of the cells from hydrocephalic rats were abnormally orientated with angles greater than 17°. The apical dendrites, although variable, were not significantly altered in cells from hydrocephalics in either vertical or horizontal extent. The results are consistent with ventricular expansion resulting in compression of the deep layers of the cortex and also with tangential forces causing disorientation of pyramidal cells.

604.14

EFFECTS OF HYDROCEPHALUS AND DECOMPRESSION ON RETINAL CELL DEVELOPMENT. E.C. Williamson, H.E. Pearson, T.J.Shickley and J.P.McAllister II. Temple University School of Medicine, Philadelphia, PA 19140.

Even after surgical decompression, infantile hydrocephalus often results in permanent neurological symptoms, including visual deficits. However, little is known about the cellular changes that may be responsible for these problems. The present study was designed to analyze the retinae of normal, severely hydrocephalic, and decompressed kittens to determine the density and size of retinal ganglion cells. Hydrocephalus was induced in 10 day old kittens by intra-cisternal injection of kaolin. Kittens were allowed to survive from 7-28 days and then sacrificed and perfused with mixed aldehydes. Animals that received ventriculoperitoneal shunts were shunted 10-15 days after the induction of hydrocephalus and then sacrificed 10-14 days after shunt placement. Retinae were flat-mounted onto glass slides and stained with cresyl violet. A 750 x 750 micron sample area was chosen 1 cm along the horizontal meridian in nasal retina, and cells were drawn using a drawing tube under 40X magnification. Cell density and cell area were determined using the Bioquant image analysis system. Total cell density was significantly increased in severely hydrocephalic animals but returned to within normal levels following decompression. On the basis of cell size, however, the glial population was significantly increased and there was a significant loss of ganglion cells in both the hydrocephalic and the shunted groups. Based on the results, we conclude that gliosis occurs as a result of cell death in the retina following hydrocephalus, and decompression is unable to reverse these effects. Supported by NS25196 and HD21527.

604.15

DYSPLASIA OF THE OLFACTORY BULB IN A RABBIT MODEL OF HYDROCEPHALUS. M. J. De Rosa, Ch. E. Olmstead#, W. J. Peacock, C. P. E. Outram, R. Gayek, H. V. Vinters, and R. S. Fisher. Departments of Pathology, Surgery/Neurosurgery, Anatomy, Laboratory Animal Medicine and Brain Research Institute. Univ. Calif. Sch. of Med., Los Angeles CA 90024

In a paradigm designed for the induction of hydrocephalus in the rabbit by the intracisternal injection of kaolin, we have demonstrated a dyslamination of the cerebral cortex, cell loss and alterations in the cytostructural composition of cortical neurons. These findings are being extended to an analysis of the olfactory bulbs, a major drainage route for cerebrospinal fluid and an area markedly affected by this manipulation. We have demonstrated a deviation from the normal layering pattern of the olfactory bulb with the most severe effects occurring in the granule cell layer. Rather than the "shingled" appearance of the granule cell layer seen in the littermate control animals, this layer in the hydrocephalic animals appears as a solid sheet of cells with no separation into the overlapping laminae commonly seen. Mitral cell and glomerular layers also appear affected by this insult. As the major developmental landmarks appear later in the olfactory bulb than in the cerebral cortex, the olfactory bulb of the kaolin induced hydrocephalic rabbit may be an interesting system for the study of neurodevelopment and developmental neuropathology.

604.16

EXPRESSION OF N-CAM IN THE REELER MOUSE CEREBELLUM. E.M. Sajdel-Sulkowska* and M. Lee Dept. Psychiatry,Harvard Medical School and Mailman Research Center, McLean Hospital, Belmont, MA 02178

During the development of CNS in rodents, the neural cell adhesion molecule (N-CAM) mRNA form containing a 30 bp VASE exon replaces RNA that lacks this exon. The resulting structural modification of N-CAM may affect neuronal interaction and migration. The objective of these studies was to compare the expression of the alternative splicing forms of N-CAM in the cerebellum of the control and reeler mice; the cerebellum of this neurological mutant is characterized by the inside-out cell layering as a result of the abnormal neuronal migration. We have used a reverse transcriptase-polymerase chain reaction (RT-PCR) method to examine the expression of the N-CAM mRNA. Both splicing forms were expressed in the control and mutant mouse cerebellum; the embryonic mouse brain tissue showed predominantly the form lacking VASE exon. Preliminary data suggests the presence of an additional form of N-CAM expressed in the mutant but not the control cerebellum. Further experiments are in progress to verify the existence of the additional splicing form and to identify the novel sequence.

604.17

EXPRESSION OF ALTERNATIVELY SPLICED FORMS OF NEUROFIBROMIN mRNA IN THE CHICK EMBRYO L. Baizer,*G.S. Ciment, K.M. Stocker, and G.L. Schafer RS Dow Neurological Sciences Institute, Good Samaritan Hospital and Medical Center and Department of Cell Biology and Anatomy, OHSU, Portland, Oregon 97209

Neurofibromatosis type 1 (NF-1) is among the most common inherited disorders. Identification and characterization of the NF-1 gene product (now known as 'neurofibromin') has revealed that it is similar in structure and function to the ras-GTPase Activator Protein (GAP). Further nucleotide sequence analysis of human neurofibromin cDNAs has indicated the existence of an alternatively spliced form of the mRNA which contains 63 additional nucleotides in the GAP-Related Domain.

To analyze the possible role(s) of the alternatively spliced forms of neurofibromin mRNA in embryonic development we have isolated the corresponding chicken cDNAs. The predicted amino acid sequence of the chicken insert, as well as the actual site of insertion, is identical to that for human neurofibromin. RNAse protection and RNA-PCR analyses were used to assess levels of expression of the alternatively spliced forms of neurofibromin in the chick embryo. Most tissues express predominantly the higher molecular weight form ('type II') throughout development. In contrast, relative amounts of the two forms are modulated dramatically in the brain between e3 and e12, with type II the major form early in development and type I later. We are currently performing in situ hybridization analysis to determine the precise localization of the two forms of mRNA. Supported by the Oregon MRF.

605

SYMPOSIUM. $GABA_B$ RECEPTORS AND THEIR ROLE IN NEUROTRANSMISSION, NEUROMODULATION AND NEUROPATHOLOGY. N.G. Bowery (Chairperson), Univ. London, U.K.; R.A. Nicoll, Univ. California; H-R. Olpe, *CIBA-Geigy, Switzerland; J. Sarvey, Uniformed Services Health Science, Washington; H. Bittiger* and K. Kuriyama, Univ. Kyoto, Japan.

Many physiological and pathological events have been attributed to $GABA_B$ receptor activation in the mammalian brain. The symposium will attempt to cover most aspects of these phenomena. N.G. Bowery will provide an overview of past and present concepts of the characteristics of $GABA_B$ receptors and will consider their role in diseased states. R.A. Nicoll will discuss the significance of $GABA_B$ receptors in the hippocampus, the brain region from which much of our knowledge of their function derives. H-R. Olpe will consider the role of $GABA_B$ site activation in neuronal excitability and plasticity within the cerebral cortex. This will be complemented by the presentation of J. Sarvey who will examine the mechanisms of long-term potentiation. H. Bittiger will introduce new $GABA_B$ receptor antagonists and will provide substantial evidence for a role for $GABA_B$ receptors in the genesis of absence seizures. Finally, K. Kuriyama will provide information about recent progress in the purification and structural analysis of the $GABA_B$ receptor.

606

SYMPOSIUM. NEUROBEHAVIORAL MECHANISMS OF SALT INTAKE BEHAVIOR. E.Stellar, Univ. of Pennsylvania. (Chairperson); B.S. McEwen, Rockefeller Univ. (Chairperson); J. Schulkin, Univ. of Penn.; R.E. Norgren, Penn. State Univ. Col. of Med., S. Nicolaidis, Col. de France; and S.J. Fluharty, Univ. of Pennsylvania.

Sodium is the major electrolyte of extracellular fluid importantly involved in virtually all aspects of nervous system function. The appetite that develops for salt during sodium deficiency is an excellent example of an innately organized, motivated behavior. This symposium will review exciting new advances in the neurobiology of this behavior. **Drs. E. Stellar and J. Schulkin** will introduce the symposium by reviewing briefly the behavioral evidence in support of the neurohormonal synergy of adrenal steroids and peptides that regulates sodium ingestion. **Dr. R.E. Norgren** will describe recent studies on the complexity of gustatory coding in the nucleus of the solitary tract and parabrachial nucleus and their modification during sodium deficiency. **Dr. S. Nicolaidis** will discuss the neurophysiological analysis of diencephalic neurons whose integrative properties are regulated both by steroids and angiotensin during sodium deficiency. **Dr. B.S. McEwen's** presentation will focus on the biochemistry and molecular biology of Type I and Type II corticosteroid receptors, as well as neuropeptide gene expression involved in the control of sodium appetite. Lastly, **Dr. S.J. Fluharty** will discuss the intracellular and behavioral functions of neuronal angiotensin receptors and the molecular mechanisms by which adrenal steroids regulate the brain's responsiveness to the peptide.

GLIA AND OTHER NON-NEURONAL CELLS VI

608.1

APOLIPOPROTEIN E IN GLIAL CELLS OF THE RAT OLFACTORY BULB: POSTNATAL DEVELOPMENT AND RESPONSE TO DEAFFERENTATION. G.E. Handelmann * and M.J. Russell. Dept. of Pharmacology, Univ. of Utah, Salt Lake City, UT 84112 and Dept. of Anesthesiology, Univ. of California-Davis, Sacramento, CA 95817.

Apolipoprotein (apo)-E, a lipid transport protein, has been proposed to play a role in nerve regeneration. We therefore studied apo-E's distribution, development, and response to injury in the olfactory system, the only region of the mammalian CNS in which true nerve regeneration is known to occur. Using single and double label immunocytochemistry, we found apo-E immunoreactivity (IR) in astrocytes in all layers of the adult olfactory bulb. Apo-E-IR was extensively colocalized with GFAP. In the olfactory nerve, apo-E was present in cell bodies and occasional fibers, possibly those of the ensheathing cells, a type of astroglia. In rat pups, apo-E was present in the olfactory nerve as early as 2 days postnatal, although very little GFAP was present. The adult pattern of apo-E distribution was reached by 3 months of age. In aged rats (1 and 2 years old), the distribution of the astrocytes was unchanged, but they contained more GFAP- and apo-E- IR and had the morphological appearance of reactive astrocytes. Similarly, deafferentation of the bulb in younger adults, induced by zinc sulfate irrigation of the nasal epithelium, also increased apo-E- and GFAP- IR in all layers. The effects were most pronounced in the glomerular layer, the site of the olfactory nerve terminals, and within the olfactory nerve. These results indicate that apo-E is synthesized by glia of the olfactory nerve and bulb, and that its synthesis is increased in response to aging and nerve injury. The increased synthesis may be elicited by a factor associated with axonal degeneration.

608.2

EXPRESSION OF β4 INTEGRIN IN RAT PERIPHERAL NERVE IS DEVELOPMENTALLY REGULATED AND AXONALLY MODULATED DURING REGENERATION. ML. Feltri[1], S. Scherer[2], L.Wrabetz[2], H. Vogelbacker [2], P. Bannerman[3*], M.Shy[4], J Kamholz[2]. [1]S.Raffaele Hospital, Milano, Italy; [2]University of Pennsylvania, [3]Children's Hospital of Philadelphia and [4]Thomas Jefferson University, Philadelphia, PA, 19104.

Integrins are a family of receptors implicated in cell adhesion and cell signaling whose ligands include extracellular matrix proteins. Myelinating Schwann cells (SC) require various basement membrane components to initiate myelination. Since expression of the β4 integrin subunit has been shown to be restricted to epithelial cells and SC, we chose to study expression of β4 integrin in postnatal sciatic nerve development and after crush injury. Using a β4 integrin cDNA from a rat peripheral nerve library, Northern blot analysis demonstrated that β4 integrin mRNA is present at low levels at postnatal day 1 and steadily increases from day 15 to at least day 90. After a crush injury, which results in Wallerian degeneration followed by prompt axonal regeneration, β4 integrin mRNA expression falls markedly by 4 days post-transection and reappears by 8 days and peaks by 12 days, paralleling the time course of axonal regeneration. Furthermore, β4 integrin mRNA expression is induced in forskolin-stimulated SC in culture, an *in vitro* model of SC-axonal signaling. We conclude that in sciatic nerve β4 integrin expression is axonally modulated, suggesting that β4 integrin may function in axon-SC interactions during myelination.

608.3

GLIAL SURFACE MOLECULES IN LESIONS AND REGENERATION: GENERATION OF A PANEL OF MONOCLONAL ANTIBODIES.
J. R. Madsen,* Dept. of Neurosurgery, Children's Hospital, Boston, MA 02115.

Several known molecular species, and probably many not yet identified, mediate neuronal-glial interactions and promote or inhibit regeneration of axonal processes. Differential interactions of primary rat central nervous system (CNS) neurons with a panel of cell lines exhibiting glial properties therefore may reveal molecular cues which affect neurite growth in the CNS. Of a panel of glial-like cell lines generated by transformation of CNS progenitor cells by retroviral infection [Snyder et al., Cell 68:33-51, 1992], two particular subclones (C27 and C17) vary significantly in their ability to support neuronal outgrowth (C17 >> C27). To look for cell surface determinants responsible for the differences in neurite growth, panels of monoclonal antibodies have been generated using protocols of "subtractive immunization."

Thus far, at least fourteen different monoclonal antibodies have been generated which recognize epitopic differences between the surfaces of the neurite-promoting C17 cell line and the neurite-inhibiting C27 cell line. Western blot analysis shows that these antibodies bind a variety of proteins in the 30 kD to 120 kD range. Immunohistological screening has revealed that several of the epitopes recognized by the antibodies increase in concentration after injury of the rat sciatic nerve or the goldfish optic nerve, two systems known to support neuronal regeneration *in vivo*. Striking histological differences between the two optic tecta of the goldfish are also observed three weeks after unilateral optic nerve crush. Less striking, but still regionally specific, changes in immunoreactivity are seen in the superior colliculus of the rat after unilateral optic nerve injury. Western blot analysis of the lesioned tissues shows up- and down-regulation of specific proteins as a result of lesioning. Two of the antibodies bind different proteins which are up- and down-regulated in the same tissue after lesioning.

These antibodies are being evaluated in neurite outgrowth assays, and may yield insight into both neurite-promoting and neurite-blocking activities of CNS glia. . Modification of these biological activities may become a therapeutic intervention for CNS injury in the future.

608.4

PERIPHERAL NERVE INJURY INDUCES SCHWANN CELLS TO EXPRESS TWO MACROPHAGE PHENOTYPES: PHAGOCYTOSIS AND THE GALACTOSE SPECIFIC LECTIN MAC-2. S. Rotshenker*, A. Saada, S. Aamar, and F. Reichert. Dept. of Anatomy & Embryology, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

There is a long standing debate whether Schwann cells (SC) play a role as phagocytes during the course of Wallerian degeneration. We examined, electron microscopically, the phagocytic activity of SC and macrophages (MO) in 3 different preparations of injured mouse peripheral nerves. One, cultures of intact nerve explants which are rich in SC but devoid of MO. Two, nerve segments undergoing degeneration in-situ, thus containig both SC and MO. Three, frozen nerves that were put back in-situ and thus became rich in MO but devoid of SC. Both MO and SC displayed phagocytic activities. This observation suggests that injury induces SC to acquire MO phenotypes. We studied, therefore, by immunocytochemistry and immmunoblot analysis, the expression of the molecules MAC-1, MAC-2, F4/80 and Fc, which characteristically are displayed by mature inflammatory murine MO. Of these, only the expression of MAC-2 was induced in the SC rich and MO poor intact nerve explants. Immunocytochemistry of dissociated nonneuronal cells further revealed that both SC and MO express MAC-2. Thus nerve injury induces SC to express two MO phenotypes: phagocytosis and MAC-2. The two may be related in that MAC-2, as a galactose specific lectin, could be instrumental in lectinophagocytosis.

608.5

EFFECT OF ANTI-GalC ON CNS MYELIN FORMATION. J. Rosenbluth*, Z. Liu, D. Guo and R. Schiff. Depts. Physiology & Rehab. Medicine, N.Y.U. School of Medicine, New York, NY 10016.

Anti-GalC hybridoma cells (Ranscht et al. PNAS 79:2709) were implanted into spinal cord dorsal columns (DC) of Cyclosporine(Cs)-treated suckling or weanling rats. Fixation after 1 wk showed numerous hybridoma cells in the DC. In the older group, whose DC were myelinated at the time of implantation, fascicles of myelinated fibers interspersed among hybridoma cells appeared largely intact, though in some cases they showed "unraveling" of outer myelin lamellae. In the younger group, the medial portion of the DC was only partially myelinated at the time of implantation, but would normally have become almost fully myelinated by the time of fixation. Here, comparable fascicles of nerve fibers interspersed among hybridoma cells remained completely devoid of myelin. The observations suggest that the Ig produced by this hybridoma has little effect on existing myelin sheaths in Cs-treated rats, after 1 wk, but interferes significantly with formation of new CNS myelin in vivo, as it does with formation of PNS myelin in vitro (Ranscht et al., J. Neurosci. 7:2936). A similar Ig-mediated mechanism could contribute to failure of remyelination in MS plaques. Supported by NIH & NMSS.

608.6

REGULATION OF A NOVEL CYCLOOXYGENASE (PROSTAGLANDIN G/H SYNTHETASE) GENE BY DEXAMETHASONE AND CALCIUM IONOPHORE IN ASTROCYTES. M. K. O'Banion*, J. C. Dusel, D. K. Miller, and P. D. Coleman. Departments of Neurology and of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642.

Prostaglandins are important mediators of many physiologic processes. Within the CNS, they may play roles in fever, the sleep/wake cycle, proprioception, and response to injury. In astrocytes, prostaglandin production is profoundly increased by calcium ionophore and decreased by glucocorticoid hormones; however the mechanisms underlying these changes are poorly understood. We have recently cloned a novel 4.1 kb cDNA encoding a cyclooxygenase that arises from a gene distinct from that for the previously cloned 2.8 kb cyclooxygenase mRNA (O'Banion *et al.* 1991. J. Biol. Chem. 266:23261; O'Banion *et al.* 1992. Proc. Natl. Acad. Sci. USA, in press). We find that both genes are expressed in primary rat astrocyte cultures and have used Northern blot analyses to characterize their regulation by calcium ionophore and glucocorticoid hormones.

In astrocytes, dexamethasone (1 μM, 4 h) reduces the level of 4.1 kb mRNA by 2-3 fold without altering the 2.8 kb mRNA level. This inhibition occurs in the presence of cycloheximide which alone increases 4.1 kb mRNA levels by nearly 10-fold without super-induction of the 2.8 kb mRNA. Calcium ionophore (A23187; 5 μM) markedly induces (>10 fold) the level of 4.1 kb mRNA within 3 h. Preliminary results suggest that the induction of 4.1 kb mRNA by ionophore is transient.

These observations are consistent with our previous studies in mouse fibroblasts and human monocytes and suggest that the 4.1 kb mRNA encodes the predominant regulated cyclooxygenase in astrocytes. [Supported by LEAD award AG09016 and RO1 AG01121 to P.D.C].

608.7

REGULATION OF TRANSFORMING GROWTH FACTOR ALPHA (TGFα) mRNA EXPRESSION IN RAT HYPOTHALAMIC ASTROCYTES BY TGFα AND ESTRADIOL (E_2). Y.J. Ma*, M. Moholt-Siebert, D.F. Hill and S.R. Ojeda. Div. Neurosci., Oregon Regional Primate Research Center, Beaverton, OR 97006

TGFα, a mitogenic polypeptide that acts via activation of epidermal growth factor receptors (EGFR), has been implicated in the neuropathological mechanism by which hypothalamic lesions induce sexual precocity and the neuroendocrine process that underlies the initiation of normal female puberty. TGFα appears to exert these effects via stimulation of luteinizing hormone releasing hormone (LHRH) secretion. Since both TGFα and EGFR expression in the hypothalamus are predominantly astroglial, and LHRH neurons are devoid of EGFR, we have postulated that TGFα facilitates LHRH release indirectly via an autocrine/paracrine-mediated activation of glial function. To begin examining this hypothesis, hypothalamic astrocytes cultured in defined medium were exposed to either TGFα (50 ng/ml) or a phorbol ester (TPA, 10 ng/ml) for various lengths of time. The effect of these agents on TGFα mRNA was assessed by RNase protection assay. TGFα mRNA was readily detected in untreated astrocytes; both TGFα and TPA increased TGFα mRNA levels to maximal values (3-4-fold increase) by 8h. Additional experiments were performed to determine if glial TGFα mRNA levels are regulated by E_2. RNase protection assays revealed the presence of E_2 receptor mRNA in cultured hypothalamic astrocytes; E_2-17β (1 nM), but not E_2-17α, induced a two-fold increase in TGFα mRNA within 8h of treatment. These results suggest that: a) TGFα can act in an autocrine fashion on hypothalamic astrocytes to enhance expression of its own gene, and b) hypothalamic astrocytes express E_2 receptors which mediate a facilitatory effect of E_2 on TGFα gene expression. (Supported by NIH Grants HD25123, HD18185 and RR00163)

608.8

DIFFERENTIAL GLIAL GENE EXPRESSION IN THE RAT BRAIN. D. G. Schaar, A. C. Sherwood, B. A. Sieber, C. F. Dreyfus and I. B. Black*. Dept. of Neuroscience and Cell Biology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 675 Hoes Lane, Piscataway, NJ 08854.

Studies conducted by this laboratory have established that in culture, local substantia nigra (SN) support cells contribute to the survival of the dopaminergic neurons selectively lost in Parkinson's Disease. Fractionation of embryonic SN glia into enriched oligodendrocyte, type I or type II astrocyte subpopulations identified the type I astrocytes as the source of the soluble neurotrophic factor(s) for SN dopaminergic neurons. These well-characterized SN glial populations have been utilized in subtractive cloning methodologies to identify genes specifically expressed by type I astrocytes.

The support cell fractionation generated 70-95% homogeneous glial subpopulations, from which total RNA was harvested. The limiting amount of RNA obtained necessitated development of a subtractive cloning technique using the polymerase chain reaction (PCR). In the library described, sequences in common between type I and II astrocytes were removed by stringent hybridization and hydroxyapatite (HAP) chromatography. Ten cDNA clones have been isolated from the type I cells which are not expressed or expressed at relatively low levels in type II astrocytes.

Nucleotide sequence analysis of the type I specific cDNA's have indicated the isolation of novel open reading frames with no significant homology to existing Genebank or EMBL sequences. In addition, two cDNA's contain novel sequences with the exception of their 3' untranslated regions which contain a 50 base pair stretch identified as a rat repetitive element (SINE). Further analysis of these type I astrocyte specific cDNA clones will give insight into the molecular basis of the type I astrocyte neurotrophic effect on SN dopaminergic neurons. In addition, subtractive cloning approaches reported here may be used to analyze other glial populations from discrete brain regions.

608.9

A SUBPOPULATION OF HIPPOCAMPAL ASTROCYTES SPECIFIC FOR THE ZINC-CONTAINING MOSSY FIBRE ZONE E. Jung, A. Schnecko, E. Braak* and T.G. Ohm. Zentrum der Morphologie, J.W.Goethe-Universität, W-6000 Frankfurt/Main 70, Germany

The projection of the zinc-containing axons of granule cells of the fascia dentata, e.g. the mossy fibres, is restricted to sectors CA3 and CA4 of the hippocampus. Serial sections of hippocampi of adult man were stained for zinc-containing fibres with a non-perfusion Timm method, while adjacent ones were stained with Darrow-red and aldehydefuchsin. In contrast to the common opinion that there is no astrocytic subtype specific for any of the hippocampal sectors, the Timm-stained areas correlate with the zone of aldehydefuchsin-positive astrocytes. The demonstration of astrocytes by means of a Gallyas-silver-stain or GFAP-immunocytochemistry reveals no morphological differences between astrocytes of CA3 and CA4 and those of CA2, CA1. Since astrocytes regulate axonal outgrowth in a region-specific manner, it is temptative to speculate that the aldehydefuchsin-positive astrocyte of sectors CA4 and CA3 might be a candidate for a specific neuron-glia interaction which restricts the outgrowth of the mossy fibres to sectors CA3 and CA4.

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608.10

IMMUNOCYTOCHEMICAL EVIDENCE FOR NERVE GROWTH FACTOR ACCUMULATION IN MICROGLIA. G.M. Gilad* and V.H. Gilad. NPB, NIMH Neuroscience Center at St. Elizabeths, Washington, DC 20032.

Nerve growth factor (NGF) can be produced by a variety of cell types, including neurons, astrocytes, Schwann cells and fibroblasts. While macrophages may produce only small amounts, their CNS counterparts, the microglia, may be stimulated to produce significant amounts of NGF. Not surprisingly, being a secretory product, immunocytochemical localization is evident only in cells that accumulate NGF. In the present study, using antibodies to NGF and to a variety of cell-specific markers, we found that accumulation of NGF immunoreactivity occurs selectively in cultured microglia, and in macrophages and microglia at the site of brain injury, but not in other cell types. It is implied that microglia and macrophages may sequester their own newly synthesized NGF or, by pinocytosis, extracellular NGF. Sequestration of NGF by these cells may assume importance after CNS injury.

608.11

MICROGLIAL PLASTICITY IN VIVO: EVIDENCE FOR THEIR RAPID AND REMOTE ACTIVATION IN EXPERIMENTAL NEUROPATHOLOGY

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DEPARTMENT OF NEUROMORPHOLOGY, MAX-PLANCK-INSTITUTE OF PSYCHIATRY, 8033 MARTINSRIED NEAR MUNICH, GERMANY

Resting microglial cells form a network of regularly spaced, ramified cells throughout the CNS. Studies on their reactions after facial nerve axotomy have suggested that they function as an intrinsic immune defense system within the CNS. To further examine this hypothesis in vivo, we have studied the microglial reactions in several experimental neuropathologies. These include retrograde reactions of motoneurons, anterograde reactions, combined anterograde and retrograde reactions and recently neurotoxin models. In addition, we have studied global ischemia and T cell-mediated autoimmune inflammation of the central and peripheral nervous system. From these results it has become evident that microglial cells (but not astrocytes) rapidly proliferate and increase the expression of several immunologically relevant molecules, such as MHC class I and II antigens. Their activation is further observed rapidly if the site of the microglial activation is quite remote from the site of the primary lesion. At the ultrastructural level, two phenomena among several other things were observed: a distinct activation of perivascular microglial cells and an involvement of activated, perineuronal microglial cells in the deafferentation (synaptic stripping) of afferent synaptic terminals from the surface of motoneurons. The latter observation in particular could be relevant for better evaluating neurological deficits in patients suffering e.g. from nerve trauma. In summary, microglial cells thus appear to be the main immuneffector cell population of the CNS.

608.12

Interleukin 6 is elevated in Human Brain Tumor Cyst Fluid.

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Radioimmunoassay for several cytokines including Tumor Necrosis Factor-alpha (TNF-α), Interleukin 1-alpha (IL-1α), Interleukin 1-Beta (IL-1β), Interleukin 2 (IL-2) and Interleukin 6 (IL-6) was carried out on tumor cyst fluid (TCF) from patients with malignant astrocytic brain tumors (n=8), serum from normal controls (n=5), serum from patients with malignant brain tumors (n=5), and cerebrospinal fluid (CSF) from 3 patients with malignant brain tumors. CSF levels of all cytokines were less than 0.1 ng/ml. TNFα, IL1α, IL1-β, and IL-2 levels were higher in serum and TCF than in CSF and were in the range between 0.2 and 1.5 ng/ml. There was no significant difference in levels of these individual cytokines between TCF and serum, suggesting an equilibrium in these factors between serum and tumor cyst fluid. In contrast, IL-6 levels were elevated an average of 50-100 fold in TCF compared to serum. These results indicate that IL-6 is enriched in TCF, suggesting a possible role of the cytokine in the pathophysiology of malignant brain tumors and/or brain response to tumor invasion.

608.13

WITHDRAWN

608.14

GAP JUNCTIONS DISAPPEAR IN CULTURED ASTROCYTES AND LEPTOMENINGIAL CELLS FOLLOWING PROTOZOAN INFECTION. A.C. Campos de Carvalho, C. Roy, E.L. Hertzberg, R.A. Corpina, H.B. Tanowitz, J.A. Kessler', L. Weiss, M. Wittner and D.C. Spray. Depts. Neuroscience, Neurology and Medicine, A. Einstein Coll. Med., Bronx, N.Y. & Fed. Univ. Rio de Janeiro, Brazil.

American trypanosomiasis [Chagas' disease caused by *Trypanosmoa cruzi* (T.c.)] and toxoplasmosis [caused by *Toxoplasma gondii* (T.g.)] are two protozoan diseases where nervous system involvement is increasingly widespread in immunosuppressed individuals. Infection of myocardial cells with T.c. results in asynchronous conduction and gap junction loss [Circ. Res (92)70:733]. To examine whether similar changes occured in cells of brain, pure cultures of leptomeningial cells or astrocytes were infected with T.c. or T.g. and junctional communication and connexin abundance and intercellular distribution were examined. For either type of cell infected with either parasite, intercellular coupling (injected Lucifer Yellow) was reduced, and was often altogether absent. Immunocytochemical studies using antibodies specific for connexin43 (in the case of astrocytes) or both connexin43 and connexin26 (for leptomeningeal cells) demonstrated that the punctate staining indicative of gap junctions was much reduced in the individual infected cells, although uninfected neighbors could display normal staining. Western blots indicated that abundance of connexin43 in infected cells was slightly increased compared to uninfected control cells. Phosphorylation state of connexin43 (inferred from electrophoretic mobility of connexin43 isoforms) was little affected. We conclude that both protozoan parasites infect both types of brain cells, resulting in a loss of intercellular communication and organized gap junctions without affecting expression or post-translational processing of the gap junction protein. Presumably, these changes result from altered targeting or assembly of the junctional protein.

PEPTIDES: RECEPTORS VI

609.1

A MELANOPHORE BASED BIOASSAY SYSTEM DESIGNED TO ANALYZE LIGAND EFFECTS ON G-PROTEIN LINKED RECEPTORS COUPLED TO PHOSPHOLIPASE C. G.F. Graminski, C.K. Jayawickreme, M.N. Potenza and M.R. Lerner*, Depts. of Cell Bio., Int. Med., & Pharm., & HHMI, Yale Univ. Sch. of Med., New Haven, CT 06510.

A melanophore based bioassay system has been developed for rapidly evaluating ligand effects on recombinant receptors coupled to the phospholipase C second-messenger pathway. The assay is based on agonist induced darkening of *Xenopus laevis* melanophores resulting from pigment dispersion. Both TPA and the Ca^{2+} ionophore A23187 are potent stimulators of pigment dispersion in *Xenopus* melanophores, suggesting that pigment dispersion is mediated via phospholipase C activation. To test this hypothesis, pigment cells were transiently transfected with the phospholipase C activating murine GRP\bombesin receptor. Treatment with bombesin and other agonists induced pigment dispersion in cells expressing the bombesin receptor but not in wild type cells. The responses were dose dependent. EC_{50} values for bombesin (0.12 nM) and other agonists such as litorin (0.15 nM) and neuromedin B (1.3 nM) were comparable to the literature values. The bombesin receptor antagonist, [D-Phe^6]Bomb$_{6-13}$ methyl ester, inhibited pigment dispersion with an IC_{50} value of 15 nM. Bombesin stimulation provoked a substantial increase of 1,4,5-IP_3 production over basal levels while no rise in cAMP was observed. This bioassay system potentially offers great utility and speed for analyzing the pharmacological potencies of agonists and antagonists associated with receptors linked to phospholipase C.

609.2

A COMPUTER-ENHANCED VIDEO ASSAY FOR THE FUNCTIONAL EXPRESSION OF RECEPTORS COUPLED TO G_s, G_i AND PHOSPHOLIPASE C. T.S. McClintock*, G.F. Graminski, M.N. Potenza, C.K. Jayawickreme, A. Roby-Shemkovitz and M.R. Lerner. Boyer Center for Molecular Medicine and Dept. of Internal Medicine, Yale Univ. Sch. Med., New Haven, CT 06510.

We have created a versatile and sensitive visual assay for the expression of receptors linked to G-proteins. Transient transfection of Xenopus melanophores with plasmids containing cDNAs for either the β_2 adrenergic (G_s), the substance P (phospholipase C), or the D_2 dopamine receptor (G_i) at dilutions of 1 plasmid in 10,000 were detectable. Computerized subtraction of video images taken pre- and poststimulation allowed the detection of individual responses in fields of 10,000 cells. Activation of phospholipase C and adenylate cyclase caused dispersion of melanosomes (organelles containing melanin) while inhibition of adenylate cyclase caused melanosome aggregation. Responding cells appear to require only a single copy of a plasmid containing a receptor cDNA. This assay is capable of conveniently screening several hundred thousand cDNA clones per day.

609.3

INDUCTION OF PKC BY ANP RECEPTOR ACTIVATION AND INVOLVEMENT OF G PROTEINS IN RAT BRAIN SLICES. A. Rathinavelu, P. Sun, G. Pavlakovic and G.E. Isom*, Dept. of Pharmacol. & Toxicol., Sch. of Pharmacy & Pharmacal Sci., Purdue Univ., W. Lafayette, IN 47907.

Atrial natriuretic peptides (ANP) are synthesized and localized in different brain areas. Natriuretic peptides bind to specific receptors and generate cGMP as the second messenger. When ANP receptors were stimulated excessively with 5 and 10 μM ANP (99-126) a marked increase in activity of protein kinase C (PKC) in the cytosolic fraction of the diencephalon occurred. The membrane PKC level was not altered significantly with treatments up to 5 μM ANP, whereas 10 μM ANP produced a slight increase. The increase in cytosolic PKC was 80% above control with 5 μM ANP treatment of the diencephalon slices and maximal induction was observed following 60 min treatment. Incubation of ANP (5 μM) with actinomycin D (10 μg/ml) or cycloheximide (20 μg/ml) blocked the induction of PKC to 90% and 84%, respectively. This indicated the increase in cytosolic PKC resulted from de novo protein synthesis. Pertusis toxin (5 ng/ml) attenuated the induction of PKC by 30% suggesting Gi proteins are involved in the ANP mediated PKC induction. cGMP levels in 0.001 and 0.01 μM ANP treated diencephalon slices increased 5.4 fold and 3.8 fold respectively compared to controls. Incubation of slices with 0.5, 1.0 and 5 μM ANP showed a non-significant increase in cGMP levels. These results suggest increases in cytosolic PKC levels alter the guanylate cyclase-coupled receptor function.

609.4

MATCHING LOCALIZATION OF IMMUNOREACTIVE VIP AND VIP RECEPTOR IN THE VISUAL CORTEX. A.Csillag[1], K.Zilles[2*], A.Schleicher[2] and F.Hajós[3]. [1]1st Dept.Anat.,Semmelweis Univ. of Med., Budapest, Hungary; [2]C.& O.Vogt Inst. for Brain Res., Univ. of Düsseldorf, Germany; [3]Univ. of Vet. Sci. Budapest, Hungary.

Vasoactive intestinal polypeptide (VIP) is present in cortical non-pyramidal neurons. The density and position of VIP immunoreactive (VIP-IR) terminals suggest a powerful regulatory role of VIP in the cortex. In the present study, the localization of VIP receptor immunoreactivity (VR-IR) was studied simultaneously with VIP-IR using double label immunocytochemistry supplemented with a quantitative analysis of distribution. A considerable spatial overlap was found between VIP-IR particles (axonal boutons) and some VR-IR cell bodies. Quantitative analysis revealed a preferential accumulation of VIP+ boutons over the cytoplasm, rather than the nuclear or pericellular region, of VR-IR cells. Ultrastructurally, VR-IR was detected in postsynaptic target structures of VIP+ boutons, including the endoplasmic reticulum and subsynaptic specialization. Thus, the neuroanatomical relations favour the existence of a functional VIP - VIP receptor interaction in the occipital cortex of the mouse.

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609.5

SR 48692, A NON-PEPTIDE ANTAGONIST OF NEUROTENSIN RECEPTORS IN HUMAN TISSUES. **W. Rostène*[1], S. Equilbey[1], D. Gully[2], C. Labbé-Jullié[3], D. Pelaprat[1], G. Le Fur[2] and P. Kitabgi[3]. [1]INSERM U.339 Hôpital St Antoine 75012 Paris, France; [2]SANOFI RECHERCHE 31036 Toulouse, France; [3]IPMC-CNRS 06560 Valbonne, France.**

SR 48692 was reported to be a potent non-peptide antagonist of Neurotensin (NT) receptors and effects in both guinea-pig and murine species (D. Gully et al.;P.Kitabgi et al.,this volume). In order to test whether this compound also had some activity in humans, homogenates from newborn or adult human cortices were prepared. Binding studies demonstrated that SR 48692 competitively inhibited specific binding of 125INT with an IC50 of around 12 nM. Autoradiographic data obtained at the level of the substantia nigra and nucleus paranigralis of normal human tissue sections demonstrated a dose-dependent inhibition of 125INT labeling with increasing concentrations of SR 48692. Similar data were obtained on 125INT binding in primary cultures of human mesencephalic neurons. In a cell line rich in NT receptors, derived from a colon carcinoma (HT29),SR 48692 competitively antagonized NT-induced intracellular calcium mobilization obtained by means of flow cytometry using indo-1 fluorescence. The observed pA2 values (mean 8.1 nM) were consistant with results obtained in binding studies (Ki 20 nM). Moreover, in this model, SR 48692 was devoid of any intrinsic agonist activity. In conclusion, this potent non-peptide antagonist of NT receptors may help to understand the putative pathological roles of NT.

609.6

REGULATION OF GALANIN BINDING SITES IN THE BED NUCLEUS OF THE STRIA TERMINALIS AND SEPTUM ACROSS PUBERTY. B. Planas*, P.E. Kolb, M.A. Raskind, and M.A. Miller. Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98195.

Galanin-like immunoreactivity in the brain increases across development in male and female rats (Gabriel et al., Peptides 10: 369-374, 1989). The increase in GAL-LI may be due to an increase in GAL gene expression induced by the pubertal rise in gonadal hormones as estrogen has been found to increase GAL-LI and GAL mRNA levels (Gabriel et al., Neuroendocrinology 51: 168-173, 1990). In order to determine whether the developmental increase in GAL-LI influences GAL binding, we have used slice binding techniques and quantitative autoradiography to compare the distribution and density of GAL binding sites in the bed nucleus of the stria terminalis (BST) and septum (S) of prepubertal and adult male rats.

^{125}I-GAL binding sites were studied in slide-mounted coronal brain sections of 24 d (n=6) and 90 d (n=6) old male rats. Slides were incubated in 0.25 nM ^{125}I-GAL at room temperature for 60 min. Specific binding was determined by the addition of 0.25 µM unlabeled GAL.

Data analysis of 8 atlas matched film autoradiograms revealed a differential distribution of ^{125}I-GAL binding with age ($p<0.0001$). The optical density of specific binding in the BSTL was significantly greater ($p<0.01$) in adult rats compared to prepubertal rats (0.42±0.01 vs. 0.34±0.02). However, many brain regions revealed a pattern of increased binding densities in prepubertal animals: BSTM (0.33±0.01 vs. 0.14±0.01), BSTV (0.33±0.02 vs. 0.15±0.02), MS (0.33±0.01 vs. 0.12±0.01), LSV (0.34±0.01 vs. 0.10±0.01), LSI (0.33±0.01 vs. 0.08±0.01). No apparent differences in GAL binding were observed in the LSD or dorsal region of the LSI.

These results indicate that the density of GAL binding sites in portions of the BST and septum changes across puberty. These differences may result from changes in GAL innervation within these brain regions.

609.7

EVIDENCE FOR SPECIFIC N-TERMINAL GALANIN FRAGMENT BINDING SITES IN THE RAT BRAIN. P.B. Hedlund*, U.-B. Finnman, N. Yanaihara and K. Fuxe. Dept. of Histology and Neurobiol., Karolinska Institutet, Box 60400, S-10401 Stockholm, Sweden.

Galanin$_{1\text{-}29}$ (GAL) is a neuropeptide with several biological activities in the brain, such as stimulation of feeding behavior and interactions with 5-HT$_{1A}$ and acetylcholine receptors. In most cases N-terminal fragments such as GAL$_{1\text{-}15}$, but not C-terminal fragments, have been shown to retain the biological activities of the native GAL$_{1\text{-}29}$ molecule.

We have analyzed the distribution of [^{125}I]GAL$_{1\text{-}15}$ (specific activity 2000 Ci/mmol) binding sites in the rat brain using quantitative receptor autoradiography. The distribution was different from [^{125}I]GAL$_{1\text{-}29}$ binding sites, but also with several overlapping regions. Most notably [^{125}I]GAL$_{1\text{-}15}$ binding sites were present in the dorsal hippocampus, the neostriatum and the neocortex, areas almost lacking [^{125}I]GAL$_{1\text{-}29}$ binding sites in the rat brain. The [^{125}I]GAL$_{1\text{-}15}$ binding was more readily displaced with GAL$_{1\text{-}15}$ than with GAL$_{1\text{-}29}$ indicating the presence of a binding site with higher affinity for the fragment. The fragment binding site was saturable with a K_d of 0.63 ± 0.02 nM and a B_{max} of 15.3 ± 1.7 fmol/mg protein in the dorsal hippocampus and a K_d of 0.44 ± 0.09 nM and a B_{max} of 15.8 ± 0.5 fmol/mg protein in the ventral limbic cortex. Non-specific binding was determined as the binding in the presence of 10 µM of GAL$_{1\text{-}15}$. The specific binding was approximately 80% at B_{max}. The present study gives evidence for the existence of specific binding sites for the GAL$_{1\text{-}15}$ fragment different from the GAL$_{1\text{-}29}$ receptor which may represent a unique GAL fragment receptor.

609.8

EVIDENCE FOR DELTA OPIOID RECEPTOR SUBTYPES L. Fang*, R. J. Knapp, M. A. Jarosinski†, V. J. Hruby† and H. I. Yamamura Dept's of Pharmacology and Chemistry†, University of Arizona, Tucson, AZ 85724.

The existence of δ opioid receptor subtypes was suggested previously in comparisons of δ agonist activity in mouse brain and mouse vas deferens (MVD); the different binding affinities in rat brain and MVD measured for [D-Ala^2, (2R, 3S)-γ^{E}Phe^4, Leu^5]enkephalin methyl ester (CP-OMe); and the differential antagonism of δ antinociception by selective irreversible antagonists *in vivo*. We have produced additional evidence for the existence of δ opioid receptor subtypes in mouse brain and vas deferens using a new δ opioid receptor selective antagonist: H$_2$N-Tyr-L-Tic-Phe-Phe-OH (TIPP). The ability of naltrindole (NTI), a selective δ antagonist, and TIPP to inhibit 100 pM [^{3}H]naltrindole binding in mouse brain and MVD were determined using a one site model. NTI has equal affinity for the sites labeled by [^{3}H]naltrindole in both tissues, whereas TIPP has 12 fold lower affinity in MVD compared to brain. These observation support the presence of different δ opioid receptor subtypes having different anatomical distributions. Supported in part by NIDA grants.

609.9

REGIONAL DISTRIBUTION OF CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTORS IN THE TREE SHREW BRAIN. E. Fuchs*, M. Weinrich and G. Flügge. German Primate Center, Göttingen, FRG.

CRF is the predominant messenger in the control of the activity of the pituitary-adrenal axis and is therefore ultimately responsible for triggering the endocrine responses to stress. In the present project we are investigating the influences of chronic stress on central nervous CRF receptors. For our studies we used tree shrews (*Tupaia belangeri*), a species which provides a useful model for studying the outcome of psychosocial stress on the central nervous system. In the first part of the study we localized and quantified the receptors in the brains of control animals by autoradiography with ^{125}I-labeled ovine CRF. It revealed the presence of receptors with a high and a low affinity component. High densities of specific CRF receptors were localized in the tractus olfactorius, the dentate gyrus, the outer layers of cortex and superior colliculi, the cerebellum, the vagal nucleus, and the pituitary (anterior part, intermediate zone). Moderate concentrations were present in the amygdala and the lateral septum. Lower densities were found in the caudate nucleus and the locus coeruleus. These results indicate species differences with respect to distribution and densities of CRF receptors. In the second part of the study we are currently investigating whether chronic psychosocial stress induces changes of CRF receptor affinities and/ or densities of these receptors.

609.10

LHRH NEURONS IN THE MEDIAL SEPTAL-DIAGONAL BAND OF BROCA-PREOPTIC AREA (mS-dbB-PO) DO NOT PROJECT DIRECTLY TO THE HIPPOCAMPUS. C.A. Dudley*, S.B. Sudderth, and R.L. Moss, Department of Physiology, UT Southwestern Medical Center, Dallas, TX, 75235-9040

Neurons containing immunoreactive LHRH are located primarily in the mS-dbB-PO complex, while autoradiographic studies have demonstrated dense concentrations of LHRH receptors in the hippocampus. The route by which LHRH reaches its receptors in the hippocampus is unknown. The present study was designed to determine if LHRH neurons in the mS-dbB-PO project directly to the hippocampal formation. The fluorescent retrograde tracer, fluorogold (FG) was injected unilaterally into 4 separate hippocampal locations in 5 ovariectomized female rats. Six additional females received similar injections of a different retrograde tracer, wheat germ agglutinin (WGA). After a 5-day survival period, the animals were sacrificed and their brains processed immunohistochemically for detection of cells containing both LHRH and FG or LHRH and WGA. As a positive control, some sections containing retrogradely labeled neurons were processed for choline acetyltransferase (CHAT) immunoreactivity. The WGA and FG injections covered targeted hippocampal sites and many retrogradely labeled cells were found in the mS-dbB-PO complex. Approximately 10% of the retrogradely labeled cells were found to contain CHAT; however, no neurons were found to co-localize LHRH and either WGA or FG. The results indicate that LHRH receptors in the hippocampus are not directly innervated by LHRH neurons in the mS-dbB-PO area and suggest that LHRH reaches its hippocampal receptors via a non-synaptic route. Supported by MH47418.

ISCHEMIA II

610.1

"THERAPEUTIC WINDOW" FOR NMDA ANTAGONIST PROTECTION AGAINST FOCAL CEREBRAL ISCHEMIA MAY BE NARROW. G.K. Steinberg*, N.M. Panahian, G.H. Sun. Dept. of Neurosurgery, Stanford University Medical Center, Stanford, CA 94305.

While NMDA antagonists have been shown to protect against focal cerebral ischemia, it is not clear how long drug therapy can be delayed and still achieve neuroprotection. Under halothane anesthesia, 55 rabbits underwent 2 hour occlusion of the left internal carotid, anterior cerebral and middle cerebral arteries followed by 4 hours of reperfusion. Rabbits were treated with either i.v normal saline (n=10) or the NMDA antagonist dextrorphan (DX) as follows (n=5/group) - 1 hour delay: 5, 10 or 15 mg/kg/hr; 2 hour delay: 7.5, 12.5, 17.5 mg/kg/hr; 4 hour delay: 10, 15 or 20 mg/kg/hr. Injury was assessed using magnetic resonance imaging for ischemic edema and histopathology for ischemic neuronal damage (IND). Protection against cortical ischemic damage was demonstrated only when DX administration was delayed 2 hours (17.5 mg/kg group: 27 ± 7% IND, 29 ± 8% edema; 12.5 mg/kg group: 28 ± 9% edema compared with normal saline control: 45 ± 7% IND, 47 ± 3% edema, p<.05). Protection against striatal IND was found only with 2 hour DX delay (7.5 mg/kg group: 37 ± 17%; 12.5 mg/kg group: 50 ± 15% vs. control: 83 ± 6%). DX treatment delayed 4 hours worsened the ischemic edema (15 mg/kg group: 70 ± 5%; 10 mg/kg group: 71 ± 9%, p<.05). DX treatment with one hour delay failed to demonstrate neuroprotection at any dose. These results suggest that NMDA antagonists may have to be given within 2-3 hours of ischemia onset to be of benefit. Lack of protection when drug was started during the ischemic period (before reperfusion) may reflect poor delivery to ischemic tissues. The "therapeutic window" for NMDA antagonist protection against focal ischemia may be relatively narrow.

610.2

NEUROPATHOLOGIC END POINTS IN EXPERIMENTAL STROKE THERAPY (MK-801): THE IMPORTANCE OF BOTH EARLY AND LATE EVALUATION. L. Persson, J. Valtysson, P. Andiné, H. Hagberg and L. Hillered*. Departments of Neurosurgery and Clinical Chemistry, University Hospital, Uppsala, Sweden.

The effect of the NMDA receptor blocker MK-801 (dizocilpine maleate) was evaluated both early (3 days) and late (28 days) after middle cerebral artery (MCA) occlusion. The aim was to determine whether or not the previously reported protective effect of MK-801 found in acute experiments remained after 28 days, i.e. when the ischemic lesion has reached its final appearance.[1]

MK-801 (0.5 mg/kg, i.v.) or isotonic saline was randomly given to rats 30 min after MCA occlusion. Infarct size was estimated from 8 histological sections of defined levels of the brain.

A 40% ($p < 0.05$) reduction of infarct size was found in MK-801 treated rats studied after 3 days. This effect was not found 28 days after MCA occlusion, where no difference in infarct size or final tissue loss (infarct volume + ipsilateral hemisphere atrophy) was seen between the MK-801 and placebo-treated rats. There was no significant effect of MK-801 on the ipsilateral hemisphere volume (reflecting edema) at 3 days.

Thus, MK-801 seemed to have a transient attenuating effect on the ischemic process itself with no apparent influence on the edema component. This study underlines the importance of including a late end point when evaluating the efficacy of neuroprotective stroke therapy. It remains to be shown whether or not multiple dose treatment with NMDA receptor blockers attenuate the final neuropathologic outcome after experimental stroke.

References: [1]L. Persson et al (1989) Stroke 20:641-645.

610.3

DIFFERENTIAL EFFECT OF MK-801 AND SEMISYNTHETIC GANGLIOSIDES ON PROTEIN KINASE C (PKC) ACTIVATION AND IMMEDIATE EARLY GENES EXPRESSION IN A PHOTOCHEMICALLY-INDUCED STROKE IN RATS. A. N. Kharlamov*, R.C. Hayes, R. Sheffield, A. Guidotti, E. Costa, and D. M. Armstrong. FGIN, Georgetown Univ. Med. School, Washington, DC 20007

In culture the protracted and abusive stimulation of glutamate (GLU) receptors results in neuronal death through a mechanism involving the persistent translocation of PKC and the destabilization of $[Ca^{2+}]_i$ homeostasis. In contrast, intermittent GLU receptor use elicits the coordinated expression of immediate early genes (IEG) acting as nuclear third messengers. In the present study the in vivo mechanism of action of GLU receptor stimulation was examined using the photochemically induced model of stroke in the rodent. Following i.v. injection of Rose Bengal, injury of the sensory/motor cortex ensues and the binding of the phorbol ester [^{3}H]PDBu as well as mRNA contents for c-*fos*, c-*jun*, *zif*268, *nur*/77 and FOS and JUN proteins were increased. [^{3}H]PDBu binding was employed as an index of PKC translocation and was observed maximally increased (i.e., 4-5 fold) in the cortex adjacent to the infarcted area 1 to 12 hours following stroke. In contrast, IEG expression extended throughout much of the cortex ipsilateral to the lesion. Importantly, the increase in [^{3}H]PDBu binding could be substantially reduced following treatment of rat with the semisynthetic gangliosides, LIGA4 and LIGA20 (35 μmol/kg, i.v., one hour before lesion). Both drugs have been shown to be blockers of PKC translocation. In addition, pretreatment with the NMDA antagonist, MK-801 (6 μmol/kg, i.v., 1 hour before lesion) nearly abolished the rise in [^{3}H]PDBu binding. MK-801 also blocked the rise in IEG although treatment with the semisynthetic gangliosides failed to affect the expression of these genes and gene products. These results *in vivo* differentiate the mode of action of MK-801 from that of semisynthetic gangliosides. Moreover, they confirm that semisynthetic gangliosides prevent the Ca^{2+}-dependent destabilization of PKC homeostasis that is also blocked by MK-801 without interfering with the Ca^{2+} mediated IEG response that is blocked by the NMDA receptor antagonist MK-801.

610.4

REVERSIBLE INACTIVATION OF CALCIUM/CALMODULIN KINASE II INDUCED BY SPINAL CORD ISCHEMIA. D.A. Shackelford, R.Y. Yeh, and J.A. Zivin*. Dept. of Neurosciences, Univ. of Calif. at San Diego, La Jolla, CA 92093-0624.

It has been proposed that ischemia-induced neuronal damage is caused by an accumulation of extracellular glutamate leading to an influx of extracellular Ca^{2+} through membrane channels disrupting Ca^{2+} homeostasis. The activity of Ca^{2+}/calmodulin-dependent protein kinase II, which is involved in regulation of neurotransmitter synthesis and release and neuronal plasticity, has been shown to decrease after ischemia in several models. In the present study, the kinetics of inactivation and fate of the protein in the rabbit spinal cord ischemia model were analyzed. The Ca^{2+}/calmodulin-dependent activity of CaM kinase II, measured by incorporation of phosphate into exogenous peptide substrates, decreased rapidly such that after 10 min of ischemia, which precedes irreveresible paraplegia, 65% and 40% of the actvity was lost from the cytosol and particulate fractions, respectively. However, analyses of CaM kinase II autophosphorylation activity by renaturation of spinal cord homogenates after Western transfer to filters, showed a decrease in autophosphorylation of the β, β' isoforms of CaM kinase in the cytosol but an increase in autophosphorylation of β, β' and α isoforms in the particulate fraction after 5-10 min of ischemia. Immunoblotting with monoclonal antibodies to the α and β subunits of CaM kinase II indicated that the subunits decreased in the cytosol and increased in the particulate fraction due to ischemia. Thus inactivation of CaM kinase II induced by ischemia correlates with loss of protein in the cytosol. However, in the particulate fraction, loss of activity is probably due to a reversible conformational change. The role of phosphorylation in inactivation and changing the subcellular distribution of the enzyme is being investigated.

610.5

ENADOLINE AFFORDS NEUROPROTECTION IN A RAT MODEL OF FOCAL ISCHEMIA.

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Various kappa opioid agonists have been shown to be neuroprotective in both global and focal models of cerebral ischemia. The present study evaluated the efficacy of the potent and selective kappa agonist enadoline (CI-977) in a rat model of chronic focal ischemia following continuous subcutaneous administration.

Under gaseous anesthesia the left middle cerebral artery of male Sprague Dawley rats was occluded proximal to its lenticulostriate branches by microbipolar coagulation. Enadoline or vehicle (saline) was administered subcutaneously 30min pre occlusion and by continuous administration starting 5min post occlusion via an osmotic minipump implanted subcutaneously in the back of the animal. Animals were sacrificed 24h post occlusion and the brains removed and rapidly frozen. 20μm sections were taken at 9 pre-selected coronal levels and stained with haematoxylin-eosin. Areas of infarction were assessed using computer-assisted image analysis and the volume of infarction derived by integration of the known stereotaxic co-ordinates of the 9 planes.

Enadoline at 0.1, 0.3, 1mg/kg plus 0.4, 1.2, 4mg/kg/day respectively, dose-dependently decreased the volume of infarction in the cerebral cortex compared to saline control animals. The greatest reduction in infarction (52%) was observed at 1mg/kg plus 4mg/kg/day. The study emphasizes the potent neuroprotective effect of enadoline in animal models of cerebral ischemia.

610.6

PEPTIDE GROWTH FACTORS ARE NEUROPROTECTIVE DURING ISCHEMIC INDUCED NITRIC OXIDE TOXICITY IN PRIMARY HIPPOCAMPAL CELL CULTURES.

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Nitric oxide synthase inhibitors prevent cell death in primary cortical cell cultures during glutamate toxicity (Dawson, V.L. et . al., Proc. Natl. Acad. Sci., 88:6368-6371, 1991). We sought to determine (a) whether nitric oxide (NO) mediates hippocampal cell death during ischemia, i.e. during anoxia and glucose deprivation; and (b) whether the peptide growth factors, basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF), can prevent hippocampal cell death during NO exposure. In a dose dependent manner, inhibition of nitric oxide synthase by N-methyl arginine rescued between 10%-50% of the cells that would normally die during an eight hour anoxic incubation . In addition, L-arginine, a precursor for NO, reversed the neuroprotective effects of N-methyl arginine during anoxia. Likewise, N-methyl arginine increased cell survival during an eight hour period of glucose deprivation, but to a lesser extent. Nitroprusside, an agent that decomposes to NO, was toxic to hippocampal cells and pretreatment of cultures with either bFGF (10ng/ml) or EGF (10ng/ml) for 24-48 hours prior to nitroprusside exposure increased survival by 28%-37%. Control experiments demonstrated that addition of equivalent concentrations of cyanide for the same time were not toxic, supporting the idea that NO was the toxic agent. This data strongly suggests that NO is necessary and sufficient to mediate hippocampal cell death during ischemia and that both bFGF and EGF are neuroprotective against NO toxicity. These insights into the mechanisms of ischemic cell death may direct future therapeutic modalities for cerebrovascular disease.

610.7

EFFECTS OF INHIBITION OF NITRIC OXIDE BIOSYNTHESIS ON FOCAL CEREBRAL INFARCTION IN RATS.

S. Yamamoto*, E.V. Golanov, S.B. Berger, and D.J. Reis. Div. of Neurobiol, Dept. Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

It has been proposed that nitric oxide (NO) synthesized *in situ* by the constitutive (Ca^{++}-dependent) form of nitric oxide synthase (NOS) may contribute to neurotoxicity associated with cerebral ischemia. We investigated whether acute or chronic inhibition of NOS by N-nitro-arginine (NNA) would modify the volume or distribution of infarctions produced by occlusion of the middle cerebral artery (MCA) in spontaneously hypertensive rats. Comparison was made with neuroprotection afforded by electrical stimulation of the cerebellar fastigial nucleus (FN) (Reis et al. *JCBFM* 11:810, 1991) or treatment with the oxazoline rilmenidine (RIL) (Maiese et al. *JCBFM* 12:53, 1992). Rats were anesthetized with halothane (2.0%) and the MCA occluded while either the FN was stimulated or RIL (0.75 mg/kg) or NNA (0.04 mg/kg/min, i.v.) administered for 1h. Rats were killed 24h later and infarct volume determined. FN stimulation and RIL reduced infarct volume by 52% ($p<0.001$; n=4) and 27% ($p<0.05$; n=4). However, NNA increased volume by 32% ($p<0.001$; n=6) unrelated to associated hypertension. Repeated administration of NNA (2.4 mg/kg, i.p.) every 4h from 4-23h after MCA occlusion had no effect on lesion size. We conclude that NO generated from constitutive NOS of neurons, glia, endothelium and/or macrophages nor the inducible form of NOS in glia (Galea et al., *Soc. Neurosci. Abstr.*, 1992) or macrophages does not contribute to cell death in focal ischemic infarction.

610.8

L-ARGININE (L-ARG) DECREASES INFARCT VOLUME AFTER MIDDLE CEREBRAL ARTERY OCCLUSION (MCAO) IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR).

E. Morikawa, Z. Huang, R.H. Price*, M.A. Moskowitz. Neurosurgery and Neurology Depts., Massachusetts General Hospital, Boston, MA 02114.

Nitric oxide (NO), a potent dilator produced by vascular endothelium is synthesized from l-Arg by NO synthase. We explored the possibility that administering l-Arg (and possibly generating NO) before and after focal ischemia might influence stroke outcome.

SHRs were subjected to distal MCAO + ipsilateral common carotid artery occlusion. L-Arg HCl (300 mg/kg) was injected i.p. 16 and 3 h before, and 5 and 120 min after right MCAO (n=9). Control rats (n=10) received saline. After 24h the resultant injury was evaluated after staining with 2,3,5-triphenyltetrazolium chloride.

Mean infarct volumes (mm^3)±SE for control and l-Arg treated rats were 147±12 and 92±17, respectively ($p<0.03$). There were no sign group differences in core and temporalis temp, MABP, brain volume and body wt or other parameters. Additional studies will be needed to determine the importance of NO to this effect.

610.9

ANTIOXIDANTS ATTENUATE DAMAGE TO CULTURED CORTICAL NEURONS INDUCED BY EXCITOTOXINS OR COMBINED OXYGEN-GLUCOSE DEPRIVATION.

J.J. Lynch III*, K. Rose and D.W. Choi. Dept. Neurol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

Antioxidant compounds can reduce CNS damage in both in vitro and in vivo models of hypoxia-ischemia. We have found that 21-aminosteroid antioxidants can reduce neuronal death induced by exposure to glutamate agonists or combined oxygen-glucose deprivation in murine cortical cell cultures.

The water soluble analog of alpha-tocopherol, trolox, produced a concentration-dependent reduction in murine cortical neuronal loss induced by a 24-hr exposure to NMDA (20 μM) or AMPA (10 μM plus 10 μM MK-801 to block NMDA receptor activation). Furthermore, trolox, vitamin C, vitamin E, and the 21-aminosteroid U74500A also augmented the neuroprotective effect of 10 μM MK801 plus 100 μM CNQX in cultures exposed to prolonged oxygen-glucose deprivation. These results support the idea that free radicals mediate some of the injury triggered by glutamate receptor overstimulation. In addition, free radicals may mediate damage induced by oxygen-glucose deprivation that is unrelated to glutamate receptor activation (at least, of ionotropic glutamate receptors).

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610.10

PREVENTION OF POST-ISCHEMIC MOLECULAR ALTERATIONS AND NEUROLOGICAL INJURY BY ACETYL-L-CARNITINE.

G. Fiskum*, Y. Liu, K.M. Myers, N.N. Kazerouni, Y.E. Bogaert, and R.E. Rosenthal. Depts. of Biochemistry and Molecular Biology and Emergency Medicine, George Washington University School of Medicine, Washington, DC 20037.

This study tested the hypothesis that post-ischemic IV administration of acetyl-L-carnitine (ALCAR) can improve cerebral energy metabolism, inhibit free radical-mediated molecular alterations and improve neurological outcome in a clinically relevant model of cardiac arrest (CA) and restoration of spontaneous circulation (ROSC). Seventy adult female beagles were anesthetized with chloralose and divided among 11 experimental groups including those where dogs were subjected to 10 min of CA followed by 30 min, 2 hr and 24 hr of ROSC in the absence and presence of post-ischemic administration of either ALCAR or acetate + carnitine at a concentration of 100 mg/kg immediately following defibrillation and 50 mg/kg q 6 hr. Although the level of total free plus esterified carnitine in the frontal cortex increased by > 100 % within 30 min following the initiation of either drug treatment relative to vehicle-treated controls only ALCAR-treated animals exhibited a significant amelioration of the abnormally elevated cortical lactate levels and lactate/pyruvate ratios present following 2 hr of ROSC. Free radical-dependent protein oxidation, as measured by the presence of dinitrophenlhydrazine-reactive carbonyl groups, is significantly elevated following 2 and 24 hr of ROSC in the absence or presence of carnitine + acetate but is not different from non-arrested controls during 24 hr reperfusion in the presence of ALCAR. These findings may help explain why ALCAR-treated, 24 hr ROSC animals exhibited significantly lower neurological deficit scores (NDS = 22.3 ± 5.2 S.E.) than those of vehicle treated controls (NDS = 48.4 ± 5.4) or than those exhibited by animals treated with carnitine + acetate (NDS = 41.0 ± 3.1) (NDS of 0 - normal; NDS of 100 = brain death).

(Supported by a grant from Sigma Tau, S.p.A.)

610.11

FELBAMATE REDUCES NEURONAL NECROSIS AND BEHAVIORAL DEFICITS INDUCED BY CARDIAC ARREST IN THE RAT. L.M. Adams, P.H. Schwartz, T.P. Palos, A.M. Morin* and C.G. Wasterlain. Epil. Res. Lab., VAMC Sepulveda, and Brain Res. Inst., UCLA, Los Angeles, CA. 90027

Felbamate, a novel anticonvulsant and putative NMDA receptor antagonist, has been shown to ameliorate hypoxic injury to the *in vitro* hippocampal slice and neuronal necrosis and infarction after hypoxia/ischemia in the neonatal rat. We tested its neuroprotective properties in an adult rat model of transient complete global cerebral ischemia induced by cardiac arrest. This model gives rise to severe neurological sequelae including myoclonus, hindlimb extensor hypertonus and severe necrosis to the hippocampus, cortex and Purkinje cell layer of the cerebellum. Rats were subjected to reversible cardiac arrest. Felbamate (1000 mg/kg p.o.) or vehicle (10 ml/kg H_2O) was administered one hour prior to cardiac arrest. Six days later the animals were given a qualitative neurological examination and were perfusion fixed for quantitative histological analysis. Felbamate reduced neurological deficits in every neurological category examined. Statistical significance was reached in the total neurological deficit score from 46.6 ± 7.4 to 67.3 ± 7.1 ($p<0.05$) (the lower the score, the more severe the deficit), facial myoclonus from 1.40 ± 0.31 to 4.14 ± 0.55 ($p<0.05$) and acoustic startle from 2.50 ± 0.60 to 4.14 ± 0.55 ($p<0.05$). In addition, Felbamate reduced the damage score in the Cortex from 2.92 ± 0.42 to 1.57 ± 0.43 ($p<0.05$) increased the number of live neurons in the CA_1 sector of the hippocampus from 154 ± 44 to 413 ± 127 ($p<0.05$). No protection was observed in the cerebellum. In conclusion Felbamate appears promising as a neuroprotective agent in global forebrain ischemia.

610.12

DELAYED INCREASES IN QUINOLINIC ACID AND SELECTIVE INDUCTION OF KYNURENINE PATHWAY ENZYMES AFTER TRANSIENT CEREBRAL ISCHEMIA. K. Saito,[1] T. S. Nowak Jr,[2] S. P. Markey,[1] and M. P. Heyes[1]* Section on Analytical Biochemistry[1], NIMH, and Stroke Branch[2], NINDS, Bethesda, MD 20892.

Accumulation of the excitotoxin and kynurenine pathway metabolite, quinolinic acid (QUIN), has been demonstrated in several brain regions following transient cerebral ischemia in the gerbil *(J. Cerebral Blood Flow Metabol.* 10: 660,1990). In the present study, proportional increases in brain indoleamine-2,3-dioxygenase (IDO) activity and QUIN levels occurred 4 d after 10 min ischemia, particularly in hippocampus and, to a lesser extent in striatum, cerebral cortex and thalamus, but not in cerebellum. Notably, these metabolic changes paralleled the degree of neuronal injury, local inflammation and macrophage infiltrates. In addition, the activities of kynureninase, kynurenine 3-hydroxylase and 3-hydroxyanthranilate-3,4-dioxygenase were also increased in hippocampus but not cerebellum. No changes kynurenic acid levels in brain and CSF, or alterations in brain kynurenine aminotransferase activity occurred. Synthesis of [$^{13}C_6$]-QUIN was demonstrated in hippocampus but not cerebellum 1 h after intracisternal administration of [$^{13}C_6$]-L-tryptophan. Increased activities of kynurenine pathway enzymes provide a mechanism to accelerate formation of QUIN from L-tryptophan within the brain. We hypothesize that these responses reflect immune stimulation within macrophage infiltrates, as well as other reactive cells, at the site of injury. Studies of the neuropathologic consequences of this delayed QUIN accumulation are warranted.

VISUAL DEVELOPMENT: CRITICAL PERIOD AND DEPRIVATION

611.1

INCREASED NGF SYNTHESIS IN RAT VISUAL CORTEX IN RESPONSE TO LIGHT-DEPRIVATION. A.A. Schoups*, R.C. Elliott, W.J. Friedman and I.B. Black. Dept. Neuroscience & Cell Biology, UMDNJ, Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Beside their well-described role in the peripheral nervous system, neurotrophins have been shown to be crucial for the development and maintenance of central neurons. To begin investigating their role in activity-dependent plasticity, we measured mRNA's for nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in the rat visual system during normal postnatal development and after dark-rearing. Northern blots as well as a fast and highly sensitive solution hybridization technique were used to determine mRNA levels.

In occipital cortex, both NGF mRNA and BDNF mRNA increased during ontogeny. Levels were low at 1 week postnatally, and increased exponentially during the second and third week after birth. Dark-rearing for three weeks following birth resulted in a marked increase in NGF mRNA, confirming recent data on a possible role of NGF in experience-dependent plasticity in the visual system. Current experiments involve: 1. Combining immunocytochemistry and in situ hybridization studies, to localize synthesis of neurotrophins and their receptors, and 2. Probing a potential role of glutamate receptors in this experience-dependent modulation of NGF synthesis.

We conclude that occipital cortex responds to light-deprivation by increased synthesis of NGF. Supported by NIH grant # HD 23315 and NS 10259.

611.2

NEUROCHEMICAL DIFFERENTIATION OF CORTICAL NEURONS IN VITRO. M.Götz* and J.Bolz, Friedrich-Miescher Labor der Max-Planck Gesellschaft, Spemannstr. 37-39, 7400 Tübingen, Germany

Neurons in the adult cerebral cortex exhibit a high degree of neurochemical diversity. At birth, however, most cortical neurons are immature and they begin to express their specific transmitters only postnatally. Which factors are involved in the determination of neurochemical phenotypes in the cortex? We studied transmitter expression of cortical neurons in slice cultures, and in dissociated cell cultures kept in medium with or without serum, from rat cortices at different developmental stages. Cells were stained after different days *in vitro* (DIV) with antisera against glutamate, GABA, or the neuropeptide VIP. Double-immunofluorescence with the neuronal marker MAP2 was used to evaluate the percentage of neurons expressing one transmitter. When slice cultures were prepared from postnatal cortices, neurons continued their neurochemical differentiation similar to the development *in vivo*. In contrast, in slice cultures prepared from E19 cortices, cells that did not complete their migration *in vitro* did not express their proper transmitter phenotypes, whereas cells that had already reached their final position differentiated properly, indicating that influences during migration of cortical neurons are crucial for neurochemical differentiation. To elucidate the time period during which these influences are required, cortical cells were dissociated either 3 days, 1 day or 1 hour after their birthdate *in vivo*. Whereas neurons labelled with BrdU 1-3 days before dissociation were found to contain glutamate or GABA after 7-10 DIV, no neurons labelled with BrdU 1 hour prior to dissociation could be double-labelled with the neurotransmitter antisera even after 14 DIV. Cells labelled with BrdU 1 day before dissociation in an acute slice preparation were also able to express their transmitters properly. These results indicate that factors in the local environment are important for the specification of neurochemical properties of cortical neurons, and that the expression of their transmitter is determined within 24 hours after their birthdate.

611.3

DEVELOPMENT OF GABA-ERGIC SUBPOPULATIONS MARKED BY NEUROPEPTIDES AND CALCIUM-BINDING PROTEINS IN KITTEN VISUAL CORTICAL AREAS. D. Hogan*, E.R. Terwilleger and N.E.J. Berman. Dept. of Anatomy and Cell Biology, Univ. of Kansas Medical Center, Kansas City, KS, 66160-7400.

The development of GABA-ergic interneurons in feline striate cortex (area 17) and extrastriate cortex (medial and lateral banks of the lateral suprasylvian sulcus; MLS and LLS) was tracked by avidin-biotin immunohistochemistry and immunofluorescent- double labelling using antibodies to GABA and to molecular markers which identify subpopulations of GABA-ergic neurons in adult mammalian primary visual cortex; i.e., neuropeptide Y (NPY), somatostatin (SOM), and the calcium-binding proteins parvalbumin (PV) and calbindin (CalD). The density of GABA-ir neurons was relatively constant during development and among visual areas. By contrast, most of the GABA-subpopulations increased in the cortex of all visual areas during development, and thus the proportion of GABA-ir neurons which were also SOM-ir, NPY-ir, PV-ir and CalD-ir rose during development. All SOM-ir cells were GABA-ergic at all ages. NPY-ir cells, though much less numerous than SOM-ir cells, were both GABA-ir and SOM -ir. Most (>98%) of the PV-ir and CalD-ir interneurons were observed to be GABA-ergic. Though SOM and CalD were not colocalized in neurons of the cortex perinatally, SOM-ir was observed in about two-thirds of the CalD-ir neurons in the subplate under MLS and LLS. In mature animals, SOM and CalD were occasionally observed to be co-expressed in neurons of supragranular cortical layers of all areas. In general, though GABA is expressed by interneurons in the cortex early during visual cortical development, their secondary molecular phenotype is not expressed until later. Supported by MH38399, BNS881997 and RCD8954894.

611.4

GROWTH-INHIBITION IN CAT VISUAL CORTEX AT THE END OF THE CRITICAL PERIOD FOR EXPERIENCE-DEPENDENT PLASTICITY, STUDIED IN VITRO C. M. Müller* and V. Schoop, Max-Planck-Institut f. Entwicklungsbiologie, 7400 Tübingen, Germany

During a restricted period of postnatal development the cat visual cortex undergoes an experience dependent modification of its circuitry including the elimination of ineffective thalamo-cortical synapses whereas effective projections can form additional contacts. We investigated whether the termination of the critical period for cortical malleability is paralleled by changes of the growth permissiveness of the cortical tissue.

Cryostat sections of unfixed visual cortex from kittens and cats aged from 2 weeks to one year were used as substrate for cultured embryonic neurons from E16-E18 rat or E6 chick cortex. After a culturing period of 1 to 3 days viable neurons were labeled with the carboxyfluoresceine-ester CFDA-AM and immediately observed with epifluorescence. Neurons readily adhere and grow on white and gray matter of visual cortex from kittens younger than 6 weeks. Thereafter neurons gradually fail to adhere on white matter and growth of neurites is significantly reduced on gray matter. This change in growth behaviour occurs between the sixth and tenth postnatal week, i.e. coincident with the termination of the critical period for cortical malleability.

It is concluded that changes in the growth permissiveness of cortical tissue contribute significantly to the termination of the critical period for cortical plasticity. As the end of the critical period coincides with the time of cortical myelination it is suggested that myelin-associated growth inhibitors (Caroni & Schwab, J.Cell Biol. 106:1281, 1988) may underly the change in growth permissiveness.

(supported by BMFT 0316902A)

611.5

THE EMERGENCE OF PINWHEEL-LIKE ORIENTATION DOMAINS IN THE VISUAL CORTEX OF KITTENS DURING THE CRITICAL PERIOD. Tobias Bonhoeffer*, Dae-Shik Kim and Wolf Singer. Max-Planck Institut für Hirnforschung, Deutschordenstrasse 46, 6000 Frankfurt 71, FRG.

It has been shown previously that iso-orientation domains in cat visual cortex are arranged in pinwheel like patterns (Bonhoeffer and Grinvald, Nature 353, 429-431). In the present study we attempted to investigate how these structures develop in the cortex of young kittens. We used optical imaging of intrinsic signals to explore the structure of iso-orientation domains in kitten visual cortex. We developed a technique which allowed us to chronically record intrinsic signals from one animal over the course of 4-8 weeks. Since the intrinsic signals recorded from kitten visual cortex proved to be much stronger than in the adult brain we attempted to record activity maps through the intact dura of the animals to minimize the risk of infection. Using this technique we found that in kittens of 4½ weeks of age pinwheels are already clearly present. Moreover, we also observed strong ocular-dominance maps, allowing us to assess the relationship between ocular-dominance and orientation maps in kittens. The orientation maps changed remarkably little between weeks four and eight postnatally. In the structures from which we were able to acquire optical data (i.e. the parts of area 17/18 on the lateral gyrus) we could not observe any new iso-orientation domains or pinwheels being formed during this period of time. This suggests that in normal kittens the basic structure of the orientation maps is formed before the age of 4-5 weeks. We are currently trying to image from the cortex of younger cats, hopefully back to the age of two weeks postnatally.

611.6

A CRITICAL PERIOD FOR THE DEVELOPMENT OF CLUSTERED HORIZONTAL CONNECTIONS IN CAT STRIATE CORTEX. M.B. Dalva, E.M. Callaway, and L.C. Katz.* Dept. of Neurobiology, Duke University Med. Center, Durham, NC 27710.

The clustered horizontal connections of layer 2/3 pyramidal neurons in cat striate cortex specifically link iso-orientation columns. The highly specific pattern of clusters emerges during the first 6 weeks of postnatal development by the selective elimination of unbranched axon collaterals to inappropriate regions and the differential growth of appropriately situated collaterals. Axon rearrangements depend on visual activity: incorrect projections are maintained in animals binocularly deprived for 6 weeks (Callaway & Katz, PNAS 88:745, 1991), and strabismic rearing alters the pattern of clusters (Lowel & Singer, Science 255:209, 1992). To determine when the development of these intrinsic connections is sensitive to activity cues, animals were deprived of patterned visual experience by binocular lid suture prior to natural eye opening. Eyes were reopened at after 6-14 weeks of deprivation to determine whether normal visual experience could restore the normal pattern of clustered connections. Small injections of red fluorescent latex microspheres were made to define patterns of horizontal connections in the deprived state; after 8-12 weeks of normal visual experience a second injection of green microspheres was made. Comparisons of the two patterns demonstrated that the crudely clustered pattern seen after six weeks of deprivation could still be refined to the normal pattern by visual experience. Preliminary results indicate that this ability to recover has disappeared by 14 weeks of deprivation. Thus, intrinsic cortical connections related to orientation, like LGN afferents related to ocular dominance, are sensitive to visual activity during a restricted period of early postnatal life. Supported by NIH grant EY07960 and the L.P. Markey Charitable Trust.

611.7

POSTNATAL DEVELOPMENT OF IPSILATERAL CORTICOCORTICAL CONNECTIONS IN THE CAT'S VISUAL CORTEX. D.J.Price*. Dept. of Physiology, Univ. Med. Sch., Edinburgh, U.K.

During the postnatal development of connections from area 17 to 18 of the cat's visual cortex, an initially highly exuberant pathway is refined mainly by axonal retraction. Previous studies suggested that the topographic organization of the early immature projection is very crude, and there is no clustering of the association neurones in area 17 as in the mature pathway.

Retrogradely transported fluorescent tracers were injected in area 18 in kittens aged 0-20 days. In some animals, axonal bifurcation in the area 17-to-18 projection was examined by co-injecting a different dye either at an adjacent point in area 18 itself, or at a topographically related point in another extrastriate cortical area. Labelling of area 17 was studied.

The results reveal a finer organization of the immature area 17-to-18 pathway than was previously suspected. Even at the earliest postnatal ages, while axons from area 17 are just penetrating area 18, there is evidence of rudimentary clusters of association neurones in area 17. Computer-modelling of the distributions of these cells suggests that the majority do send axons to topographically related regions of area 18, while a smaller number project extremely divergently. Few double-labelled cells were seen, indicating a low incidence of bifurcation in kittens, as in adults.

611.8

DEVELOPMENT OF CONNECTIONS IN VISUAL CORTICAL AREAS OF MACAQUE MONKEYS. T.A. Coogan and D.C. Van Essen* Div. Biology, Caltech, Pasadena CA 91125

The development of connections within and between areas V1 and V2 was studied by placing crystals of Di-I in fixed tissue taken from animals at two stages of development: the day of birth and embryonic day 133 (E133), 30 days after the latest generated neurons in V1 are born (Rakic, '74). At E133 labeled axons from V1 are confined to white matter below V2, where they ramify to some degree, but by birth the V1-V2 projection has reached layer 4. No labeled subplate neurons were seen after dye injections at either age. Intrinsic V1 connections are quite extensive at E133; the same layers are involved at this stage as at maturity, and extend approximately the same distances, with the exception of layer 1, where fibers extend over 10mm from the injection site, even crossing the V1/V2 border. However, at E133 there is no evidence of clustering of connections in superficial layers of V1, whereas these connections are clearly clustered at birth. In the feedback direction, the V2 to V1 projection has entered the grey matter by E133. At this stage the projection arises principally from deep layer cells, each of which has a dendrite which extends into layer 1. At birth the V2 to V1 projection comes predominantly from superficial layer cells. These observations suggest that the modular arrangement of connections in superficial layers of V1 is sculpted from a more uniform state; and that the feedback projection begins to innervate its target before the forward projection does so.

611.9

NATURALLY-STRABISMIC PRIMATE LACKS INTRINSIC HORIZONTAL CONNECTIONS FOR BINOCULAR VISION IN STRIATE CORTEX. L. Tychsen*[1] and A. Burkhalter[2]. Depts. Ophthalmology[1] and Neurosurgery[2], Washington Univ. Sch. of Med., St.Louis, MO 63110.

Although it has been postulated that strabismus in human infants is associated with maldevelopment of connections in the visual cortex, no anatomic data from naturally-strabismic primates has been reported. We have now shown abnormalities of horizontal connections in visual area V1 in a macaque monkey with infantile-onset strabismus. The monkey developed esotropia 6 weeks after birth and showed ocular motor behaviors that characterize early-onset esotropia in human infants.

In V1 of normal primates blobs and interblobs of left eye columns connect to blobs and interblobs of left and right eye columns (Livingstone and Hubel, 1984). To examine whether this organization is present also in naturally-strabismic animals, we injected biotinylated dextran amine (BDA) into left or right eye columns of V1. Ocular dominance columns were made visible by laserablating the left optic nerve head 5 days before the injection. After 3 more days the animal was sacrificed and alternating sections were stained for BDA and cytochrome oxidase (CO). Injections confined to columns driven by the left eye revealed connections only to left eye columns and skipped right eye columns (and vice versa). Connections along the horizontal axis of the visuotopic map were longer than connections along to the vertical axis. The total extent along the long axis was ~16 columns (~5° of the central visual field) and ~8 columns along the short axis.

These results show that anatomic alterations in strabismic primate correspond to behavioral deficits in binocularity, and suggest that as in cat (Löwel and Singer, 1992), correlated input from the 2 eyes is important for the normal development of intracortical connections.

611.10

LABELING OF OCULAR DOMINANCE COLUMNS IN AMBLYOPIC MACAQUE MONKEYS USING CYTOCHROME OXIDASE HISTOCHEMISTRY. M.P. Stryker* & J.C. Horton. Departments of Physiology & Ophthalmology, UCSF, San Francisco, CA 94143-0444.

Two monkeys underwent suture of the right eyelids at 1 week of age. A year later, ^{3}[H]-proline was injected into single geniculate lamina. Autoradiographs revealed shrunken ocular dominance columns belonging to the amblyopic eye, but remarkably, cytochrome oxidase (CO) activity in layer IVC appeared completely homogeneous. In layers II,III alternating dark and light rows of blobs were visible. The light rows fit in register with deprived eye dominance columns in IVC. To label the eye dominance columns in IVC using CO, a third monkey was raised with the right eyelids sutured. At 21 months the left occipital lobe was excised. No CO columns were visible in layer IVC, confirming the two prior experiments. Next ^{3}[H]-proline was injected into the left eye. A week later the left eye was enucleated and the right eye was re-opened; 2 weeks later sections from the remaining occipital lobe were processed for either CO or autoradiography. In sections reacted for CO we observed thin, dark columns alternating with wide, pale columns. Comparison with adjacent autoradiographs established that the thin, dark columns belong to the deprived, right eye. These findings indicate that early monocular suture in macaques produces uniform CO activity in layer IVC of striate cortex, but visual deprivation combined with subsequent enucleation causes a pattern to emerge of shrunken (amblyopic eye) columns alternating with expanded (normal eye) columns. (Supported by grants from NEI & National Children's Eye Care Foundation)

611.11

ANISOMETROPIA INDUCES AMBLYOPIA WITHOUT SHRINKAGE OF OCULAR DOMINANCE COLUMNS IN HUMAN STRIATE CORTEX. J.C. Horton* & M.P. Stryker. Departments of Ophthalmology & Physiology, UCSF, San Francisco, CA 94143-0350.

In macaques raised with unilateral eyelid suture, cytochrome oxidase activity is homogeneous in layer IVC of striate cortex (Stryker & Horton, 1992). Subsequent enucleation of the normal eye causes thin, dark columns alternating with wide, pale columns to appear in layer IVC. The thin, dark columns are the ocular dominance columns of the amblyopic eye. In an analogous clinical case, we examined the pattern of cytochrome oxidase activity in a 53-year-old man with amblyopia in the left eye (20/400 acuity), who became blind in his normal right eye 3 months before death. The amblyopia was due to a difference of 6 diopters in the refractive power of the two eyes (anisometropia), first detected at age 5.

The mosaic of ocular dominance columns in each striate cortex was reconstructed from serial flattened sections reacted for cytochrome oxidase activity. Surprisingly, no shrinkage of the ocular dominance columns serving the amblyopic eye was observed. Dark stripes occupied 48% of the column area in the left cortex and 54% of the column area in the right cortex. Slightly more cortex was filled in each side by the contralateral eye, reflecting the normal attenuation of ipsilateral eye columns in peripheral binocular cortex. We conclude that ocular dominance columns in layer IVC of human striate cortex do not shrink in anisometropia, implying a different cortical basis for this form of amblyopia.

(Supported by grants from NEI & National Children's Eye Care Foundation)

611.12

EFFECTS OF ARTIFICIAL STRABISMUS ON MACAQUE MT.

J. Anthony Movshon* and Lynne Kiorpes.
Howard Hughes Medical Institute and Center for Neural Science, New York University, New York 10003.

Tyschen and Lisberger (1986, *J. Neurosci.*) observed a nasal-ward bias in smooth pursuit eye movements and visual motion judgements in humans with esotropic (convergent) strabismus, suggesting that these individuals had abnormal visual motion processing. Subsequently Walton and Lisberger (1989, *ARVO*) found similar oculomotor abnormalities in monkeys raised with artificial strabismus, and raised the possibility that abnormal mechanisms of pursuit initiation might underlie these changes.

Motion signals in cortical area MT support pursuit eye movements. We therefore used suitable targets to assess the direction and speed preferences of MT neurons in anesthetized adult macaque monkeys given surgical esotropia in infancy. We found no unusual asymmetries in the motion-related responses of these cells. We did observe a sharp reduction in binocular interaction in MT cells, similar to that seen in V1. Because MT cells receive a convergent input from large areas of V1 and V2, this suggests that the functional organization of cortico-cortical connections can be altered by abnormal experience in a manner reminiscent of the well-known changes seen in thalamocortical connections. These changes do not seem to affect visual motion signals, so we conclude that pursuit anomalies in strabismus reflect changes in the generation of oculomotor commands rather than in the visual processing of motion information.

DRUGS OF ABUSE: COCAINE AND OTHER STIMULANTS

612.1

PRENATAL COCAINE EXPOSURE ALTERS DOPAMINE TRANSPORTER BINDING IN ADULT MICE. G.A. Pritchard*, J.J. Byrnes, L.G. Miller. Dept. of Pharmacology and Experimental Therapeutics and Neuroscience Program, Tufts Univ. School of Medicine, Boston, MA 02111.

Prenatal cocaine administration is associated with persistent behavioral effects, but the neurochemical basis for these effects is uncertain. We exposed pregnant mice to cocaine, 10 mg/kg, during days 14-21 of gestation. At 6 weeks of age, offspring were evaluated for motor activity and dopamine transporter binding in striatum in vivo using WIN 35,428. No differences in motor activity were observed among untreated, vehicle-exposed, or cocaine-exposed mice. Similarly, no change in activity was noted in response to chronic cocaine among the exposure groups. However, total dopamine transporter binding in striatum was significantly reduced by approximately 20% in cocaine-exposed mice compared to the other groups. No change was observed in nonspecific binding evaluated in cerebellum. Specific binding in striatum was thus reduced significantly by approximately 25%. These data indicate that prenatal cocaine exposure is not associated with changes in motor activity untreated or in response to chronic cocaine, but is associated with alterations in dopamine transporter binding in mature offspring.

612.2

REGULATION OF DOPAMINE RECEPTORS mRNA EXPRESSION IN THE RAT BRAIN BY COCAINE. C.Spyraki(1,3), A. Prikhozhan(1), G.W.Huntley(1) and S.C.Sealfon*(1,2) 1.Fishberg Center for Research for Neurobiology and 2.Department of Neurology,The Mount Sinai School of Medicine, NY, NY 10029, 3. Lab.of Pharmacology,Medical School,University of Crete, GR.

The mesolimbic/mesocortical DAergic pathways and the DA receptor subtypes, pharmacologically defined as D1/D2, appear to mediate the reinforcing properties of cocaine, on both behavioural and biochemical grounds. The present study focuses on possible regulation of D1 and D2 receptor gene expression in rat brain by cocaine treatment, using in situ hybridization (ISH). Adult male Wistar rats were treated with intraperitoneal cocaine administered either for one (0; 5.0; 10.0; 20.0; 40.0 mg/kg) or for 15 days (0; 10.0; 20 mg/kg). Twenty four hrs after the last injection, the animals were deeply anaesthetized and perfused. The brains were frozen and 25μm thick sections were taken through the accumbens,striatum and midbrain with a sliding microtome. The floating sections were processed for ISH with 35S-radiolabeled rat D1 and D2 cRNA probes. Quantitative ISH, performed on film autoradiograms with a computer assisted densitometer, was used to examine changes in the levels of D1 and D2 mRNA in nucleus accumbens (ACC), olfactory tubercle (TO), caudate nucleus (CP), ventral tegmental area (VTA) and substantia nigra(SN). The results show that D1 mRNA expression was affected by neither acute nor chronic cocaine treatment at the ACC, TO and CP. Similarly no changes were revealed in the D2 mRNA levels at the ACC, CP, and SN. An average of 25% decrease(statistical significance 95%) in D2 mRNA density was measured at TO in animals treated with single injections of cocaine at the high doses(20 and 40 mg/kg). A tendency for decrease with the same regimen was observed in VTA. Within the limitations of the experimental paradigm used in this study, the data suggest that the regulation of DA receptor gene expression by cocaine is not robust and manifests dose, receptor subtype and anatomical specificity. (supported by the Aaron Diamond Foundation)

612.3

COCAINE INCREASES ACCUMBENS SEROTONIN RELEASE CONCURRENTLY WITH DOPAMINE RELEASE IN PSYCHOSTIMULANT REINFORCEMENT. P.A. Broderick*, R.T. Wechsler, F.T. Phelan, and F. Eng. Dept. Pharmacol., CUNY Med. Sch. (J-910) & CUNY Grad. Sch., Convent Ave, W. 138th St, NY, 10031,USA.

Ultrastructural evidence from light and electron microscopic studies shows that serotonin (5-HT) plays an important role in the regulation of dopaminergic (DA-ergic) ventral tegmental (VTA) neuronal circuitry (Herve *et al, Brain Res.* 435:71; 1987). *In vivo* neurochemical studies show that a colocalized, DA-ergic and 5-HT-ergic response to cocaine occurs in VTA, suggesting that 5-HT may be a relay or a gating mechanism for DA (Broderick, P.A., *Pharmacol. Biochem. & Behav.* 42[4]; 1992). This paper assesses the concurrent effects of intraperitoneal (IP) cocaine (10 mg/kg) on DA and 5-HT release in nucleus accumbens (NAcc) of freely moving and behaving, male, Sprague-Dawley rats with *in vivo* electrochemistry (voltammetry). DA and 5-HT were detected separately and sequentially within 10-15 seconds with stearate microelectrodes (cf. Broderick P.A., *Brain Res.* 495:115; 1989, for microelectrode preparation). Activity patterns were studied with infrared photocell beam detection. The results show that (IP) cocaine increased DA and 5-HT release. Concomitantly, the cocaine-induced psychostimulant behaviors, hyperactivity, rearing and stereotypy were increased. Agoraphobic behavior (the tendency to avoid the center of the cage) was inhibited; thus, an acute anxiolytic effect was seen. Cocaine-induced DA and 5-HT release highly correlated with behavior. The data show that cocaine comodulates 5-HT with DA in NAcc, the terminal neurons of the VTA-DA-ergic pathway. [Supp: NIDA R01 DA 04755-01 and PSC-CUNY Awards RF 6-69201 and RF 6-61188].

612.4

AJ76, DOPAMINE AUTORECEPTOR ANTAGONIST, MODULATES ACCUMBENS DOPAMINE AND SEROTONIN IN COCAINE-INDUCED PSYCHOSTIMULANT BEHAVIOR. F.T. Phelan*, R.T. Wechsler, F. Eng, M.F. Piercey[1] and P.A. Broderick. Dept. Pharmacol., CUNY Med. Sch., Convent Ave. & W. 138th St., NY, 10031, USA, CNS Res., Upjohn Co.[1], Kalamazoo, MI 49007, USA.

AJ76 is a centrally acting dopamine (DA) receptor antagonist which produces weak behavioral stimulation via inhibition of presynaptic DA autoreceptors (Svensson *et al, Naunyn-Schmeid. Arch. Pharmacol.* 334:234; 1986). AJ76 reversed the electrophysiological effects of cocaine (Hoffman *et al. Neurosci. Abstr.* 17:681; 1991) and is itself not self-administered (Roberts, D.C., pers. comm.). The present paper assesses the neurochemical and behavioral profiles of AJ76 in combination with cocaine, using *in vivo* electrochemistry and activity pattern analysis. Specific details for *in vivo* electrochemistry (voltammetry) are published (Broderick, P.A., *Brain Res.* 495:115; 1989). Stearate indicator microelectrodes were stereotaxically implanted in nucleus accumbens of male, Sprague Dawley rats under Na pentobarbital anesthesia; 9-15 days of recovery were allowed. Time course studies show that, although AJ76 significantly increased DA and 5-HT release *per se* (Eng *et al*, this meeting), AJ76 pretreatment down-modulates cocaine-induced DA and 5-HT release concurrently with down-modulating locomotor activity, rearing and stereotypy. Cocaine's acute anxiolytic activity was not down-modulated. The unusual behavioral profile of AJ76, involving biogenic amines subserving cocaine reinforcement, offers a new approach to cocaine pharmacotherapeutics. [Supp: NIDA R01 DA 04755-01, The Upjohn Co. RF 7-76207 & PSC/CUNY RF 6-69201 & RF 6-61188 Awards to P.A. Broderick].

612.5

MESENCEPHALIC NEURONAL CELL CULTURE AS A MODEL OF DOPAMINE TRANSPORTER ONTOGENESIS. M. Valchár and I. Hanbauer*. Lab. of Chem. Pharmacol., NHLBI, Bethesda, MD 20892.

In primary cultures of ventral mesencephalon (CVMN) from 14 day old rat embryos, [^{3}H]DA uptake, sensitive to nanomolar concentrations of cocaine and its congeners, is expressed as soon as neurites are formed. Using [^{3}H]WIN 35,428 as a ligand, we found no specific binding sites for [^{3}H]WIN 35,428 in the absence or presence of Na^+ in membrane preparations of cells cultured 5-6 days. However, in intact cells low affinity binding sites of [^{3}H]WIN 35,428] (K_D=336,000nM) were found in the cytosol of CVMN. The cytosolic [^{3}H]WIN 35,428 binding was sodium-independent, and was displaced by micromolar concentrations of DA uptake inhibitors. When CVMN were cultured for 12-20 days both high (K_D=57 nM) and low affinity binding sites (K_D=105,776 nM) were expressed in intact cells. The high affinity binding site of [^{3}H]WIN 35,428 was sodium-dependent, and was displaced by nanomolar concentrations of cocaine or mazindol. Membrane preparations from CVMN, cultured for 12-20 days, also contained sodium-dependent, high affinity binding sites of [^{3}H]WIN 35,428. Pharmacological studies indicate a relationship between the high affinity [^{3}H]WIN 35,428 binding sites and the DA transporter complex, whereas the cytosolic [^{3}H]WIN 35,428 binding sites were functionally unrelated.

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612.6

THE COCAINE BINDING SITE: MODULATION BY VARIOUS IONS AND CHANNEL BLOCKERS. J.W. Boja* and T.A. Kopajtic. NIDA Addiction Research Center, P.O. Box 5180, Baltimore, MD 21224

Several studies have suggested that certain calcium channel modulators may affect the behavioral effects of cocaine. However, no study has examined the effects of various ion channel modulators directly upon the binding of cocaine to the dopamine transporter. The effects of NaCl, KCl and Ca_2Cl and their respective channel modulators had upon the striatal cocaine binding site were determined using the cocaine analog [^{3}H]WIN 35,428. The addition of NaCl had no effect upon specific binding however, Ca_2Cl and to a lesser extent KCl decreased binding. Various Na^+, K^+, Cl^- and Ca^{2+} channel blockers were also tested for their ability to inhibit specific [^{3}H]WIN 35,428 binding. Most of the Na^+ channel blockers tested were of moderate potency, the exceptions being benzamil and flunarizine which displayed higher potency. Both of these agents are also reported to have activity at the Ca^{2+} channel. The K^+ channel blockers were of low and moderate potency while the Cl^- channel blockers had no effect. Of the Ca^{2+} channel blockers tested only pimozide demonstrated high potency. This was postulated to be due to its ability to act upon both L and T-type channels. These results suggest that the Ca^{2+} channel blockers deserve further study as useful therapeutic potential in the treatment of cocaine addiction.

612.7

NEUROANATOMIC AND MOLECULAR SPECIFICITY OF C-FOS INDUCTION BY COCAINE IN DEVELOPING RAT BRAIN. Barry E. Kosofsky*, and Steven E. Hyman. Molecular Neurobiology Laboratory, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114.

A class of immediate early genes (eg., the IEG c-fos) that act as transcription factors, may subserve one mechanism by which substances of abuse alter programs of neural gene expression. We have injected saline (vehicle control) or cocaine (30 mg/kg, ip) in to rat pups (P8, P15, P28) and adults and sacrificed animals acutely (@45 minutes for mRNA (northern) and in situ hybridization histochemistry analyses, and @120 minutes for immunohistochemical analysis) to determine the ontogeny and spatial distribution of cocaine induced IEG activation. On P8, cocaine induces c-Fos in striatal patches, in a few scattered globus pallidus neurons, in layer VI neocortical neurons, but not in anterior cingulate cortex. On P15, cocaine induced striatal c-Fos is more diffuse, with a lateral predominance. In globus pallidus there is a high density of c-Fos immunoreactive (IR) neurons. In neocortex there is augmentation of significant basal c-Fos expression after cocaine treatment, particularly evident in infragranular layers. Anterior cingulate cortex, which is minimally activated on P8 or P15, has a high density of c-Fos IR neurons in cocaine exposed P28 and adult animals. In globus pallidus of P28 and adult rats exposed to cocaine there are no c-Fos IR neurons. Striatal induction of c-Fos by cocaine on P28 and in the adult is densest medially. Both basal and cocaine induced c-Fos IR in neocortex is markedly decreased in P28 and adults when compared to P15 animals. Analysis of adjacent sections in cocaine treated animals at each age suggests that neither presynaptic markers (dopamine innervation), nor post-synaptic markers (D1 and D2 dopamine receptor mRNA distribution) nor intrinsic neuronal markers (DARP-32, Substance P) are entirely predictive of the spatial and temporal specificity of cocaine induction of c-Fos IR described. We conclude that cocaine induces c-fos mRNA and c-Fos protein with a significant degree of spatial selectivity, and temporal specificity. We suggest that cocaine induced alterations in gene expression during critical developmental periods may irrevocably alter CNS form and function, with differential regional, temporal, and cellular vulnerabilities.

612.8

EFFECTS OF REPEATED COCAINE ADMINISTRATION ON 5-HT_{1A} RECEPTORS IN RAT BRAIN. J. I. Javaid*, S. K. Sahni, S. C. Pandey and J. M. Davis. Illinois State Psychiatric Institute, University of Illinois at Chicago, Chicago, IL 60612.

Repeated administration of cocaine causes augmentation in behavioral effects (behavioral sensitization) induced by acute administration. However, the specific adaptive neurochemical mechanisms underlying this behavioral sensitization are not well-understood. Electrophysiological studies have suggested that the effects of cocaine on serotonergic function may be modulated by 5-HT_{1A} receptor subtype. In the present studies we examined the changes in the binding of [^{3}H]-8-hydroxy-2-(di-N-propylamino) tetralin (8-OH-DPAT, 5-HT_{1A} selective ligand) in rat cortex and hippocampus after repeated cocaine administration. Cocaine (10 mg/Kg) was administered once daily by intraperitoneal injection for a total of 9 injections over 11 days (5 injections with 2 days off, followed by 4 injections). Four groups [saline (S)/S; cocaine (C)/S; S/C; C/C] of animals were pretreated with 8 injections of S or C followed by the 9th injection as S or C "challenge". The animals were sacrificed by decapitation 24 hours after the last injection, brains removed immediately and dissected into cortices and hippocampi for [^{3}H]-8-OH-DPAT binding. There were no statistically significant differences in either the number of binding sites (B_{MAX}) or the affinity (K_D) of DPAT binding in cortex and hippocampus from various groups. These results suggest that behavioral sensitization with repeated cocaine administration is not associated with changes in either pre- or postsynaptic 5-HT_{1A} receptors.

612.9

THE ROLE OF DA, NE, AND 5-HT NEURONAL SYSTEMS IN THE DISCRIMINATIVE STIMULUS EFFECTS OF COCAINE. A. K. Singha* and J. B. Appel. Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina, Columbia, S.C. 29208.

To elucidate the role of dopaminergic, adrenergic, and serotonergic neuronal systems in the stimulus effects of cocaine, rats (n = 32) were trained to discriminate i.p. injections of cocaine (10 mg/kg) from saline (1 ml/kg) in a two-lever, drug discrimination paradigm. Various doses of compounds which have been reported to act selectively at DA, NE, or 5-HT receptor sites were then given in combination with the training drug. The results indicated that the DA (D_1) antagonist SCH 39166 (0.01-0.2 mg/kg) blocked the effects of cocaine in a dose-dependent manner. Neither the α_1 NE antagonist prazosin (0.05-0.2 mg/kg), the α_2 antagonist idazoxan (1.25-5.0 mg/kg), the non-selective 5-HT antagonist metergoline (5.0-20.0 mg/kg), nor the 5-HT_2 antagonist ketanserin (0.06-0.2 mg/kg) had significant effects on the cocaine cue. These data support the hypothesis that DA, particularly D_1 receptors, are involved in the discriminable and, perhaps, other subjective effects of cocaine.

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612.10

IN VITRO AND IN VIVO LABELING OF DOPAMINE AND SEROTONIN TRANSPORTERS WITH β-CIT. R.B. Innis*, M. Laruelle, Y. Zea-Ponce, S.S. Zoghbi, R.T. Malison, M.S. Al-Tikriti, G. Wisniewski, E.H. Sybirska, E.O. Smith, J.L. Neumeyer, R.A. Milius, D.S. Charney, P.B. Hoffer, R.M. Baldwin. Yale University. /VA Medical Center, West Haven, CT 06516 and Research Biochemicals, Inc., Natick, MA 01760

Methyl 3ß-(4-iodophenyl)tropane-2ß-carboxylate (β-CIT or RTI-55) displayed high affinity for both dopamine (DA) uptake sites labeled with [^{3}H]CFT (IC50 = 1.6 nM) in monkey striatum homogenates and serotonin (5-HT) uptake sites labeled with [^{3}H]paroxetine in rat cortical membranes (3.8 nM). Saturation studies performed with [^{125}I]β-CIT showed the presence of high and low affinity sites (K_{high} = 0.74 nM, K_{low} = 34 nM, $Bmax_{high}$ = 140 pmol/g tissue, $Bmax_{low}$ = 760 pmol/g tissue) in rat striatum, while binding in cortex was best fit with a one-site model (Kd=1.7 nM, Bmax=67 pmol/g tissue). *In vivo* [^{123}I]β-CIT binding was studied in baboons with SPECT scanning (n=39). Striatal activity increased slowly, reached a plateau level at 140 ± 10 min and remained constant until the end of the experiment (330 ± 8 min). Midbrain activity peaked at 47 ± 2 min and declined with a rate of 14 ± 1% /hr. At 300 min, the striatal to cerebellar ratio was 7.3 ± 1 and the midbrain to cerebellar ratio was 2.3 ± 0.4, with cortical activity similar to that in cerebellum. *In vivo* displacement studies with selective monoamine inhibitors GBR12909, citalopram, and maprotiline showed that striatal activity was associated with DA transporters, and midbrain activity with 5-HT transporters. Since DA and 5-HT uptake sites displayed different uptake kinetics and anatomical localization, [^{123}I]β-CIT is a potentially useful SPECT ligand to visualize both sites.

612.11

AMPHETAMINE-INDUCED DOPAMINE RELEASE: A CELL CULTURE MODEL. K. F. Farrell, D.L. Conant and S. P. Berger*. Dept. of Psychiatry, Univ. California San Francisco and Veterans Affairs Medical Center, San Francisco, CA 94121.

Amphetamine derivatives and other substrates of the catecholamine transporter such as tyramine are believed to induce release of dopamine by a carrier mediated "exchange diffusion". Uptake of amphetamine derivatives by the catecholamine transporter is thought to be coupled with a nonexocytic release of dopamine. An important assumption in this "exchange diffusion model" is that specificity of these agents is derived from their ability to act as substrates of the dopamine transporter. Therefore, a good correlation is expected between the competitive inhibition of dopamine uptake and dopamine release. Pheochromocytoma (PC12) cells have been used extensively to characterize the exocytic release of dopamine and we have begun to study PC12 cells as a model of nonexocytic dopamine release.

Our findings on the stereospecific release of dopamine from PC12 cells by amphetamine derivatives is supported by earlier reports that tyramine is believed to release dopamine via a carrier mediated mechanism. Likewise, consistent with the nonexocytic exchange diffusion model, the ability of amphetamine derivatives to release dopamine is calcium independent . However, there appears to be a poor correlation between the inhibition of dopamine reuptake and the ability to induce release. We have observed a rank order of fenfluramine > d-amphetamine > l-amphetamine > tyramine in the ability to release dopamine and d-amphetamine = l-amphetamine > tyramine > fenfluramine for inhibition of uptake. Inasmuch as tyramine has also been reported to bind to and be a substrate of the vesicular dopamine transporter, we examined whether the transport of catecholamines into storage vesicles is a potential source of specificity for dopamine release. An identical rank order was observed in the ability of amphetamine derivatives to inhibit [^{3}H]ketanserin binding to vesicles as was earlier found to release dopamine. These results suggest that the vesicular transporter may play a critical role in the mechanism of amphetamine-induced dopamine release from PC12 cells.

612.12

DAILY POST-SESSION COCAINE ADMINISTRATION IMPAIRS ACQUISITION OF A POSITIVELY-MOTIVATED AUTOSHAPED LEVER-TOUCH RESPONSE IN RATS. P.H. Janak*, W.A. Rodriguez and J.L. Martinez, Jr. Dept. of Psychology, Univ. of California, Berkeley, CA 94720.

The effects of daily peripheral (IP) post-session injection of cocaine (COC) on the development of an autoshaped lever-touch response were investigated. Male Sprague-Dawley rats received 10 daily pairings of a retractable lever (CS) and food delivery (US). Reinforcement delivery was not contingent upon a response; however, if the subjects contacted the lever during CS presentation, then reinforcement was delivered immediately. COC, at a dose of 5.55 mg/kg [$F(1,30)=5.21$, $p<.03$], but not 2.75 mg/kg [$F(1,30)=0.18$, $p>.5$], impaired acquisition of the lever-touch response. COC's effect on lever-touch acquisition depended upon the time of drug administration relative to the conditioning session, as injection of COC 3 hrs after each session did not affect response acquisition. In addition, COC's effect depended on explicit CS-US pairing as post-session COC administration did not alter responding when the presentation of both the CS and the US were uncorrelated. These results indicate that the post-session administration of COC can alter the retention of a positively-motivated conditioned response, as seen by impaired acquisition of the lever-touch response. (Supported by DA06192 and DA05375.)

EXCITATORY AMINO ACIDS: PHARMACOLOGY VI

613.1

POLYAMINE AGONISTS, ANTAGONISTS AND INVERSE AGONISTS REDUCE NMDA RECEPTOR SINGLE-CHANNEL CURRENTS. D. M. Rock* and R. L. Macdonald#. Neuroscience Program* and Depts. of Neurology and Physiology#, University of Michigan, Ann Arbor, MI 48104.

Polyamines modulate the binding of open channel blockers to the NMDA receptor subtype of postsynaptic glutamate receptor. Using voltage-clamp and single-channel recording techniques, we previously have shown that spermine (SP) has multiple actions on NMDA receptor currents. At low concentrations SP increased opening frequency to enhance NMDA receptor current, but at higher concentrations SP produced a voltage-dependent reduction in the amplitude and average open time of NMDA receptor single-channel openings.

The related polyamines, arcaine (ARC), spermidine (SD), diethylenetriamine (DET), putrescine (PUT) and diaminodecane (DA-10), all produced a voltage-dependent reduction in NMDA receptor current. Reduction in amplitude of NMDA receptor single channel openings by SD, DET and PUT was similar to SP and the concentration dependence of the reduction was related to charge at physiological pH. The highly charged polyamine SP (+4) reduced the amplitude of single channel openings at concentrations lower than PUT (+2). Reduction of NMDA receptor currents by ARC and DA-10 however was different. The reduction of NMDA receptor single-channel currents by ARC was associated with increased open channel noise and DA-10 produced a flickery block of single-channel currents. Reductions of NMDA currents by SP and DA-10 were not reversed by the polyamine antagonist DET.

These data suggest that polyamines may reduce NMDA receptor current by voltage-dependent effects on NMDA single-channel amplitude and average open time and that the polyamine reduction of NMDA receptor current appears to have a pharmacological profile different from that described for polyamines in receptor binding assays.

613.2

POSITIVE ALLOSTERIC MODULATION OF NON-NMDA RECEPTORS IN HIPPOCAMPAL RAT BRAIN SLICES. M. Bertolino*, M. DiBella', M.Baraldi', S. Vicini and E. Costa. FGIN, Georgetown University, Washington, DC, 'School of Pharmacy, Modena University, Italy.

Excitatory synaptic transmission in the mammalian brain is due to the activation of non-NMDA agonist-sensitive glutamate receptors, and the termination of the synaptic currents may be related to the fast desensitization of these receptors (Trussel, L. and Fischbach, G. Neuron 3:209-218, 1989; Vyklicky, L. et al. Neuron 7:971-984, 1991). Therefore, drugs that potentiate the action of non-NMDA receptors can potentiate synaptic transmission and related functions (such as long term potentiation, etc.). We investigated whole-cell currents elicited by bath application of L-glutamate in CA1 pyramidal neurons in thin (250 μM) slices of adult rat hippocampus using the patch-clamp technique. L-glutamate (50 μM) was applied in the presence of TTX (0.5 μM) in order to block action potentials and evoked synaptic activity and Dizolcipine (MK-801, 5 μM) to block non-competitively NMDA-sensitive glutamate receptor activation. Combined application of L-glutamate with the "nootropic" compound aniracetam (1 mM) produced ionic current 144 ± 15% greater than L-glutamate alone (mean ± SE, n= 5 neurons). Similarly, the benzothiadiazide Diazoxide (1 mM) potentiated the L-glutamate current by 145 ± 31% (n= 5), while cyclothiazide (a potent thiazide derivative) at 50 μM enhanced the L-glutamate current by 288 ± 80% (n= 4). We were interested in characterizing the potency and efficacy of other diazoxide derivatives to identify more potent and selective compounds. To this aim we synthesized diazoxide derivatives by various groups substitution. Cyclothiazide is not the only derivative that is more potent than diazoxide. In fact, ring halogenation in position six and seven also plays an important role in the positive modulation of hippocampal glutamate receptors.

613.3

2-PHOSPHONOETHYLPHENYLALANINE DERIVATIVES AS NOVEL ANTAGONISTS OF KAINIC ACID/AMPA RECEPTORS. E.W. Karbon*, D.L. Bednar, M.A. Bailey, S. A. Borosky, R.A. Zubrowski, J.W. Ferkany, Z. Huang and G.S. Hamilton. Nova Pharmaceutical Corporation, Baltimore, MD 21224.

A series of substituted 2-phosphonoethylphenylalanines were synthesized and tested for their ability to interact with kainic acid (KA)/AMPA receptors in rat brain poly (A$^+$) RNA-injected *Xenopus* oocytes and excitatory amino acid receptor binding sites in rat brain membranes. Of the four monomethyl substituted derivatives tested, only the 5-methyl compound potently inhibited KA-induced currents (K_i = 13 μM). 5-Trifluoromethyl (K_i = 10 μm) and 5-ethyl (K_i = 17 μM) derivatives had potencies comparable to 5-methyl, whereas 5-*t*-butyl (K_i = 29 μM), 5-methoxy (K_i = 67 μM) and 5-phenyl (K_i = 139 μM) were less potent. 3,5-Dimethyl (K_i = 12 μM) was substantially more potent than 3,6-dimethyl (K_i = 220 μM). Within the 5-halo series, the rank order of potency was 5-I (K_i = 3 μM) > 5-Br (K_i = 10 μM) > 5-Cl (K_i = 15 μM) > 5-F (K_i = 68 μM) >> 5-H (K_i > 200 μM). Reduction of the aromatic ring resulted in a complete loss of activity for the 5-methyl compound.

Test compounds exhibited comparable potency in inhibiting KA-induced currents and [^{3}H]CNQX binding in rat brain membranes, but were weak inhibitors of [^{3}H]glutamate binding. Some compounds exhibited moderate (K_i = 40-100 μM) affinity for strychnine-insensitive [^{3}H]glycine binding sites. The 5-methyl compound blocked the KA-induced reduction in striatal choline acetyltransferase activity, and both the 5-methyl and 5-I compounds inhibited KA-induced cytotoxicity in cultured rat cortical neurons.

The findings demonstrate that selected 2-phosphonoethyl-phenylalanine derivatives are antagonists of KA/AMPA receptors. As representatives of a new chemical class of KA/AMPA antagonist, these compounds should be useful in further defining the physiological roles of KA/AMPA receptors.

613.4

RECEPTOR BINDING AND ELECTROPHYSIOLOGICAL PROPERTIES OF KAINIC ACID/AMPA RECEPTOR AGONISTS AND ANTAGONISTS. D.L. Bednar*, R.A. Zubrowski, M.A. Bailey and E.W. Karbon, Nova Pharmaceutical Corporation, Baltimore, MD 21224.

[^{3}H]CNQX binding assays and rat brain poly (A$^+$) RNA-injected *Xenopus* oocytes were used to characterize the biochemical and electrophysiological properties of several kainic acid (KA)/AMPA receptor agonists and antagonists. The rank order of potency for inhibition of [^{3}H]CNQX binding in rat cerebral cortical membranes was NBQX > CNQX > (S)-5-nitrowillardiine (NW) > (S)-5-fluorowillardiine (FW) > KA = (RS)-AMPA = (S)-5-iodowillardiine (IW) > L-BOAA. Inclusion of KSCN (100 mM) resulted in 80-, 20- and 2-fold increases in the potency of the agonist ligands AMPA, L-BOAA and KA, respectively, whereas the potency of the antagonists, NBQX and CNQX, was reduced. Amongst the agonist willardiines, the greatest increase in potency was observed with FW (20-fold), followed by IW (6-fold) and NW (4-fold). Aniracetam (1 mM), a compound which reduces KA/AMPA receptor desensitization, had no effect on AMPA potency either in the absence or presence of KSCN.

In *Xenopus* oocytes, KA produced large, non-desensitizing inward currents which were similar to those elicited by IW. In contrast, AMPA, L-BOAA and FW produced small, desensitizing responses. In the presence of either AMPA, L-BOAA or FW, KA-induced currents were markedly reduced. Likewise, responses to IW were attenuated by 50% in the presence of either AMPA or FW.

These findings suggest that in the presence of KSCN, KA/AMPA receptors adopt a conformation with greatly increased affinity for desensitizing, but not non-desensitizing agonists. Furthermore, an inverse relationship was observed between the magnitude of the KSCN-induced increase in agonist potency and the magnitude of agonist-induced steady-state currents.

613.5

THE 2,3-BENZODIAZEPINE GYKI 52466 SELECTIVELY BLOCKS AMPA/KAINATE RECEPTORS BY A NOVEL NON-COMPETITIVE MECHANISM.

S.D. Donevan* and M.A. Rogawski, Epilepsy Research Branch, NINDS, NIH, Bethesda MD 20892.

Excessive activation of non-NMDA (AMPA/kainate) excitatory amino acid receptors may play a role in the pathogenesis of epilepsy and various neurodegenerative disorders. Recent studies have demonstrated that the 2,3-benzodiazepine GYKI 52466 antagonizes non-NMDA excitatory amino acid receptor responses (Tarnawa et al., 1990; Ourdouz & Durand, 1991). In the present study we compared the effects of GYKI 52466 with that of the quinoxaline NBQX on currents evoked by AMPA and kainate in whole cell recordings from cultured rat hippocampal neurons. GYKI 52466 caused a concentration-dependent block of inward currents evoked by AMPA (IC_{50}=7.5±0.4 μM) and kainate (11±1 μM), but was inactive against currents evoked by NMDA or GABA. The EC_{50} values for kainate in the presence of 10 and 30 μM GYKI 52466 (169±2 μM and 202±18 μM, respectively) were similar to control (173±16 μM), whereas GYKI 52466 produced a concentration-dependent reduction in the maximal current evoked by kainate. In contrast, NBQX caused a concentration-dependent rightward shift in the kainate concentration-response curve with no change in the maximal response to kainate. Thus GYKI 52466 is a non-competitive antagonist, whereas NBQX acts in a competitive fashion at the glutamate recognition site. GYKI 52466 did not appear to block via an open channel mechanism as there was no voltage- or use-dependence to its actions. These results demonstrate that GYKI 52466 selectively inhibits AMPA/kainate responses by a novel noncompetitive mechanism. Noncompetitive non-NMDA antagonists such as GYKI 52466 could offer advantages over competitive blockers in the treatment of neurological disorders, particularly in situations where high levels of glutamate would render the competitive antagonists relatively ineffective.

613.6

STEREOSELECTIVE EFFECTS OF AMOA ON NON-NMDA RECEPTORS EXPRESSED IN XENOPUS OOCYTES

P.Wahl*, B. Nielsen, P.Krogsgaard-Larsen, J.J.Hansen A.Schousboe & R. Miledi PharmaBiotec Research Center, The Royal Danish School of Pharmacy, Copenhagen, Denmark, and Laboratory of Cellular and Molecular Neurobiology, UC Irvine CA 92717, USA.

There are pharmacological and therapeutic interests in compounds capable of blocking subtypes of central glutamate receptors. Pharmacological charaterization of the action of the novel non-N-methyl-D-aspartate (non-NMDA) antagonist AMOA (2-amino-3-[3-(carboxymethoxy)-5-methylisoxazol-4-yl]propionate) on glutamate receptors was investigated in Xenopus oocytes injected with mouse brain mRNA. AMOA (150 uM) produced a nearly parallel shift to the right of the dose-response curve for kainate induced currents. AMOA was found to have two different effects on AMPA receptors: (1) currents elicited by low concentrations of AMPA (6 uM) were inhibited by AMOA with an IC_{50} value of 160 +/- 19 uM; (2) currents elicited by high concentrations of AMPA (100 uM) were potentiated with an IC_{50} value of 88 +/- 22 uM. The maximal potentiating effect of AMOA on AMPA currents was around 170%. Quantitative analysis showed that the apparent K_d value of AMOA with respect to its potentiation of AMPA responses was about 15-fold lower that its K_i value for inhibition of AMPA responses. This suggests that the ability of AMOA to potentiate or inhibit AMPA responses may be mediated by different sites. Nonetheless, the two opposing effects of AMOA on AMPA responses are specific for the L-configuration of AMOA. This unusual antagonistic/agonistic property of AMOA may explain its unusual properties with regard to antagonism of non-NMDA receptor mediated events previously described.

613.7

NMDA RECEPTOR SPLICE VARIANTS DIFFER IN THEIR RESPONSE TO ETHANOL. S.N. Treistman*, H. Bayley, V.V. Koltchin, and V. Anantharam. Dept. of Pharmacology, Univ. of Mass. Medical School, Worcester, MA 01655 and Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

A number of laboratories have reported the NMDA receptor to be very sensitive to ethanol, and the reduction of current through this receptor/channel may underlie some of the behavioral effects of ethanol. Using RT-PCR, we have isolated four splice variants of the NMDA receptor from rat brain (see V. Anantharam abstract, this meeting). The variants, in which each of two cassettes (21 and 37 amino acids) are present or absent, have been expressed in *Xenopus* oocytes, and their response to ethanol has been examined. In all of the splice variants 25-100 mM ethanol reduces the inward current evoked by 100 μM NMDA. However, the magnitude of this reduction differs among the different variants. The rank-ordering of the variants according to the reduction of NMDA-evoked current in the presence of ethanol, with the most sensitive first, is: NMDAR1-LL > NMDAR1-LS > NMDAR1-SL > NMDAR1-SS. In addition to differences in their ethanol sensitivity, we find that the apparent desensitization of the variants differs, with NMDAR1-LL showing significantly greater desensitization than NMDAR1-SS. We are currently determining the relationship between the apparent desensitization of the receptor and its response to ethanol. Work supported by ADAMHA grant AA05542.

613.8

NON-SELECTIVE ACTIONS OF ETHANOL AS AN EXCITATORY AMINO ACID (EAA) ANTAGONIST ON RAT SPINAL NEURONES IN VIVO. D. Lodge*, S.N. Davies and M.G. Jones. Royal Veterinary College, London NW1, UK.

Recent reports have indicated that ethanol selectively reduces N-methyl-D-aspartate (NMDA) induced excitation in several neuronal preparations. To examine this action of ethanol in vivo, we studied the effects of both local pressure ejection and systemic administration of ethanol on responses of spinal and brainstem neurones in anaesthetised rats to iontophoretic administration of NMDA, quisqualate, kainate and AMPA.

Pressure ejection of ethanol produced variable effects on responses to EAAs, sometimes with reductions in spike height, but selective antagonism of NMDA was not observed. Intravenous ethanol up to 4g/kg reduced effects of all EAAs on some neurones and the higher doses were sometimes accompanied by respiratory and/or cardiovascular effects but no preferential reduction of responses to NMDA were observed.

In the same protocol, LY274614 (1mg/kg), ketamine (5mg/kg) and (-)-b-cyclazocine (0.25mg/kg) blocked responses to NMDA whereas GYKI52466 (10mg/kg) and NBQX (5mg/kg) blocked those to AMPA/kainate.

In conclusion, no evidence was found to support the view that ethanol is a selective NMDA antagonist in vivo.

613.9

AGENTS WHICH ANTAGONIZE THE NMDA RECEPTOR-CHANNEL COMPLEX IN VIVO ALSO CAUSE DISTURBANCES OF MOTOR COORDINATION. A. J. Carter* and R. E. Miller, Dept. of Pharmacology, Boehringer Ingelheim KG, W-6507 Ingelheim, Germany.

We have investigated the relationship between functional antagonism of the NMDA receptor-channel complex in vivo and disturbances of motor coordination. Antagonism of the NMDA receptor-channel complex was assessed by measuring the ability of various compounds to inhibit NMDA-induced lethality in mice. Disturbances of motor coordination were measured by the rotarod technique. Noncompetitive NMDA antagonists of both arylcyclohexamine (PCP, (+)MK-801, (-)MK-801, ketamine) and benzamorphan (dextrorphan, N-allylnormetazocine) structure, competitive antagonists (CGP 37849, CGP 39551) and a glycine site antagonist (L-687,414) inhibited NMDA-induced lethality after subcutaneous administration. These compounds also interfered with motor coordination. There was an excellent correlation (r=0.8) between the two tests. Several other antagonists of the glycine site (7-chlorokynurenic acid, 5,7-dichlorokynurenic acid, ACPC and (+)HA-966) did not inhibit NMDA-induced lethality after systemic administration of doses up to 100 mg/kg and did not interfere with motor coordination. We conclude that any compound which antagonizes the NMDA receptor in vivo, irrespective of whether it is a noncompetitive, competitive or glycine site antagonist, will disturb motor coordination.

613.10

EFFECTS OF THE NMDA ANTAGONIST, KETAMINE, IN HEALTHY HUMAN SUBJECTS. J.H. Krystal*, L.P. Karper, J.P. Seibyl, R. Delaney, G. Freeman, G.R. Heninger, M.B. Bowers, J.D. Bremner, D.S. Charney. Schizophrenia Biological Research Center West Haven VA Med. Cntr., West Haven, CT 06516.

Non-competitive NMDA antagonists, such as PCP, are thought to produce a schizophrenia-like syndrome in humans. This hypothesis was formally evaluated in humans using ketamine. METHODS: Healthy subjects (n=18) completed 3 test days in a randomized order, under double-blind conditions: placebo, ketamine 0.1 mg/kg, ketamine 0.5 mg/kg. All drugs were infused i.v. over 40 minutes. RESULTS: The Mini-Mental Status Examination was not effected by either ketamine dose. Positive and negative symptoms of schizophrenia assessed by the BPRS, perceptual changes assessed by the Perceptual Aberration subscale of the Psychosis Proneness scale, and dissociative symptoms assessed by the Clinician Administered Dissociation Scale, all showed dose-related increases. Frontal lobe function assessed by Wisconsin Card Sort perseverative errors and by assessment of verbal fluency showed dose-related impairment. Ketamine also produced dose-dependent impairment in delayed but not immediate recall of object names. Ketamine also dose-dependently increased plasma cortisol and prolactin and suppressed plasma HVA. IMPLICATIONS: Ketamine transiently produced a broad range of the symptoms and neuropsychological deficits characteristic of schizophrenic patients. These data place further emphasis of evaluations of excitatory amino acid function in schizophrenia.

613.11

SOMATOSTATIN CONTAINING DENTATE HILAR NEURONS IN PRIMARY CULTURE EXPRESS Ca^{2+} PERMEABLE KAINATE RECEPTORS. Simon J. Gibbons* and Richard J. Miller. Dept Pharmacol. and Physiol. Sci., University of Chicago, 947 E. 58th Street, Chicago, IL 60637. USA.

We have studied the excitatory amino acid receptor pharmacology in a population of hippocampal GABA-containing neurons in primary culture. Neurons were dissociated from the dentate gyrus of 5 day old rats and cultured in a defined serum free medium over a feeding layer of astrocytes. These cultures are highly enriched (>85%) in neurons which express immunoreactivity for somatostatin-14 and GABA but not the Ca^{2+} binding proteins parvalbumin or calbindin D28K. Together with the morphological appearance of the cells, this information suggests that they represent a population of neurons which are particularly sensitive to ischaemic insults in vivo and are exceptionally vulnerable in human limbic epilepsy (Freund et al. (1992) Brain Res. Bull. 28 27, deLanerolle et al. (1987) Epilepsia 28 600).

Using fura-2 based microfluorimetry non-NMDA glutamate receptor-mediated rises in $[Ca^{2+}]_i$ were observed (EC50 values for AMPA = 11.5μM and Kainate = 55.8μM, n=6 to 8). In the absence of extracellular Mg^{2+}, with 10μM glycine added to the perfusate, responses to 10μM NMDA were also observed (mean $\Delta[Ca^{2+}]_i$ = 172nM, n = 5). Although some cells have caffeine sensitive intracellular stores, no metabotropic glutamate receptor-activated $[Ca^{2+}]_i$ rises have been detected. In Na^+ free medium, kainate caused concentration dependent rises in $[Ca^{2+}]_i$ (EC50 = 373μM). This effect was reduced by CNQX. In addition, kainate mediated uptake of Co^{2+} was observed in these cells using a silver staining technique. Thus it appears that dentate gyrus hilar neurons have Ca^{2+} permeable kainate receptors which may contribute to the selective vulnerability of these cells in vivo.

SJG is supported by a long term fellowship from the Human Frontier Science Program.

613.12

NMDA DIFFERENTIALLY STIMULATES SOMATOSTATIN (SS) BUT NOT NEUROPEPTIDE Y (NPY) GENE EXPRESSION IN CORTICAL SS/NPY PRODUCING NEURONS. Y.C. Patel, A. Warszynska, G. Kent, J.-L. Liu, D.N. Papachristou, and S.C. Patel*. Fraser Labs, McGill University, Montreal, Quebec, and Newington VAMC, CT.

SS and NPY are coproduced in a subpopulation of neurons that are selectively resistant to NMDA neurotoxicity. We have previously reported that quinolinic acid (QA) and NMDA augment SS-mRNA in cultured fetal rat cortical neurons. Here we have examined coregulation of SS and NPY gene expression by these 2 agents in this system and compared their effects with those of forskolin (F) and PMA known to activate SS and NPY gene transcription by cAMP or protein kinase-C (PKC)-dependent mechanisms. Cultures were treated with different agents for up to 24 h. mRNA for SS and NPY was determined by Northern analysis with cRNA probes.

	SS-mRNA (fold increase)		NPY mRNA (fold increase)
QA 5 mM	3.9^+		1.1
NMDA 5 mM	5.0^+	$^+p < 0.05$	0.65
forskolin 10 uM	4.5^+	vs control	1.6^+
PMA 0.4 uM	1.5^+		2.9^+

QA and NMDA stimulated mRNA for SS but not for NPY. In contrast, F and PMA augmented both SS and NPY mRNA. In time-course studies NMDA/QA induction of SS-mRNA occurred after a delay of 16 h. Transcriptional regulation of the SS gene was examined by acute transfection of cortical cultures with an SS promoter-CAT construct. CAT activity was stimulated 3-4 fold with F but not by NMDA or QA. These data show differential NMDA stimulation of SS but not NPY mRNA. NMDA stimulation of SS gene expression does not require activation of the cAMP or PKC signalling pathways. It is not transcriptionally mediated and is likely posttranscriptional as also suggested by the delay in SS-mRNA induction.

PROCESS OUTGROWTH, GROWTH CONES AND SPROUTING VIII

614.1

MAPPING OF CYTOSOLIC CALCIUM CONCENTRATION IN GROWING AXONS OF RAT SENSORY NEURONES. S.R. Bolsover, M.R. Duchen* and A. Amato. Physiology Department, University College London.

Previous work using the calcium indicator Fura-2 has suggested the presence of steady-state gradients of cytosolic free calcium concentration ($[Ca^{++}]_i$) in various types of nerve cells that are extending axons or dendrites. These measurements were subject to possible errors caused by uptake of Fura-2 into intracellular organelles. We have therefore used dextran-conjugated Fura-2, which is not taken up into organelles, to measure $[Ca^{++}]_i$ in the axons and growth cones of sensory neurons in culture.

After injection with Fura-2 dextran (MW ≈10,000, Molecular Probes) dorsal root ganglion cells from adult rats were incubated in Hams F14 medium supplemented with 4% Ultraser G (Gibco) in an atmosphere of 5% CO_2 and 95% air. $[Ca^{++}]_i$ was imaged in 31 growth cones which were displaying a full repertoire of behaviours including active forward growth. Mean growth cone $[Ca^{++}]_i$ was 225±44nM, n=31. No steady-state gradients of $[Ca^{++}]_i$ were observed, either within the growth cone (leading edge $[Ca^{++}]_i$ minus mean growth cone $[Ca^{++}]_i$ = 29±32nM, n=30) or along the axon (soma $[Ca^{++}]_i$ minus growth cone $[Ca^{++}]_i$ = 88±87nM, n=30). Taken together with similar findings in mouse neuroblastoma cells (Silver *et al.*, 1989) these data strongly refute the model that steady-state $[Ca^{++}]_i$ gradients are a general feature of growing neurites.

Goldberg and Burmeister (1989) described three stages in the advance of axons of *Aplysia* neurons: protrusion, engorgement and consolidation. In rat sensory neurones $[Ca^{++}]_i$ was identical in regions of the growth cone undergoing each of these behaviours. Thus spontaneous changes in $[Ca^{++}]_i$ do not appear to trigger these behaviours in isolated neurones. However, an increase of $[Ca^{++}]_i$ to >400nM, whether spontaneous or induced by A23187, caused growth cone retraction.

REFERENCES: Goldberg, DJ. and Burmeister, D.W. (1989) Trends in Neurosciences 12, 503-506; Silver, R.A., Lamb, A.G. and Bolsover, S.R. (1989) Journal of Neuroscience 7, 3588-3599.

614.2

SPATIAL GRADIENTS OF CYTOSOLIC CALCIUM DURING DEPOLARIZATION OF DEVELOPING SENSORY NEURONES. F.A. Al-Mohanna, A. Amato and S.R. Bolsover*. Physiology Department, University College London

Previous work using mouse neuroblastoma cells (Silver *et al.*, 1990) has shown that depolarization produces marked spatial gradients of cytosolic free calcium concentration ($[Ca^{++}]_i$). Little or no $[Ca^{++}]_i$ change was seen in the neurite, while $[Ca^{++}]_i$ changes in the growth cone were restricted to a small number of spatially restricted hotspots. We have now examined whether similar $[Ca^{++}]_i$ gradients are set up by depolarization of primary sensory neurones. As before, we used the fall of Fura-2 fluorescence during steady illumination with 380nm light to measure $[Ca^{++}]_i$ changes with a time resolution of 60msec.

During the first day after plating on a laminin substrate, dorsal root ganglion cells isolated from adult rats extend large (up to 40µm across) growth cones connected to the cell body by broad, short neurites. When cells at this early stage of axon outgrowth were whole-cell patch clamped and depolarized, $[Ca^{++}]_i$ increased in all regions of the cell, so that no spatial gradients of $[Ca^{++}]_i$ developed in the neurites and growth cones.

After two or more days in culture, cells exhibited the more familiar appearance of long narrow axons terminated by small (up to 20µm across) growth cones. Since these cells are not suitable for voltage clamp, we examined $[Ca^{++}]_i$ changes during a train of five action potentials delivered during a 50msec period. Unlike the situation in the early growth cones, depolarization produced a clear $[Ca^{++}]_i$ gradient in the growth cones, with the leading edge of the growth cone showing a greater $[Ca^{++}]_i$ increase than the more proximal region of the growth cone. These results raise the possibility that electrical activity could selectively activate calcium-dependent processes at the growth cone leading edge.

REFERENCE: Silver, R.A., Lamb, A.G. and Bolsover, S.R. (1990) Nature 343, 751-754.

614.3

GROWTH CONE COLLAPSE, A DETERMINANT OF SYNAPSE SPECIFICITY. N.I. Syed*, K. Lukowiak and A.G.M. Bulloch. Departments of Physiology and Anatomy, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

A model system for examining synaptogenesis is provided by the large, identified dopamine neuron (RPeD1) of the adult mollusc *Lymnaea stagnalis*. This interneuron exhibits robust sprouting and synaptogenesis with appropriate targets *in vitro*. Behavioral analysis of the growth cones from both RPeD1 and the appropriate targets indicates mutual attraction. We present evidence that this attraction of target cell growth cones towards RPeD1 growth cones prior to synaptogenesis is mediated via transmitter/receptor interaction. We also demonstrate that RPeD1 cell growth cones contain and release dopamine and that the target cell growth cones respond appropriately to the exogenous application of dopamine. However, when cultured with inappropriate target cells RPeD1 does not form synapses, rather its growth cone induces the collapse of approaching target growth cones either upon contact or even before filopodial contact is made. Utilizing time-lapse video microscopy we present evidence that this contact and non-contact mediated collapse of inappropriate target cell growth cones by RPeD1 is mediated via transmitter receptor interaction involving second messenger pathways other than Ca^{2+}. Our experiments suggest that the release of dopamine from RPeD1 growth cones and the presence of appropriate receptor on the target may provide the basis for cell-cell recognition during synaptogenesis in this system.

614.4

DEPOLARIZATION TRIGGERED NEURITE RETRACTION OF LEECH CELLS IN CULTURE IS SUBSTRATE AND CALCIUM DEPENDENT. M.D. Neely* and M. Gesemann, Biocenter Univ. Basel, 4056 Basel, Switzerland

The substrate upon which a leech neuron is placed influences not only its pattern of growth and the distribution of Ca^{2+} channels on its surface, but also the degree of retraction following electrical stimulation (S. Grumbacher-Reinert and J. Nicholls, 1992, J.Exp.Biol. 167:1-14). To investigate how substrates regulate neurite outgrowth, the effects of depolarization (by raised extracellular K^+) were analyzed in cells grown on leech extracellular matrix (ECM) and the plant lectin Concavalin A (Con A). In cells on ECM, K^+ depolarization, like electrical stimulation, led to neurite retraction. This retraction was inhibited by Mg^{2+} and therefore appeared to be a Ca^{2+} mediated effect. Cells grown on Con A did not respond with retraction of their neurites after depolarization. Scanning electron microscopy of growth cones on ECM revealed filopodial and lamellipodial retraction following high K^+ exposure, while growth cones on Con A showed no change after the same treatment. Analysis of the effect of depolarization on the cytoskeleton revealed changes in the microfilaments in cells on ECM. No alteration in microfilamental organization after high K^+ application was observed in cells on Con A. These results demonstrate the key role of substrate molecules in defining the final form of a neuron.

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614.5

SERUM LYSOPHOSPHATIDATES CAUSE RECEPTOR MEDIATED NEURITE RETRACTION IN PC12 CELLS. G. Tigyi, D. L. Dyer & R. Miledi*. Lab. of Cellular and Molecular Neurobiology, Dept. of Psychobiology, University of California, Irvine, CA 92717 Lysophosphatidic acids (LPA's), produced during blood coagulation and bound to serum albumin were found to be the principal serum component causing neurite retraction in NGF differentiated PC12 cells. LPA's purified from albumin, or of synthetic origin, elicited neurite retraction with an ED_{50} in the micromolar range. Application of LPA's caused the intracellular level of IP3 to increase ~10 fold within 7.5 min. Monitoring intracellular free [Ca^{2+}] levels with Fura2 ratio-imaging revealed a concomitant transient increase from the resting level (<100nM) to 300 nM. Neurite retraction was abolished in the absence of extracellular Ca^{2+}. Divalent cation blockers of Ca^{2+} channels had a similar effect; however, organic blockers of the different classes of voltage gated Ca^{2+}-channels were ineffective. This suggests the involvement of a non-voltage activated Ca^{2+}-influx mechanism. Bradykinin and carbachol activated similar second messenger events without causing neurite retraction. Thus, increased PIP2 turnover and a transient rise in Ca^{2+} are not sufficient for neurite retraction by LPA. Neurite retraction was also prevented by pretreatment with cholera toxin, suggesting the involvement of a G_s-mediated, cAMP-dependent pathway. It is concluded that LPA-induced neurite retraction is not due to an increase in [Ca^{2+}] alone, but depends on interactions of different second messenger pathways. Supported by grants BNS-9010398 and NS-23284.

614.6

SINGLE CELL LASER INACTIVATION OF FASCICLIN II PERTURBS AXONOGENESIS IN THE GRASSHOPPER LIMB BUD. J.L. Schnipper, M.S. Shankland* and D.G. Jay. Dept. Cell. & Dev. Biol., Harvard Univ., Cambridge, MA 02138 and Dept. Anat. & Cell Biol., Harvard Med. Sch. Boston, MA 02115.

Fasciclin II is a neural cell adhesion molecule that is structurally similar to NCAM and is expressed by the Til pioneer neurons during axonogenesis. Previously, we showed that chromophore assisted laser inactivation (CALI) directed against fasciclin II results in a perturbation of axonogenesis but not axon adhesion of the Til neurons (Booth et al., Neurosci. Abstr. 17: 14, 1991). Recently, single cell CALI has been developed, in which the laser is focused on one cell (Sydor et al., Neurosci Abstr. 17: 14, 1991). We now show that single cell CALI against fasciclin II on the Til neurons perturbs axonogenesis with high efficacy and reveals a three-hour time period during which CALI will cause this perturbation.

Laser irradiation of Til neurons in whole-mounted grasshopper embryos at the 30% stage of development incubated with malachite green-labeled anti-fasciclin II blocked axon initiation (15/18). No significant disruption in axonogenesis was seen in non-irradiated contralateral limbs (n=47), or in embryos incubated with dye-labeled anti-fasciclin I regardless of laser irradiation (n=15). Single-cell CALI against fasciclin II had no effect on axon adhesion (n=20), in contrast with CALI against fasciclin I. Also, single cell CALI against fasciclin II was only effective between complete Til cell emergence from the epithelium and these cells becoming tear-shaped. This stage is coincident with the localization of cytoskeletal elements and organelles to the proximal pole from which the growth cone will emerge (Lefcort and Bentley, J. Cell Biol. 108: 1737, 1989). The higher efficacy of single cell CALI over large scale CALI in perturbing axonogenesis may be due to greater antibody accessibility, more precise aiming of the laser, and the ability to perform CALI at precise stages based on Til cell morphology. This last feature also enabled us to pinpoint the narrow time window during which CALI is effective in perturbing axonogenesis.

614.7

GLYCOSAMINOGLYCANS AS MEDIATORS OF RETINAL GANGLION CELL BODY AND AXON POLARITY. P. A. Brittis* and J. Silver. Dept. of Neurosciences, CWRU, Cleveland, OH 44106

In the developing vertebrate retina differentiating retinal ganglion cells retain a radial configuration while maintaining primitive endfeet attachments at both the ventricular and vitreal surfaces of the retinal neuroepithelium. We have previously shown that early in retinal development, only the vitreal endfeet are emersed in a dense matrix of chondroitin sulfate (CS).The perturbation of the glycosaminoglycan (GAG) side chains with chondroitinase ABC in whole developing retinas disrupts several stages of retinal ganglion cell differentiation such as the temporal control of gangliogenesis, cell soma polarization, and the control over the invariance of axonal direction (Brittis et al. *Science*, 1992).

After whole rat retinas at E.13 were cultured and exposed to free chondroitin-6-sulfate GAG chains for 48 hours, the exogenous CS was distributed in high concentrations around the ventricular endfeet. In direct contrast to control retinas and retinas treated with a chondroitin sulfate proteoglycan in solution, all of the retinal ganglion cell bodies and axons in the GAG treated eyes were found within this novel layer of CS matrix. This repolarization was concentration dependent and was marked by the retraction of the vitreal end feet followed by the relocation of the retinal ganglion cell bodies to the ventricular surface of the neuroepithelium. This newly polarized optic fiber layer was anatomically indistinguishable from a normal nerve fiber layer except that the individual axons were not oriented in any particular direction.

We show that the ability of immature retinal ganglion cells to initiate axons is not restricted to the vitreal endfoot and that the ventricular endfoot also has this potential. We have also found that cell body and axon polarity in intact retinas can be induced by the location and concentration of the GAG component of the extracellular milieu.

614.8

NCAM, L1 AND N-CADHERIN INFLUENCE NEURITE OUTGROWTH FROM RETINAL NON-PROJECTION NEURONS. I. Kljavin*, Neurosci. Res. Group, U. Calgary; C. Lagenaur, Dept. Neurobio. Anat. and Cell Sci, U. Pittsburgh, J. Bixby, Dept Pharmacology, U. Miami Sch. Med, T. Reh, Dept. Bio. Structure, U. Washington.

NCAM, N-cadherin and L1 are cell adhesion molecules now known to be important for promoting axon growth from projection neurons, eg. retinal ganglion cells. However, the CAMs important for regulating axon outgrowth from non-projection neurons, like amacrine and photoreceptor cells, are not known. Such local circuit neurons extend their neurites on cellular surfaces not normally encountered by the ganglion cell axons, to terminate on neighboring cells. Therefore, we compared neurite outgrowth from rat ganglion cells, amacrine cells and rods in vitro on immunopurified forms of NCAM, L1 and N-cadherin. Single isolated early postnatal ganglion cells grow neurites on all three cell adhesion molecules. NCAM and N-cadherin strongly promote neurite outgrowth from either early (P3) or late (P10) postnatal amacrine cells, while L1, promotes neurite outgrowth from only a small percentage of the amacrine cells. None of these cell adhesion molecules support neurite outgrowth from early postnatal rods, but, surprisingly, NCAM stimulated vigorous neurite extension from rods isolated from postnatal day 10. These results show that NCAM, N-cadherin, and L1 can promote neurite outgrowth from local circuit neurons, but the use of any particular CAM is dependent on the cell type and the developmental period. Supported by NIH NS 30305; Sigma Xi.

614.9

PATTERNS OF GROWTH CONE CONTACT WITH DIFFERENT ADHESIVE SUBSTRATES AS DETERMINED BY INTERFERENCE REFLECTION MICROSCOPY J.A. Drazba*, C.L. Smith, and V. Lemmon, Laboratory of Neurobiology, Laboratory of Neural Control, NINDS, NIH, Bethesda, MD 20892 and Dept. of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH, 44106

We examined dynamic changes in the closeness of growth cone contacts with laminin, L1/8D9, or N-cadherin substrates by measuring reflection intensities in images obtained with time-lapse interference reflection microscopy (IRM). Growth cones from both chick retinal ganglion cells and sympathetic neurons were observed. The closeness of contact of individual growth cone membrane patches on each substrate modulated rapidly. However, there were marked differences in the overall extent of growth cone contacts to different substrates. Growth cones on laminin had the fewest areas of close contact, growth cones on L1 had the most, and those on N-Cadherin were intermediate between these two. Measurements of growth cones were compared to those of fibroblasts, which exhibited a much broader range of reflection intensities. The focal contacts of fibroblasts were closer than any of the contacts of growth cones. The IRM profiles of growth cones observed in these studies reveal quantitative differences in the closeness of growth cone adhesion to different substrate molecules and correlate with reported differences in growth rate and morphology observed on these substrates.

614.10

GROWTH CONES ARE ACTIVELY INFLUENCED BY SUBSTRATE-BOUND ADHESION MOLECULES.
S.M. Burden*, H.R. Payne, and V. Lemmon, Dept. of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH, 44106

As axons advance to appropriate target tissues during development, their growth cones encounter a variety of cell adhesion molecules (CAMs) and extracellular matrix molecules (ECMs). Purified CAMs and ECMs influence neurite outgrowth *in vitro* and are thought to have a similar function *in vivo*. We previously utilized scanning electron microscopy to compare morphological characteristics of retinal ganglion cell (RGC) growth cones on several substrates. We found that growth cone lamellipodial area and number of filopodia per growth cone are affected by the substrate bound adhesion molecule (Payne, et.al.,(1992) Cell Motil. and Cytoskel. 21:65-73). In this report, we use timelapse videomicroscopy to examine dynamic transformations of RGC growth cones as they progress from L1/8D9, N-cadherin, or laminin onto a different substrate. Contact made by the leading edge of a growth cone with a new substrate causes a rapid and dramatic alteration in growth cone morphology. Frequently, the changes encompass the entire growth cone including those regions not in direct contact with the new substrate. These studies demonstrate that growth cones are actively affected by the substrate, probably through a transmembrane signalling mechanism.

614.11

INTERFERENCE REFLECTION MICROSCOPIC (IRM) STUDY OF CHANGES IN GROWTH CONE-SUBSTRATUM CLOSE CONTACTS AT BOUNDARIES BETWEEN LAMININ AND FIBRONECTIN. T. M. Gomez* and P. C. Letourneau, The University of Minnesota, Minneapolis, Minnesota 55455.

Growth cones of chick dorsal root ganglia (DRG) neurons exhibit a variety of behaviors in culture at a boundary between glass-adsorbed laminin (LMN) and fibronectin (FN) (Gomez et al., Soc. Neurosci. Abst. 17:738, 1991). Growth cones that encounter LMN while migrating on FN usually cross onto LMN, often with an increased rate of neurite elongation and altered morphology. Reciprocally, growth cones that encounter FN while migrating on LMN often reorient their direction of migration and maintain association with LMN. It is not known whether differences in substratum adhesion contribute to the behavior of DRG growth cones at LMN-FN boundaries.

To address this question, growth cone contact with LMN and FN treated substrata was assessed using IRM. Our results confirm a previous report that DRG growth cones express closer contacts when growing on FN than on LMN (Gundersen, J. Neurosci. Res. 21:298-306, 1988). A new finding, however, using time-lapse IRM, was that the nature of contacts of a single growth cone changes rapidly as it passes from one substratum onto another, such that a growth cone in contact with both substrata can show two patterns of attachment. Growth cones accelerate and expand their lammelipodia as they migrate onto LMN from FN and simultaneously lose many close contacts, acquiring an IRM pattern typical for migration on LMN alone. Alternatively, growth cones that extend onto FN from LMN increase their total area of close contact and acquire an IRM pattern typical of migration on FN alone.

These data suggest that: 1) the extent of adherence of a growth cone to LMN or FN does not necessarily predict the choice of substratum and; 2) the pattern of close contacts expressed by a growth cone, as revealed by IRM, can change locally as a growth cone extends from LMN onto FN or from FN onto LMN. Supported by NIH grants HD19950 (PCL,TMG) and EY07133 (TMG).

REGENERATION V

615.1

EXPRESSION OF L2/HNK-1 IN REINNERVATED PERIPHERAL NERVE. T.M.Brushart*, R.Martini, and M.Schachner. Depts. of Orthopaedics & Neurology, Johns Hopkins, Balt., MD, and Swiss Federal Institute, Zurich, Switz.

The cell adhesion molecule-associated carbohydrate epitope L2/HNK-1 is abundant in peripheral motor nerve, but scarce in sensory nerve (Martini et al'92). These experiments evaluate the expression of L2 by denervated motor and sensory pathways after reinnervation by motor or sensory axons. Surgeries were performed on the femoral nerves of adult C57Bl6 mice, in which motor and sensory axons intermingle proximally, but are segregated distally into sensory and motor branches. Six groups were prepared: 1) distal sensory and motor branch repair 2) crossed distal repair, sensory to motor, motor to sensory 3) proximal trunk repair 4) correct intercalated graft-sensory graft in sensory branch, motor graft in motor branch 5) incorrect intercalated graft-sensory in motor, motor in sensory. Sensory axons did not induce L2 expression in sensory or motor pathways. Motor axons induced L2 in some sensory pathways, but correctly reinnervated motor pathways expressed L2 more vigorously. Regenerating axons thus alter pathway characteristics, yet pathways retain evidence of their previous axonal associations. The strong interaction of motor axons with old motor pathways suggests that L2 expression is associated with preferential reinnervation of motor pathways by regenerating motor axons (Brushart '88,'90).

615.2

EXPRESSION OF mRNAs FOR NGF, BDNF, NT-3, *trk*, *trk*B, AND *trk*C IN SPINAL CORD SCAR TISSUE IN THE ADULT RAT AND CAT. Frisén, J., Verge, V.M.K., Cullheim*, S., Fried, K., Hökfelt, T., and Risling, M. Departments of Anatomy, and Histology and Neurobiology, Karolinska Institute, S-104 01 Stockholm, Sweden.

Lesioned CNS axons sprout into scar tissue formed at the injury, but fail to regrow for any significant distance. It is not fully understod by which mechanisms the scar tissue assists sprouting. We have examined expression of neurotrophins and their receptors in two different types of spinal cord lesions, with in situ hybridization and immunohistochemical techniques. In adult rats the dorsal funiculus was cut at the mid-thoracic level and a longitudinal incision was made rostral to the transection in order to extend the lesion. In adult cats a longitudinal incision was made in the lumbar ventral funiculus which results in intramedullary axotomy of motoneurons. The scar tissue that forms after these lesions is known to assist and sustain longterm axonal sprouting and lack blood-brain barrier function. Elevated expression of mRNA for nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3) was seen predominantly around the border of the scar and in surrounding neuropil and white matter, after both types of lesions, with some expression within the dorsal funiculus scar tissue. In the ventral funiculus lesioned animals labelled cells were also seen in the motor nucleus neuropil. Low affinity NGF-receptor (LNGFr) mRNA was increased in the scar tissue after both types of lesions, and immunoelectron microscopy localized LNGFr-immunoreactivity mainly to axons and perivascular cells. Preliminary results regarding localization of mRNAs for the *trk*-family show all three *trk*s (*trk*, *trk*B, and *trk*C) to be increased in the scar border, neuropil and white matter ipsilateral to the lesion. In addition, both full-length and truncated forms of *trk*B were also abundantly expressed within the scar tissue. The expression of mRNA for these trophic factors and receptors could be of importance for the permissiveness to axonal sprouting in spinal cord scar tissue. Macrophages which have admittance to the scar tissue, in the absence of blood-brain barrier, may be involved in the regulation of the studied mRNAs.

615.3

CNS SCARRING IN DEVELOPING AND MATURE ANIMALS EXAMINED WITH ANTIBODIES TO GFAP, G-CAM, NCAM AND THE 11-59 AND TED15 ANTIGENS. J.D. Peduzzi*, T.B. Grayson, and E.E. Geisert, Jr. Department of Physiological Optics (J.D.P.) and Department of Cell Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294.

A major barrier to CNS regeneration is the glial scar that forms following injury. Astrocyte maturation correlates with the lack of axonal regeneration. We used several antibodies to examine scar formation in developing rat brain.

Cortical lesions were made on postnatal day 2 (P2), P9, P18 and adult rat. The animals were deeply anesthetized and sacrificed 10 days later. Brains were processed for immunohistochemistry using antibodies to GFAP; NCAM; G-CAM (a recently identified adhesion molecule - Geisert,E.E., T.P. Murphy, M.H. Irwin & H. Larjava, NS Lett. 133:262, 1991); 11-59 antigen (involved in neuron-glia interactions); and TED15 antigen (a brain-specific keratan sulfate proteoglycan - Geisert, E.E., R.C. Williams & D.J. Bidanset, Brain Res. 571:165, 1992).

A scar was consistently observed following a stab wound at P9 or older. Injuries at P2 gave variable results: some animals formed scars and others had no reactive gliosis. Intense G-CAM and TED15 but little NCAM or 11-59 staining was found at the scar's edge. This suggests that G-CAM and TED15 are involved in CNS response to injury. Supported by Spinal Cord Society & Whitehall Foundation.

615.4

NGF RECEPTOR EXPRESSION ALONG THE PNS/CNS DORSAL ROOT AXON PATHWAY. A.M. Avellino, K. C. Andrus, and M. Kliot*. Dept. of Neurological Surgery, Univ. of WA. and Seattle VAMC, Seattle, WA. 98108.

Expression of NGF and its low affinity receptor (NGFr) by Schwann cells increases dramatically in peripheral nerve distal to an axonal injury and then decreases back to negligible baseline levels following successful axonal regeneration (Johnson & Taniuchi, 1987). In contrast, oligodendrocytes and astrocytes do not express NGFr and also either inhibit or do not support comparable axonal growth (Snider & Johnson, 1989). These findings suggest that the expression and distribution of NGFr may explain, at least in part, the preferential ability of the adult mammalian PNS to promote axonal growth as compared to the CNS. To test this hypothesis, we have studied the expression of NGFr along the PNS/CNS pathway traversed by dorsal root sensory fibers.

Using a polyclonal antibody to the NGFr (provided by M. Bothwell), we have studied its expression both normally and following dorsal root transection. Tissue was cut in the parasaggittal plane so as to visualize the dorsal root and its entry zone (DREZ) as well as the spinal cord in the same section. Immunohistochemical staining has demonstrated the following findings: 1) NGFr is normally present, at low levels, in the PNS but not the CNS portion of the dorsal root pathway with an abrupt transition occuring at the DREZ, 2) ventral roots demonstrate very little if any NGFr staining normally, and 3) following dorsal root transection, NGFr staining increases dramatically in its PNS but not CNS portion. These results suggest that the absence of NGFr expression within the CNS portion of the dorsal root pathway following axonal injury may contribute to the regenerative failure observed following a dorsal root crush injury (Liuzzi & Lasek,1987/Stensaas et al.,1987/Kliot et al.,1990).

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615.5

CHANGES IN EXPRESSION OF JUN AND KROX PROTEINS AND IN NITRIC OXIDE METABOLISM FOLLOWING TRANSECTION OF THE MEDIAL FOREBRAIN BUNDLE IN THE RAT CNS. T. Herdegen , S. Brecht, R. Bravo[1] and M. Zimmermann, II. Physiol. Institut, D-6900 Heidelberg; [1]Bristol-Myers Squibb Pharm. Inst, Princeton, USA.

In anaesthetized SD rats, the medial forebrain bundle (MFB) was unilaterally transected by a 1 mm blade via craniotomy in a stereotaxic frame at Bregma -2.0 and expression of JUN, FOS and KROX nuclear proteins was assessed by immunocytochemistry. This resulted in axotomy of neurons located in the medial thalamus, ncl. parafascicularis of thalamus, ncl. mammilaris, ventral tegmentum and substantia nigra. Neuronal cell nuclei in these areas showed an ipsilateral expression of c-JUN, JUN D and KROX-24 (syn. Zif/268, EGR-1, NGFI-A). The proteins appeared within 12-24 h and had a maximal expression after 48 h. JUN D and KROX-24 declined after 30 days whereas c-JUN still persisted on a submaximal level for up to 60 days (the longest investigated period). c-FOS, FOS B, JUN B and KROX-20 (syn. EGR-2) proteins were not induced. Further investigations will elucidate dependence of expression on period of survival, age and length of the proximal nerve stumps. MFB lesion also induced changes in the nitric-oxide metabolism in axotomized neurons such as increase of activity of nitric-oxide synthase. Supp. by DFG.

615.6

ACETYLCHOLINESTERASE IN DENERVATED AND REGENERATING FROG MUSCLES. B. Haesaert and L. Anglister*. Anatomy Dept., Hebrew Univ. Med. Sch., Jerusalem 91010, Israel.

Acetylcholinesterase (AChE) in muscle appears as globular (G) and asymmetric (A) molecular forms, differing in structure, solubility and location. The enzyme is highly concentrated at neuromuscular junctions. Our studies, carried out on frog muscles, were aimed to determine the effect of denervation on AChE produced by myofibers. In cutaneus pectoris muscles the major globular forms, distinguished by their sedimentation coefficients, are 4-6S AChE (G_1, G_2, 50-60%) and 10.8S (G_4, 10-15%). The major asymmetric form is a 17.5S AChE (A_{12}, 15-20%). Following denervation and irreversible inactivation of all original AChE in the muscles, the myofibers have newly produced AChE at junctional areas, that was predominantly G_4-AChE. The A_{12} form was still produced at 2 wks, but was practically undetectable at 4 wks after denervation. Histochemical examination showed no appearance of active AChE on the myofiber surface at the denervated synaptic sites. However, in contrast, regenerating myofibers showed production of high concentrations of A-AChE at 4 wks after denervation combined with muscle damage. Moreover, active newly made AChE accumulated on the surface at the denervated synaptic sites. The results show that denervation causes inhibition of the production and/or assembly of A-AChE and of the appearance of the synaptic AChE. This inhibition does not occur if muscles are regenerating. In all cases the level of intacellular G4-AChE is elevated, suggesting its possible role as precurser or degradation product. (Supported by grants from BSF and Bruno Goldberg Foundation).

615.7

CHRONIC NERVE LIGATION LEADS TO ENHANCED $GABA_A$ RECEPTOR-INDUCED CONDUCTANCES IN A SUBCLASS OF RAT DORSAL ROOT GANGLION NEURONS. D.L. Eng*, G.B. Richerson, J.D. Kocsis, Dept. Neurology, Yale Med. Sch., New Haven, CT. 06510; and VAMC, W. Haven, CT. 06516.

Chronic ligation of the rat sciatic nerve elicits both a reduction in primary afferent depolarization and a decrement in GABA-induced depolarization of the L4 and L5 dorsal roots. The aim of the present study was to examine electrophysiological properties of $GABA_A$ receptors on dorsal root ganglion (DRG) neurons following chronic axonal injury. Sciatic nerves were disconnected from their targets by ligation and transection. L4 and L5 DRG neurons were cultured in 2-4 wks. Whole cell patch clamp recordings were obtained from 20-42 μm neurons within a day after plating. Patch electrodes (1-3 Mohm) were made from borosilicate glass and filled with 140 mM KCl-based solution. GABA (100 μM) was applied to the entire cell body by rapid pressure microinjection to capture the peak response prior to desensitization. GABA-induced current was measured from voltage clamp experiments using test potentials of -60, -80, -100 mV (>80% series resistance comp.) to calculate slope conductances. In neurons with diameters of 34-42 μm, whole cell conductances of 116 ± 111 nS(n=31) and 446 ± 295 nS(n=26) ($p < 0.005$) were recorded from control and ligated groups, respectively. Muscimol elicited a similar response as GABA, and the responses were blocked by bicuculline. There was no difference in GABA-mediated conductance between control and ligated groups in the 20-32 μm neurons. All injured neurons with large GABA conductances had short duration action potentials with no notable inflections. Control neurons had a variety of action potential types. These data indicate that peripheral nerve ligation leads to a substantial increase in $GABA_A$ induced inward current in a subset of DRG neurons (34-42 μm dia). Given that dorsal root axonal GABA-sensitivity is reduced following a similar nerve injury, it is suggested that GABA receptor synthesis may continue after chronic nerve injury, but that receptor transport is impaired to the central terminals. Supported by the VA and NIH.

615.8

NEURONAL INDUCTION OF A PC12 NEURITE PROMOTING ACTIVITY. L.M. Bolin* and E.M. Shooter, Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Peripheral nerve Schwann cells provide trophic support to regenerating neurons in injured sciatic nerve. An *in vitro* paradigm in which cells could interact through diffusible substances but not through cell contact was designed to study the neuron-Schwann cell trophic milieu. Adult rat Schwann cells isolated 30hr post-crush were cocultured with chick E8 dorsal root ganglion neurons. Neurons respond to this coculture by neurite extention. The neurite promoting activity present in the coculture conditioned medium (CM) can be transferred to naive DRG cells and elicit a similar neurite extension. This was true for E8 retina and PC12 cells but did not occur when conditioned media (CM) from cocultures with E8 liver or L cells was transferred. The same results were obtained when iSC cells (a spontaneously immortal Schwann cell clone) were cocultured. CM from primary Schwann cells or iSC grown alone has no effect on PC12 cells. The fact that this activity results from neuron-Schwann cell coculture suggests a neuronal induction. Antisera raised against NGF did not inhibit the PC12 neurite extension in response to 20% iSC-PC12 coculture CM. Incubation of the coculture CM with heparin sepharose resulted in a loss of activity that could be restored by elution in 1M NaCl. This heparin binding is characteristic of FGF-1 (acidic FGF) which promotes neurite extension in PC12 cells. It is tempting to speculate that it is the neuron-stimulated Schwann cells which produce this heparin-binding neurite promoting activity, because these Schwann cells were isolated from the injured sciatic nerve and may be maximally responsive to neuronal signals.

615.9

RAPID AND EXTENSIVE GROWTH OF NEURITES ACROSS LESIONS OF IMMATURE MAMMALIAN SPINAL CORD IN CULTURE. J.M. Treherne*, G. Knott, J.G. Nicholls and N.R. Saunders Biocenter, University of Basel, CH-4056 Basel, Switzerland

The isolated central nervous systems of newly born opossums and 15-day rat embryos survive in culture for 7 days or longer. Our earlier work has shown that lesions to the spinal cord *in vitro* are followed, after 3-5 days, by restoration of conduction and by profuse fiber outgrowth. Experiments have now been made in which we follow with video microscopy the trajectories of axons stained by the carbocyanine dye (DiI) in living preparations. By 2 days after injury, neurites entered and traversed the lesion; by 4-days, neurites were observed several mm beyond the crush. These morphological measurements correlate well with the restoration of conduction measured by recording electrically from injured preparations. With the CNS *in vitro* it is possible to analyze how the rate and the pattern of growth are influenced by such factors as: the age of the animal, the extent of myelination, the level of electrical activity and the chemical environment. Our results demonstrate the dramatic repair of CNS that can be accomplished at early stages of development, compared with that seen in older animals. Supported by a grant from the Swiss National Fund No. 31-30047-90.

615.10

THE INFLUENCE OF SCHWANN CELLS ON EARLY PERIPHERAL NERVE REGENERATION STUDIED BY CHRONICALLY IMPLANTED ELECTRODES IN THE CAT. K. Fugleholm*, C. Krarup, Dept. of Neurophysiology, Inst. of Med. Physiology, Panum Inst., Univ. of Copenhagen, Copenhagen, Denmark, DK 2200 N.

I 21 cats, implanted silicone cuff and patch electrodes with multiple contacts were used to examine the influence of Schwann cells on the rate of elongation after Wallerian degeneration. The right tibial nerve was crushed or cut and resutured and in the other leg in addition frozen for 20 mm distal to the lesion. Regeneration was followed by weekly recordings of electrically evoked responses from the axonal sprouts until reinnervation of the plantar muscles occurred after 42-84 days. The action potentials from single regenerating fibers which had conduction velocities and amplitudes of 0.5-3 m/s and 0.15-0.5 μV respectively. The spatial relation of the regenerating axons to the stimulation electrodes was examined by electron microscopy showing the presence of axons when an action potential was recordable. The serial electrophysiological observations suggested that the rate of elongation was 3-4 mm/day after crush alone and similar after crush+freezing. However after section and resuture, freezing caused an initial phase of very slow regeneration suggesting that the presence of Schwann cells was critical when the continuity of bands of Büngner had been disrupted. After the initial slow phase, the elongation rate seemed to be independent of freezing.

615.11

ABDUCENS INTERNUCLEAR NEURONS SURVIVE AND REGAIN NORMAL DISCHARGE CHARACTERISTICS FOLLOWING LOSS OF THEIR TARGET MOTONEURONS. R.R. de la Cruz, A.M. Pastor and J.M. Delgado-García* Lab. de Neurociencia. Univ. de Sevilla, 41012-Sevilla, Spain.

The highly specific projection of abducens internuclear neurons (ABD Ints) onto the contralateral medial rectus motoneurons (MR Mns) offers a good model to evaluate the morpho-physiological consequences of target removal on adult CNS neurons. MR Mns were killed by the injection of a cytotoxin into the MR muscle. Following target removal, the overall firing activity of ABD Ints appeared markedly reduced. The neuronal sensitivity to both eye position and velocity showed values significantly lower than controls. ABD Ints also showed a significant reduction in the amplitude of their excitatory and inhibitory synaptic potentials of vestibular origin. However, after this initial period, ABD Ints exhibited, from one month up to one year, discharge properties and synaptic potentials that did not differ from controls. A morphological study demonstrated the absence of cell death in the ABD Ints population, but a progressive decrease in the density of their axonal terminals within their normal area of distribution. Therefore, these adult CNS neurons survive long-term target removal with the maintenance of appropriate physiological signals.

615.12

TOWARDS PULSE PROCESSING NEURAL COMPUTERS FOR BIDIRECTIONAL COMMUNICATION WITH THE NERVOUS SYSTEM.
R. Eckmiller* and M. Jansen. Dept. Biophysics, University of Düsseldorf (FRG)

Monitoring and control of signal time courses of the human nervous system becomes increasingly important for diagnostic and functional repair purposes (e.g.: cochlear implants, EEG, FES). However, recording from or stimulation of nervous tissue currently does not allow for an adequate communication with the nervous system as an information processing structure by means of multiple spike trains at the single neuron level.

Neurotechnology is gradually emerging for development of multicontact neural interfaces (MNI) and adaptive neural computers (ANC) as the two key components for communication with the nervous system by means of asynchronous spike trains via a multi-channel (with 10^2-10^3 single neuron contacts) neural interface.

This paper reports on the development and test of an ANC prototype consisting of 32 electronic neuron analogs and 64 synapse analogs with adaptive weights and adaptive delays (Jansen et al. In: 2nd Int. Conf. Microel. of Neural Netw. Munich, Oct. 91). In brief, each of the biology-inspired neuron analogs generates single impulses (1 ms) once its membrane potential (as definded by the asynchronous input of EPSPs and IPSPs from corresponding synapses) reaches the adjustable threshold. In conjunction with a PC to monitor neural parameters in real time and to specify various initial network topologies (via electronic switch arrays), a number of selected applications for pattern recognition, adaptive filtering, and motor control in real time have been successfully tested. With these experiments, some main features of weight- and/or delay adaptation with embedded learning rules (using 'training neurons' and presynaptic synapses) were studied.

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DEGENERATIVE DISEASE: ALZHEIMER'S—β-AMYLOID VII

616.1

IDENTIFICATION AND ANALYSIS OF AN AMYLOID PRECURSOR-LIKE PROTEIN LOCALIZED ON CHROMOSOME 19 W.Wasco, B.T.Hyman*, and R.E.Tanzi, Dept. of Neurology, MGH, Boston, MA.

We have isolated a cDNA from a mouse brain library that encodes a protein whose predicted amino acid sequence is 42% identical to the amyloid β protein precursor (APP). This 653 amino acid amyloid precursor-like protein (APLP) is similar to the APP and the *Drosophila* APPL genes in overall structure and amino acid sequence. The strongest homology occurs in the cysteine-rich, acidic-rich, glycosylated, and cytoplasmic domains. These data suggest that APP is part of a highly conserved gene family. The APLP cDNA hybridizes to two messages of approximately 2.4 and 1.6 kb that are present in mouse brain and neuroblastoma cells. APLP has been mapped to human chromosome 19q13.2-cen, the same region in which a late-onset familial Alzheimer disease (FAD) locus has been provisionally assigned. In situ hybridization and immunohistochemical studies show APP and APLP to be expressed in similar human brain regions and cell subpopulations. We are currently testing APLP as a candidate gene for late-onset FAD by standard genetic linkage and mutation analysis. Additionally, we are screening the human genome for other spliced forms of APLP and additional members of the APP gene family.

616.2

TRANSMITTER DEPENDENT INDUCTION OF β-APP mRNA. V. Haroutunian*, S.T. Ahlers[1], P.A. Shea[1], N. Girenkova, K.L. Davis, and W.C. Wallace. The Mount Sinai School of Medicine, NY and [1]Naval Medical Research Institute, Bethesda, MD.

Previous studies have indicated that lesions of the nucleus basalis of Meynert (nbM) induce β-APP synthesis and β-APP mRNA in the ipsilateral cortex. The induction is rapid (within 1hr) and long-lasting (at least 45 days). We now report on the nature of the stimulus which triggers the β-APP response. Anesthetized rats were implanted with cannulae directed at the nbM and received an infusion of 20% lidocaine. In some of the rats cortical acetylcholine (ACh) release was measured by **in vivo** microdialysis prior to, during, and for 90 minutes following lidocaine infusion. These rats were then sacrificed. The remaining rats were re-anesthetized one week later and recovery of ACh release was measured without further lidocaine infusions. Infusion of lidocaine into the nbM led to a rapid reduction (approximately 50%) in cortical ACh release. ACh release had returned to pre-lidocaine infusion levels when measured one week later. Analysis of cortical β-APP mRNA by Northern blot indicated that β-APP was induced immediately after lidocaine infusion when cortical ACh release was reduced by approximately 50%, but not one week after lidocaine infusion when cortical ACh release had normalized. These results indicate that β-APP mRNA is reversibly induced when cortical transmitter levels drop below a certain threshold and provide a possible mechanism for β-APP induction in neurodegenerative disease.

616.3

TWO AP-1 SITES ARE NECESSARY FOR THE PROMOTER ACTIVITY OF APP GENE IN ASTROCYTE CELLS. D. K. Lahiri* and C.Nall. Lab of Molecular Neurogenetics, Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN-46202

Beta-protein (4.2kDa molecular weight) is generated by proteolytic cleavage of larger amyloid precursor proteins (APP) encoded by a gene on chromosome 21. The apparent over expression of the APP gene in certain areas of the brain in Alzheimer disease indicate that abnormalities in gene regulation might be an important factor in the neuropathology. The control of transcription is mediated by different DNA regulatory elements (*cis-acting*) present in the promoter of the gene. There are about 26 DNA motifs, present in the 5'-flanking region of the APP gene, through which various cell-type specific factors (*trans-acting*) can exert their influence on transcription. Recombinant plasmids containing different parts of the promoter region of the APP gene linked to a reporter gene were constructed. Sequences derived from the 5'-flanking region were tested for their effects on the promoter activity by transient expression in PC12 (chromaffin), IMR-32 (neuronal) and C6 (astrocyte) cells. The amyloid gene promoter has two sites, one at -345 and one at -38, which contain a consensus sequence recognized by transcription factor AP-1/*fos*. Since this factor is induced by phorbol esters and retinoic acids, their effects on the activity of the APP gene promoter were examined. A differential stimulation in the basal activity of the promoter was observed in the cell extracts prepared from the phorbol-ester treated astrocyte and neuronal cells. The deletion plasmids were used to determine if the two AP-1 sites act independently or cooperatively. Results of three separate transfections, one using a plasmid containing both AP-1 sites and two others using deletion plasmids containing either of the two sites, suggest that both AP-1 sites are essential for the stimulation in C6 cells. Gel shift and DNase I foot printing assays are being employed to characterize the trans-acting factors in C6 and IMR cells. Thanks to Drs. B. Ghetti, H. Hendrie, J. Nurnberger and N. Robakis.

616.4

CHARACTERIZATION OF A NUCLEAR FACTOR BINDING DOMAIN WITHIN A REGION OF THE APP PROMOTER THAT IS ESSENTIAL FOR ITS ACTIVITY.
W.W. Quitschke* and D.Y. Goldgaber, *Department of Psychiatry, State University of New York at Stony Brook, Stony Brook, NY 11794-8101.*

The major component of amyloid depositions is the amyloid beta protein, which is a truncated form of the larger amyloid precursor proteins (APP). The promoter of the APP gene was analyzed for its ability to direct cell type specific expression. The APP promoter and selected deletions were placed 5' to the reporter gene chloramphenicol acetyl transferase. The promoter deletions were transfected into different cell lines that showed variant levels of endogenous APP transcripts. Transient transfection assays showed that 96 base pairs 5' to the transcriptional start site are sufficient for full cell type specific promoter activity.

A nuclear factor that binds to this region in a sequence specific manner was identified by mobility shift electrophoresis, DNase footprinting, and methylation interference. The DNase protected domain extends from position -31 to -51 upstream from the main transcriptional start site (+1). Mutations within this domain revealed a sequence of twelve base pairs that is crucial for factor binding. This sequence overlaps with the consensus sequences for transcription factors AP-1 and AP-4. However, competition experiments suggest that the nuclear factor that binds to the APP promoter is distinct from both AP-1 and AP-4. In addition, factor binding to the characterized recognition sequence is observed in nuclear extracts originating from human, mouse, and rat cells, suggesting a high degree of conservation.

616.5

REGULATION OF APP SPLICING AND NEUROTROPHIN LEVELS IN THE ADULT RAT HIPPOCAMPUS BY RETINOIC ACID. T. Giordano*, J. B. Pan, L. Monteggia, and S. Watanabe. Neuroscience Research, Abbott Laboratories, Abbott Park, IL 60064.

It has recently been reported that retinoic acid (RA) is capable of increasing the levels and altering the splicing ratio of APP in cultured SH-SY5Y cells. We have observed a similar effect in these cells. To determine whether this effect is present in vivo, aged (20-22 month old) male Wistar rats were injected (i.p. at 1 ml/kg body weight, q.d.) with saline, vehicle (DMSO), or low (64 µg/kg body weight) or high (640 µg/kg body weight) dosages of RA in DMSO for 2 weeks. The abundance of each of the three major APP species and a control RNA, cyclophilin, were measured by rtPCR. In the saline injected rats, APP-695 represented approximately 90% of the total APP measured. DMSO treated rats exhibited a 10X increase in total APP ($p<0.005$) relative to cyclophilin and an increase in the level of APP-695 to 94% of the total APP. Treatment of RA in DMSO decreased the accumulation of total APP relative to cyclophilin at both the low (4X; $p<0.01$) and high (5X; $p<0.05$) dosages when compared to DMSO treated rats. Furthermore, the level of APP-695 decreased to 82% with low dosage of RA and 75% at high dosage of the total APP transcripts. In addition we did not detect a significant change in either NGF, NT-3, or BDNF transcripts following low or high dosage RA administration relative to cyclophilin RNA nor did we observe a change in ChAT activity at either of the dosages tested. In conclusion, the effects of RA on APP RNA observed in vivo cells are not indicative of changes in cultured SH-SY5Y.

616.6

A RAT MODEL TO STUDY THE EFFECTS OF BAP-CONTAINING AMYLOID IN BRAIN. A.D. Snow, R. Sekiguchi, D. Nochlin*, K. Kimata, W.A. Schreier and D.G. Morgan. Dept. of Neuropath., Univ. of Washington, Seattle, WA 98195; Inst. for Molecular Science of Medicine, Aichi Med. Univ., Japan & Div. of Neurogerontology, USC-Los Angeles, CA 90089.

Accumulation of amyloid containing beta-amyloid protein (BAP) in the brain is a diagnostic feature of patients with Alzheimer's disease. An animal model is needed to study the formation and subsequent consequences of amyloid deposition in the brain. In the present study, 3 month old Sprague-Dawley rats (4 animals/group) received continuous infusion (for 1 week) directly into hippocampus of 1) saline, 2) BAP (residues 1-40), 3) different amyloid plaque co-components, or 4) BAP (1-40) plus different amyloid plaque co-components. Congo red and thioflavin S positive extracellular amyloid deposits were observed only in the group given BAP (1-40) plus a specific amyloid plaque co-component. This study therefore provides a rapid in vivo animal model to study amyloid accumulation in brain, and further indicates that a specific co-component in necessary for amyloid accumulation and its persistence in brain. Support by NIH grants #AG05136, #AG7892, Ad Res.Prog.of the Am. Health Fdn, the French Fdn. for AD Res.,& Gliatech Inc.

616.7

TRANSGENIC MOUSE STUDIES OF ALZHEIMER AMYLOID PRECURSOR (AAP) PROTEINS AND DERIVATIVES. B.D.Greenberg[1]*, S.M.Ali[1], S.Siedlak[2], P.A.Gonzalez-DeWhitt[1], S.Kuentzel[1], R.A.Altman[1], H.G.Polites[1], J.M.Glendening[1], D.E.Lowery[1], P.Cras[3], T.J.Raub[1] and G.Perry[2]. [1]The Upjohn Company, Kalamazoo, MI 49001; [2]Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106; [3]Born-Bunge Foundation, University of Antwerp, Belgium.

We are attempting to generate transgenic mouse models for Alzheimer-type amyloidogenesis based on overexpression of AAP protein derivatives in appropriate brain regions. A C-terminal AAP-695 segment containing the entire β protein domain (Glu593-Asn695) has been expressed under the control of a chimeric metallothionein-growth hormone promoter system (Swanson et al. Nature **317**:363 [1985]), with AAP sequences replacing the growth hormone coding sequence described in that paper. This transgene is abundantly expressed in transfected HeLa cells, producing novel dense inclusions associated with the endoplasmic reticulum. In extracts of whole brain, Northern analyses reveal that transgene mRNA levels exceed those of the endogenous mRNA up to six-fold. *In situ* hybridization indicates that the transgene is expressed in pyramidal neurons within cortex and hippocampus. Immunocytochemical analyses with several antibodies to AAP epitopes N-terminal to Glu593, reveal elevated intraneuronal accumulation of endogenous AAP protein in these brain regions in the transgenic mice. In cortex, this immunostaining is significantly more abundant than in control mice and nontransgenic littermates, with p values ranging from <.05 to <.0001 depending upon the antibody used. These mice may therefore provide useful models to study the effects of chronic AAP overexpression on brain pathology and amyloid formation.

616.8

MORE TRANSGENIC MOUSE STUDIES OF ALZHEIMER AMYLOID PRECURSOR (AAP) PROTEINS AND DERIVATIVES. S.M.Ali, P.A.Gonzalez-DeWhitt, R.A.Altman*, T.M.Pohlad, H.G.Polites, S.Kuentzel, T.J.Raub and B.D.Greenberg. The Upjohn Company, Kalamazoo, MI 49001.

We are attempting to generate transgenic mouse models for Alzheimer-type amyloidogenesis based on overexpression of AAP protein derivatives in appropriate brain regions. The mouse AAP gene promoter has been used to direct the expression of the full length human AAP-695 cDNA, and the region encoding the N-terminal 639 residues. The latter contains the complete β-protein domain at its C-terminus. One set of constructs (MP-SAR) utilizes the 3'-untranslated region of rat growth hormone, and another (LBM-SAR) utilizes approximately 1500 bases of the mouse AAP gene 3'-untranslated region. Both sets of transgenes are abundantly expressed in transfected HeLa and mouse LTK- cells. Media conditioned by HeLa cells expressing the MP-SAR constructs contain in excess of 200 ug AAP protein per liter. AAP production by LBM-SAR transfectants has not yet been quantitated. Transgene expression has been revealed by Northern blot analysis of whole-brain homogenates from MP-SAR mice in several independent founder lines. *In situ* hybridization analyses are underway. Microinjections of LBM-SAR constructs into fertilized mouse oocytes have just begun. The current status of these studies will be reviewed.

616.9

EFFECTS OF ALZHEIMER-ASSOCIATED MUTATIONS ON A HUMAN BRAIN APP RNA-BINDING PROTEIN: . R.E.Tanzi*, W.H.Pettingill, W.Wasco, and B.T.Hyman. Dept. of Neurology, Mass. General Hospital, Boston, MA 02114.

Three single basepair substitutions in exon 17 of the APP gene have been reported in affected individuals of ten families with familial Alzheimer's disease. All three mutations are predicted to destabilize a stem-loop structure in the APP mRNA which occurs in the 3' end of the sequence encoding the amyloidogenic βA4 domain (nucleotides 1906 and 1924; APP695 sequence). The stem-loop resembles iron-responsive elements (IREs) present in the 5' and 3' untranslated regions of the mRNAs for ferritin and transferrin receptor, respectively. The stem-loops in these messages control translation in response to iron concentration via RNA-binding proteins. We have shown that the IRE-like stem-loop in APP message binds a novel human brain RNA-binding protein. Preliminary studies indicate that this binding is abolished when the APP717(V->I) is introduced into the stem. We are also testing the effects of the other two mutations on the RNA-protein interaction. Data will be presented on our attempts to isolate and clone the APP RNA-binding protein. Additionally, the effects of the Alzheimer disease mutations on APP mRNA stability and translation are being assessed.

616.10

BIOLOGICAL ACTIVITY OF THE AMYLOID β/A4-PROTEIN PRECURSOR I: FUNCTIONAL MAPPING OF GROWTH PROMOTING ACTIVITY. J.-M. Roch*, H. Ninomya, D. Otero and T. Saitoh. Dept. of Neurosciences, University of California San Diego, La Jolla, CA 92093, USA.

The secreted form of the amyloid β/A4-protein precursor (APP) is involved in the growth regulation of fibroblasts (Saitoh et al., Cell 58:615-622, 1989). Recently, we have shown that the region of the secreted form of APP-695 necessary for this growth regulation is contained within a 40 amino acid domain which is adjacent and C-terminal to the KPI insertion site of APP-751. This active site begins at Thr296 and extends to Met335 (Roch et al., J. Biol. Chem. 267:2214-2221, 1992). To define more precisely the site of activity, we synthesized several peptides spanning the 40 amino acid domain. In addition, we also prepared bacteria-made APP-751 (secreted form), as well as mutant forms of APP-695 and -751 (secreted forms) . These deletion mutants were lacking the majority of the 40 amino acid domain. Each of the peptides, as well as the bacteria-made APP variants were tested in our biological assay on A-1 fibroblasts (a cell line that produces very low APP levels and is dependent on exogenous APP in the medium for normal growth). Our results narrow down the active site of the secreted form of APP-695 from the domain of 40 amino acids to a region of 17 residues starting at Ala319 and extending to Met335.

616.11

BIOLOGICAL ACTIVITY OF THE AMYLOID β/A4 PROTEIN PRECURSOR. II: NEUROTROPHIC EFFECT OF AN APP PEPTIDE ON A RAT BRAIN NEUROBLASTOMA CELL LINE.

L.-W. Jin*, E. Masliah, H. Ninomiya, J.-M. Roch, D. A. Otero, D. Schubert+, and T. Saitoh. Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093 and+ The Salk Institute for Biological Studies, San Diego, CA92138.

We previously demonstrated that secreted forms of amyloid β/A4 protein precursor (APP) promote growth of fibroblasts (Cell, 58:615, 1989), and adhesion of PC12 cells to substratum (Neuron, 3:689, 1989). The growth promoting activity of APP is fully represented by a synthetic 17-mer peptide corresponding to Ala319-Met335 of APP-695 (Roch et al., reported in this meeting). In order to investigate the effect of the 17-mer APP peptid on neuronal cells, we have chosen B103, a neuronal cell line derived from rat brain that does not express APP. The neuronal phenotype of B103 has been documented (Nature, 249:224, 1974). We found that the rat CNS neuroblastoma cultures treated with 17-mer APP peptide for 20 hours had longer neurites and more neurite-bearing cells. The significant effect was seen at 10 nM to 200 nM, with maximal effect at 100 nM. The peptide with sequence reverse to that of 17-mer did not have any effect at the same concentration range. This result demonstrates that a small stretch of sequence in the secreted form of APP-695 has both growth promoting and neurotrophic activities.

616.12

BIOLOGICAL ACTIVITY OF THE AMYLOID β/A4-PROTEIN PRECURSOR. III: BINDING OF IODINATED 17-MER PEPTIDE (ALA319-MET335 OF APP-695) TO RAT NEUROBLASTOMA (B103) CELL MONOLAYER.

H. Ninomiya, L.-W. Jin, J.-M. Roch, D. Otero and T. Saitoh.* Dept. of Neurosciences, University of California San Diego, La Jolla, CA 92093, USA.

A synthetic 17-mer peptide corresponding to Ala319-Met335 of APP-695 has a neurotrophic effect on rat neuroblastoma (B103) cells (L.-W. Jin et al., reported in this meeting). As an initial attempt to find out a possible mechanism for this effect, we employed the binding assay of ^{125}I-17-mer on B103 cell monolayer. (1) The binding was time-dependent and saturable with Kd values of 20±5 nM and Bmax values of 80±8 fmole/10^6 cells (mean±SEM, n=4). (2) Both unlabeled 17-mer and secreted form of APP-695 fully displaced the binding while reverse-sequence 17-mer did not. (3) C-terminal 11 amino acid-long peptide of the 17-mer fully displaced the binding while N-terminal 8 amino acid-long peptide did not. (4) Neither the addition of heparin nor heparan sulfate in the assay medium had any effect on the binding. These results suggest the presence of novel binding sites on B103 cell monolayer recognized by a specific domain of the secreted form of APP.

REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS

617.1

DISTRIBUTION OF MELATONIN RECEPTORS IN THE BRAIN AND RETINA OF THE LIZARD.

A.F. Wiechmann* and C.R. Wirsig-Wiechmann. Depts. of Neurobiology & Anatomy and Ophthalmology, Bowman Gray School of Medicine, Winston-Salem, NC 27157.

Melatonin binding sites were identified in the brain and retina of the lizard *Anolis carolinensis* using *in vitro* autoradiography and computer-generated color imaging. Radioactive labelling was observed in areas which receive primary, secondary, and tertiary visual input: the superficial layers of the optic tectum, lateral geniculate nucleus, nucleus rotundus, dorsal ventricular ridge, striatum, and interpeduncular nucleus. Other areas that demonstrated binding included the medial habenular nucleus, medial cortex, dorsal cortex, mammillary nucleus, and septum. In the retina, melatonin binding was localized in the inner plexiform layer.

An asymmetry of melatonin binding was seen in the diencephalon: a high degree of melatonin binding was present in the left medial habenular nucleus, and no binding was observed in the habenulum on the right side of the brain.

This study demonstrates that melatonin receptor binding sites are widely distributed in the forebrain and midbrain of the lizard, and are very prominent in areas of the nervous system which are associated with visual processing. Since the light-sensitive parietal eye projects to the left medial habenulum in the lizard but not to the right habenulum, these observations suggest that the left habenulum is under dual control (neuronal and hormonal) of the parietal eye/pineal complex, and that melatonin may play a significant role in neural processing of visual information. *(Supported by NIH grants EY08006 and NS27586)*

617.2

EXPRESSION OF THE GLUCOCORTICOID RECEPTOR (GR) IN THE TREE SHREW BRAIN BY *IN SITU* HYBRIDIZATION.

O. Jöhren[1], H. Haack[2], G. Flügge[1], F.W. Schürmann[3]* and E. Fuchs[1]. [1]German Primate Center, [2]Max-Planck-Inst. Biophys. Chem. and [3]Zool. Inst. University, 3400 Göttingen, FRG.

In the brain, glucocorticoids act via two types of intracellular receptors, the mineralocorticoid and the glucocorticoid receptor (GR). Among other processes, hippocampal GRs are involved in the regulation of the HPA-axis activity. Studying the neurophysiological consequenses of psychosocial stress in tree shrews (*Tupaia belangeri*), we recently demonstrated degeneration of hippocampal pyramidal neurons in chronically stressed males probably due to elevated glucocorticoids (1). To better understand these processes on the cellular level, we cloned the tree shrew GR for constructing a probe for *in situ* hybridization histochemistry (ISHH). Tree shrew genomic DNA was amplified by polymerase chain reaction and DNA fragments were subsequently cloned. Dideoxy sequence analysis of the cloned DNA fragment revealed 92% (human) and 88% (rat, mouse) homology compared to the corresponding cDNA sequences. Linearized vectors containing the tree shrew GR DNA fragment were used to generate ^{35}S-labeled sense and antisense RNA probes. ISHH experiments with this specific probe showed high levels of GR-mRNA in the hippocampal formation. Currently we are investigating the regional distribution of GR-mRNA in the three shrew brain. These data will be the basis for further studies on regulation GR-numbers under chronic psychosocial stress.

1) H. Uno, G. Flügge, C. Thieme, O. Jöhren, E. Fuchs, Soc. Neurosci. Abstr. 17 (1991): 129

617.3

STRATEGIES FOR THE PRODUCTION OF EXTREMELY HIGH TITRE ANTISERA AGAINST SMALL NEUROTRANSMITTER MOLECULES.

D.V. Pow, D. K. Crook, I.C. Gynther* and D.I. Vaney. Vision, Touch and Hearing Research Centre, University of Queensland, Brisbane 4072, Australia.

We have devised a simple new technique which permits the rapid production in 1-2 months of extremely high titre polyclonal antisera against a wide variety of small molecules including the transmitters glycine, glutamate, and GABA. These antibodies are used routinely for post-embedding immunocytochemistry of resin-embedded sections at dilutions of 1 in a million, rather than a typical dilution of 1 in a thousand for conventionally prepared antisera, indicating that we have achieved a 100- to 1000-fold increase in titre. The high titres of these antisera, which are due to the dramatic expansion of lymphocyte clones secreting high affinity antibodies, have a number of distinct advantages, principally the dramatic reduction of non-specific background labelling. To achieve this result we made use of the observation that particulate antigens, of a size likely to be encountered by the immune system (such as viruses), are very effective antigens. We have coupled antigens to suitably sized gold particles, along with a non-specific stimulator of the immune system, muramyl dipeptide. This combination, when injected, elicits massive and rapid immune responses in all animals we have studied to date. We suggest that our methodology may have much more widespread application than indicated here; it should facilitate greater efficiency of monoclonal antibody production by virtue of expanding the lymphocyte clones of interest prior to fusion and may well, with appropriate modifications, represent a potent alternative modality for human immunisations.

617.4

DISTRIBUTION OF THE KAINATE CLASS GLUTAMATE RECEPTOR SUBUNITS (GluR5-GluR7) IN THE TEMPORAL CORTEX OF THE MACAQUE: LOCALIZATION TO IDENTIFIED PROJECTION NEURONS OF THE ENTORHINAL CORTEX.

P.F. Good[1]*, T. Moran[2], S.W. Rogers[3], S. Heinemann[4], and J.H. Morrison[1]; [1]Fishberg Rsrch. Ctr. for Neurobiology, and [2]Hybridoma Facility, Mt. Sinai Med. Ctr., NY, NY 10029, [3]University of Colorado Med. Ctr., Denver, CO. 80262, and [4]Salk Institute for Biological Studies, La Jolla CA 92037

Glutamate is considered to be the predominant excitatory neurotransmitter in the central nervous system. Specificity of synaptic circuitry and physiological response to this ubiquitous neurotransmitter is conferred by receptor diversity. We have generated monoclonal antibodies to the kainate (K) class (GluR5-GluR7) glutamate receptor subunits. Analysis of the distribution of the K class subunits in the temporal cortex of the cynomolgus monkey demonstrates that it is heavily concentrated in the hippocampus and entorhinal cortex. Antibodies to the K class subunits label the soma and dendrites of pyramidal neurons within all CA fields and subiculum, as well as the dendrites of the granule cells of the hippocampus. The soma of the granule cells are conspicuous by their absence of immunoreaction for the K subunits. In the entorhinal cortex, soma and apical dendrites of neurons within layers V and VI extending to superficial layers were prominently labeled while only the soma and sparse proximal dendrites of layer II and III cells exhibited labeling. This dense pattern of labelling was also present in the perirhinal region while the predominantly unimodal visual region of the inferior temporal cortex was sparsely labeled. The polymodal region within the superior temporal sulcus was heavily labeled as was the insula.

Neurons of layer V of the entorhinal cortex identified by retrograde labeling with fast blue as projecting to STS were filled with lucifer yellow. Double labelling with antibodies to the K class subunits has demonstrated that these neurons express these subunits. Immunoreactivity is present on the soma and primary dendritic shaft of these neurons but is excluded from distal dendrites. Supported by NIH grant AG06647 and American Health Assistance Foundation (AHAF).

617.5

DISTRIBUTION OF GLUTAMATE RECEPTOR SUBUNIT PROTEINS IN MONKEY NEOCORTEX. J.H. Morrison[1]*, J.C. Vickers[1], G.W. Huntley[1], P.F. Good[1], W.G. Janssen[1], N. Archin[1], T. Moran[2], S.W. Rogers[3], and S.F. Heinemann[4]; [1]The Fishberg Research Center for Neurobiology, and [2]Hybridoma Facility, Mount Sinai School of Medicine, New York, NY 10029, [3]University of Colorado Medical Center, [4]The Salk Institute for Biological Studies.

The non-NMDA glutamate receptor subunits have been classified into an AMPA/kainate (A/K) class (GluR1-GluR4) and a kainate (K) class (GluR5-GluR7). We recently developed monoclonal antibodies (MAb) to several of these subunits, and have used them in combination with polyclonal antibodies (PAb, provided by R.J. Wenthold) to characterize glutamate receptor profiles of specific regions, layers and cell types in primate neocortex. Neurons containing the K class subunits are widely distributed in primate neocortex, yet exhibit a high degree of regional and laminar specificity. MAbs to K class subunits used in combination with PAbs to A/K class subunits suggest that K class subunits are present in pyramidal cells that represent a subclass of the neocortical cells immunoreactive for A/K subunits GluR1 and GluR2/3. In addition, K subunits are present in some pyramidal cell soma, but are particularly abundant in primary apical dendrites of pyramidal cells. Often the labeled apical dendrites coalesce into dendritic bundles, and appear to originate primarily from layer V. Double labeling studies with antibodies to various cytoskeletal proteins demonstrate that the K subunits are often localized to a particular portion of the primary dendrite, suggesting that K class subunits may exhibit preferential laminar localization along a given apical dendrite, allowing for association with particular afferents with preferred laminar targets. Electron microscopic studies confirm the dendritic localization of K subunits, and suggest that these subunits may be preferentially distributed in primary dendritic shafts as opposed to spines. In summary, a high degree of specificity exists in respect to the glutamate receptor profile of neocortical neurons, and complex colocalization patterns exist suggesting that individual neurons may contain K/A and K subunits with differential somatodendritic distribution. Supported by NIH Grant AGO6647 and the American Health Assistance Foundation (AHAF).

617.6

DETECTION OF Y1-NEUROPEPTIDE Y BINDING SITES IN RAT BASILAR ARTERY BY QUANTITATIVE RECEPTOR AUTORADIOGRAPHY. E.S. Corp* and J.A. McQuade. Bourne Laboratory, New York Hospital-Cornell Medical Center, White Plains, N.Y. 10605.

We employed *in vitro* quantitative autoradiography to measure parameters of binding over the basilar artery (BA) visualized in slide-mounted sections of rat medulla labeled with ^{125}I-peptide YY (PYY, 0.05 nM). Affinity, capacity and specificity of the labeled sites were determined using NPY, PYY and subtype-selective ligands.

Assuming a single site fit, nonlinear regression analysis (LIGAND) resolved NPY and PYY binding with equal affinity and capacity: (NPY-K_i = 0.8 nM, B_{max} = 10 fmol/mg; PYY-K_d = 1.3 nM, B_{max} = 14 fmol/mg). The occupied sites were predominately (> 75%) **Y1** (Pro^{34} NPY K_i = 4.4 nM). Based on the inhibitory potency of NPY$^{13-36}$, **Y2** occupancy was < 25% (KD_i = 141).

Vasoconstrictive effects of NPY are well known and these data suggest that cerebral vascular NPY receptors are type-**Y1**, like their counterpart in peripheral vascular smooth muscle.

This rank order of potency is similar to the rank order of potency for these same agonists to stimulate feeding following fourth icv administration. Although it is unlikely that NPY acts at the BA to induce food intake, an action on cerebral arterioles can't be ruled out. Alternatively, NPY and PYY may act at vascular-type receptors expressed in neural elements as yet undetected by current methods.

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617.7

DYNAMIC AND EQUILIBRIUM SPECT MEASUREMENT OF HUMAN BENZODIAZEPINE RECEPTOR BINDING POTENTIAL A. Abi-Dargham, J.P. Seibyl, M. Laruelle, Z. Rattner, S. Zoghbi, R.M. Baldwin, G. Wiesnewski, Y. Zea Ponce, E. O. Smith, D.S. Charney*, P.B. Hoffer and R.B. Innis. Yale University/VA Medical Center, West Haven, CT 06516.

SPECT imaging with [^{123}I]iomazenil was performed in healthy volunteers under dynamic and equilibrium conditions to measure the benzodiazepine binding potential (BP = B_{max}/K_d). In the dynamic paradigm, four subjects were injected with 10 to 16 mCi of [^{123}I]iomazenil (SA ~180000 Ci/mmol) in a single iv bolus. Continuous blood sampling was performed and free parent compound measured. Brain acquisitions were obtained every 2 min on the ASPECT device, reconstructed with the photopeak window, attenuation corrected, and resliced to a standard orientation using external fiducial markers. Brain and blood data were fit to a three compartment model to estimate transfer rate constants, K1 to k4. BP was calculated as $K_1*k_3/k_2*k_4*f_1$, with f_1 = the measured free fraction in the blood. In the equilibrium paradigm, a continuous i.v. infusion after an initial bolus of the radiotracer lead to a steady state concentration in the plasma which was sustained for 8 hours allowing equilibrium to occur at the receptor site. Under equilibrium conditions with negligible receptor occupancy, $B_{max}/K_d = B/F$, where B is the specific binding at equilibrium and F is the free radioligand concentration in the blood. Two subjects were given 10 mCi with a bolus to hourly infusion ratio of 3 to 4. The BP values for the occipital pole obtained with equilibrium (184±29; n=2) and dynamic (151±39; n=5) approaches were in relative good agreement supporting the validity of both methods.

617.8

IN VITRO AND EX VIVO AUTORADIOGRAPHY OF α_2 ADRENOCEPTORS WITH [^{3}H]ATIPAMEZOLE. T. F. Budinger, C. A. Mathis* and A. Biegon. Lawrence Berkeley Laboratory, Univ. of California, Berkeley, CA 94720.

The feasibility of using [^{3}H]atipamezole as an in vitro and in vivo imaging agent for brain a_2 adrenoceptors was examined with ex vivo autoradiography in the rat and in vitro autoradiography in rat and human brain postmortem. In vitro auotradiography was performed by applying 0.5nM radioligand to 20μ rat or 40μ human brain sections for 1 hour at room temperature, followed by 2 X 10 min wash in ice cold incubation buffer. Nonspecific binding was determined in the presence of 10μM unlabeled clonidine. Sections were dried and apposed to tritium sensitive film for 4 weeks. A computerized image analysis system was employed to measure standards and regions of interest and to compute regional specific binding. Distribution of specific [^{3}H]atipamezole binding in rat and human brain was compatible with the known distribution of a_2 adrenoceptors as demonstrated by in vitro autoradiographic studies using agonists and the antagonist idazoxan but not rauwolscine. Following the tail vein injection of 100μCi [^{3}H]atipamezole (60 Ci/mmole), the ligand readily crossed the blood-brain-barrier and accumulated in the rat brain, with a brain:blood ratio of 5:1 and 0.6% injected dose/gr brain at 5 min. The distribution of activity at 1 h following injection was similar to the distribution of a_2 adrenocepotrs observed in vitro. Coinjection of Idazoxan 2mg/kg produced a significant reduction in brain activity in vivo. Thus, appropriately labeled atipamezole shows promise as an imaging agent for human a_2 adrenoceptors with high resolution positron emission tomography (PET).

617.9

MOUSE BRAIN LOCALIZATION OF NEW [F-18]BENZO-VESAMICOL TRACER IS NOT CHANGED BY PRE-TREATMENT WITH SIGMA LIGANDS HALDOL, 3-PPP, OR E2020. GK Mulholland*, PS Sherman, MR Kilbourn, Y-W Jung, KA Frey, DM Wieland and DE Kuhl. Division of Nuclear Medicine, Department of Internal Medicine, University of Michigan School of Medicine, Ann Arbor, MI 48109.

Radiolabeled benzovesamicol analogs studied in these laboratories as potential tracers for Alzheimers disease (AD) show a localization pattern *in vivo* consistent with cholinergic density in the normal rodent or monkey brain. However, recent reports that vesamicol (VES) itself binds to non-cholinergic sites in addition to the ACh vesicular transporter (Hicks *J Neurochem.* 57:509, 1991), and a lack of correlation between [3H]VES binding and ChAT levels in postmortem AD brains (Kish *Neurosci Let.* 117:347,1990 Ruberg, *Neurosci.* 35:327, 1990) raise questions about the cholinergic specificity of benzovesamicols. The potent inhibition of [3H]VES binding *in vitro* by haldol (IC_{50} 43 nM, Altar, *Synapse* 2:486,1988) points to possible interactions of VES and sigma sites. We wanted to know if brain localization of a promising new F-18 labeled benzovesamicol FEOBV ((-)-fluoroethoxybenzovesamicol) would be affected by pretreatment with CNS drugs reported to have high affinity for the sigma receptor (Koe, Soc Neurosci Abs 17:, S133.3, 1991). Haldol (5 μmol/kg), (+)-3-PPP (20 μmol/kg), or E-2020 (10 μmol/kg) were administered intraperitoneally 60 min prior to IV injection of high specific activity FEOBV (30 μCi, 15 pmol). Animals (n=3 per data point) were sacrificed 5, 45 and 240 min post tracer. Brain regions were dissected and counted for radioactivity. Control animals showed total brain uptake of 1.5% of injected dose, and a striatum: cortex: hippocampus: cerebellum ratio of 9:3:3:1, at 240 min. In spite of the large doses, none of the pretreatments produced significant change in regional distribution from control values at the time points examined. In terms of total brain uptake only the haldol group showed a slight depression in early global FEOBV levels that can be ascribed to reduced blood flow and tracer delivery. Thus it is concluded that, unlike vesamicol, FEOBV and possibly similar benzovesamicol analogs do not interact at sigma binding sites *in vivo*.

617.10

EXPRESSION OF AN ALTERNATIVELY SPLICED VARIANT OF THE DOPAMINE D3 RECEPTOR IN HUMAN BRAIN. C. Schmauss, H. Haroutunian & L.M. Refolo*. Dept. Psychiatry, Mount Sinai Medical School, New York, NY 10029.

The amplification of the human dopamine D_3-encoded cDNAs (D_{3A}) by reverse transcriptase/polymerase-chain reaction resulted in the additional amplification of a dopamine D_3-like mRNA sequence (D_{3B}). A comparison of the nucleotide sequences of both D_{3A}- and D_{3B}-encoded cDNAs revealed that they are identical, with the exeption of 98 nuclotides that are found in the third cytoplasmic domain of cDNA that encodes D_{3A}, but not in cDNA that encodes D_{3B}. This results in a differently predicted amino acid sequence of the carboxyl terminal region of the putative D_{3B} receptor. Inclusion or exclusion of the 98 nucleotide-long sequence is apparently regulated by alternative splicing. In postmortem brains obtained from schizophrenic patients, D_{3A}- and D_{3B}-encoded mRNAs are co-expressed in the olfactory bulb, prefrontal and temporal cortex, hypothalamus and nucleus accumbens, while in the parietal cortex, hippocampus, substantia nigra, pons, cerebellum and pituitary only D_{3B}-encoded mRNA is expressed. D_{3A}-encoded mRNA is the predominant form found in the caudate.

We have began to examine the distribution of D_{3A}- and D_{3B}-encoded mRNAs in postmortem brains of patients with Alzheimer's disease (AD) (age and postmortem interval matched). These preliminary data indicate that the expression of D_{3B}-encoded mRNA is similar in AD and schizophrenia, but, in contrast to schizophrenia, the expression of D_{3A}-encoded mRNA could also be detected in the parietal cortex and the hippocampus of AD brains. Whether these results reflect individual variations in the ability to process D_{3A}-encoded mRNA in different brain regions or are disease-related is currently under investigation. Furthermore, we will present results of experiments that aim at characterizing the pharmacological characteristics of the putative D_{3B} receptor.

618.1

NEURITE OUTGROWTH IN PRIMARY CULTURES OF *DROSOPHILA* PHOTORECEPTOR AND OPTIC LOBE CELLS. Chinglu Li* and I.A. Meinertzhagen. Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

The visual system of *Drosophila melanogaster* has been widely used in developmental neurobiology, for which successful *in vitro* cultures of eye imaginal discs and optic lobe neurons offer a powerful prospective tool. We have established a primary culture system, with which, for the first time, we have obtained constant neuronal differentiation from both optic lobe and eye imaginal discs. Late third-instar larval or white prepupal wild-type *Drosophila melanogaster* raised at 25°C were surface sterilized, and the eye imaginal discs and the lateral poles of the supraesophageal hemispheres (containing the optic lobes) dissected out under sterile conditions. Eye discs were partially dissociated by mechanical trituration, while optic lobe cells were dissociated by trituration after 10min in 0.25% trypsin. Preparations were culured in Nunc dishes coated with poly-L-lysine at 25°C in a humid chamber using the bicarbonate-free Leibovitz L-15 medium supplemented with 10% fetal bovine serum. Neurite outgrowth was observed in both cultures within a few hours of initiating the culture. The cells are immunoreactive to anti-HRP, which recognizes an epitope on insect neurons. Optic lobe cells differentiated as single cells with bifurcated fibers, or as clusters with neurites that faciculate together. Sometimes ganglion cells about 5μm in diameter clustered around a larger round cell (diameter ~10μm), presumed to be their stem cell neuroblast. Some processes from these neurons had clear growth cones and varicosities. Cultures have been maintained up to 30d. For successful culture of eye imaginal discs it is important to dissociate the discs only partially. Disc fragments usually reseal into vesicles of various sizes. Healthy neurite outgrowth is usually observed from those vesicles or clusters, although single cells with long fibers are also seen; growth cones can be seen in some cells. Anti-HRP indicates that neuronal differentiation occurs in these eye disc fragments, which also give rise to non-neuronal cells.

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618.2

ELECTRICAL PROPERTIES OF PEPTIDERGIC NEURONS ARE UNCHANGED WITH TIME IN CULTURE. D.E.R. Meyers and I.M. Cooke*, Békésy Lab. of Neurobiol., Univ. of Hawaii, Honolulu, HI 96822, USA.

After 1 d in defined culture, regenerating crustacean (*Cardisoma Carnifex*) peptidergic neurons show veiling or branching morphologies. Veiling cells are routinely immunopositive for crustacean hyperglycemic hormone (CHH), fire over-shooting spikes and express Na, Ca and K currents. Branching neurons having spherical somata <25 μm diam. and fine processes that are often varicose are CHH negative, do not fire over-shooting spikes; under voltage clamp (VC) they are dominated by K current (Cooke et al., PNAS **86**:402, 1989; Meyers et al., J. Neurophysiol. **67**:1301, 1992). We have now recorded from these types and larger branching neurons after 5 - 6 d in culture. Using whole-cell patch recording (pipette, 300 mM K^+; bath, crab saline), 3 of 4 veiling but only 1 of 5 branching cells fired over-shooting spikes. Under VC, all 6 veiling cells tested showed inward (max. 0.3 - 1.9 nA) and outward (1.1 - 3.9 nA, V_c 0 mV, V_h -40 mV) current, while only 3 of 12 branching cells did so (<0.5 nA). The mean soma diam. of these cells (44 ± 6, $\bar{x}\pm$SD, μm) was significantly larger ($F(1,7) = 17.6$, $P = .004$) than that of the others (25 ± 6 μm). Max. outward current at 0 mV (V_h -40 mV) in the 12 cells was 0.6 - 3.5 nA. Sixteen branching cells were examined in solutions designed to isolate I_{Ca}. Ten generated I_{Ca} (max. 334 ± 328 pA). The mean soma diam. of these cells (37 ± 9 μm) was significantly larger ($F(1,15)$, $P = .02$) than that of cells devoid of I_{Ca} (26 ± 7 μm). We conclude that the electrical properties of the cells previously studied at 24 h do not change substantially over the following 5 d. The large-diam. branching cells which generate I_{Ca} were not targeted in our earlier study as they could not be distinguished from veiling cells that had not produced a lamellipodium by 24 h.

Supported by NSF BNS-8910432 and the Univ. of Hawaii Foundation.

618.3

CALCIUM TRANSIENTS IN RESPONSE TO CONDITIONING FACTORS IN REGENERATING *HELISOMA* NEURONS. D.L. Kania* and C.S. Cohan Dept. of Anatomical Sciences, SUNY at Buffalo, Buffalo, N.Y. 14214

Previous studies have shown a correlation between neurite elongation and intracellular calcium levels in *Helisoma* neurons (Cohan and Kater, 1987). This study examined whether the initiation of outgrowth from a cut axon stump was associated with changes in intracellular calcium levels in response to the presence of conditioning factors.

Identified neuron, B19, was removed from the buccal ganglia with an attached piece of axon and plated into defined culture medium. Cells were injected with Fura-2 and calcium levels were monitored at the distal axon before and at various times after the addition of conditioning factors. In the absence of conditioning factors, no regenerative outgrowth was observed and calcium levels remained constant. However, the addition of conditioning factors resulted in the extension of new neurites from the distal axon together with cyclical elevations in intracellular calcium that were first detected at 8-12 hours. Approximately 50% of the cells exhibited calcium transients. These transient elevations had peak amplitudes that were 20-30% above basal calcium levels and had a period of about 10 minutes. Transients were blocked by lanthanum indicating that the calcium originated from influx across the plasma membrane. Blocking calcium transients with lanthanum did not inhibit the initiation of outgrowth. However, the rate and extent of outgrowth was significantly lower.

These data indicate that application of conditioning factors to *Helisoma* neurons induces calcium transients. While the calcium transients do not appear to be directly related to the initiation of outgrowth, they do appear to have some affect on the rate and extent of outgrowth in these neurons.

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618.4

RAPID REGULATION OF $[Ca^{2+}]_i$ CHARACTERIZES NEURONS SHOWING OUTGROWTH IN DEFINED CULTURE. R. A. Graf*, Békésy Lab. of Neurobiol. and Dept. of Zool., U. of Hawaii, Honolulu, HI 96822.

There are conflicting observations on the relations between $[Ca^{2+}]_i$ and outgrowth from cultured neurons. This report provides observations pointing to the capability for Ca^{2+} homeostasis as an important attribute of neurons showing outgrowth. Peptidergic neurons from the X-organ - sinus gland neurosecretory system of land crabs (*Cardisoma carnifex*) when cultured in defined medium show immediate outgrowth having several characteristic patterns including veiling (associated with immunoreactivity to crustacean hyperglycemic hormone antisera) and various types of branching (Cooke, et al. 1989, PNAS **86**:402). A few neurons show little regeneration. Here I compare the $[Ca^{2+}]_i$ responses of cells lacking outgrowth with extensively-regenerated cells (from same culture) after application of depolarizing $[K^+]_o$ (5X normal; 1-7 min). $[Ca^{2+}]_i$ was determined by imaging with fura-2/AM using the ratiometric equation with $R_{min}=0.4$, $R_{max}=6.0$, and $K_d=800$ nM. In both morphological classes, basal $[Ca^{2+}]_i$ ranged from 50-200 nM and increased at least 200 nM in response to $[K^+]_o$, in processes as well as somata. Time courses of $[Ca^{2+}]_i$ increases of neurons with and without outgrowth were similar (peak within 1-2 min), but $[Ca^{2+}]_i$ declined to within 100 nM of basal levels only in cells exhibiting outgrowth. In non-growing neurons it remained high. New $[Ca^{2+}]_i$ steady state levels were established within 20 min. The cell's ability to regulate perturbations of $[Ca^{2+}]_i$ may be critical to neuronal outgrowth.

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618.5

THE ROLE OF EXTRACELLULAR MATRIX MOLECULES AND MICROGLIA IN REGENERATION OF THE LEECH CNS. L. M. Masuda-Nakagawa, D. Brodbeck and J.G. Nicholls* Biocenter, University of Basel, Basel, Switzerland, 4056

The aim of our experiments is to investigate how substrate molecules of the extracellular matrix (ECM) influence the growth and regeneration of nerve cells. Earlier work has shown that the growth of leech neurons in culture depends on substrate molecules, each substrate producing a characteristic growth pattern. In particular a laminin-like molecule induces slender, straight and relatively unbranched processes. *In vivo*, laminin appears in the regenerating CNS closely associated with regenerating fibers. One question is whether the laminin is newly synthesized or displaced from other sites. Metabolic labeling of regenerating CNS in culture with ^{35}S-methionine indicates that laminin synthesis increases one week after a lesion. Tests have been made to identify the cellular components that make the laminin. Two findings implicate microglial cells: (1) the accumulation of laminin correlates with the migration of microglial cells to the crush site. (ii) microglial cells in culture show immunoreactivity to laminin by immunofluorescence staining. To clarify further the association of laminin with microglial cells immunolabeling is being studied by electronmicroscopy. Our evidence suggests that these wandering macrophage like cells may play a key role in regeneration of the leech CNS in vivo. Supported by Swiss National Fund Grant No. 31-27 814.89.

618.6

MODULATION OF BOTH AN NCAM-LIKE ADHESION MOLECULE ON THE SURFACE OF *APLYSIA* NEURONS AND NEURITE FASCICULATION BY 5-HT AND FMRFAMIDE. N. Peter, B. Aranoff, F. Wu and S. Schacher*. Ctr. Neurobiol. & Behav., Columbia Univ. Coll. of P & S, NYSPI, N.Y., N.Y. 10032.

A group of neuron-specific membrane glycoproteins in *Aplysia*, apCAM, was characterized recently and belongs to the same family of cell adhesion molecules as NCAM. Mabs recognizing apCAM can alter the degree of neurite fasciculation of most cells, including sensory and motor cells mediating the gill-withdrawal reflex. The level of expression of these molecules on the surface of the sensory cells, but not the motor cell L7, is down-regulated by applications of 5-HT that evoke long-term synaptic facilitation and structural changes in sensorimotor synapses in-vitro. We therefore examined 1) whether the transmitter FMRFamide, that produces long-lasting changes in sensorimotor connections accompanied by structural changes in the sensory cells, can modulate the level of apCAM, and 2) whether the modulation of apCAM is correlated with transmitter-induced changes in neurite-neurite interaction. Applications of FMRFamide that evoke long-term structural and functional plasticity modulate the level of apCAM on the surface of target cell L7, but not presynaptic sensory cells. As reported for 5-HT effects on sensory cells, the down-regulation of apCAM on L7 by FMRFamide may involve its internalization. Cell-specific changes in the pattern of neurite interactions are evoked by the transmitters and parallel the growth pattern of cells treated with Mabs to apCAM. 5-HT produces a significant reduction in the frequency of fasciculation of sensory cell growth cones with neurites of other sensory cells compared to control or FMRFamide applications. In parallel, FMRFamide reduces significantly the frequency of fasciculation by L7 growth cones on other neurites of L7 compared to control or 5-HT applications. These results suggest that the down-regulation of apCAM on the surface of sensory or motor cells by the respective neurotransmitters may contribute to the transmitter-induced changes in the interactions between homologous cells. These cell-specific changes may participate in the structural modulations accompanying long-term facilitation or inhibition of the sensorimotor synapse evoked by 5-HT or FMRFamide, respectively.

618.7

EVIDENCE FOR A ROLE FOR TYROSINE PHOSPHORYLATION IN REGULATING THE PERIPHERAL ACTIN NETWORK OF THE GROWTH CONE. D.-Y. Wu* and D. J. Goldberg, Center for Neurobiology and Behavior, Columbia University College of Physicians and Surgeons, New York, NY 10032

The network of actin filaments in the periphery of the growth cone is specialized in its organization and activities and plays a critical role in the formation and movement of protrusive structures (filopodia, veils and lamellipodia). The facts that 1) binding of growth factors (such as NGF) to receptor protein-tyrosine kinases apparently rapidly and locally initiates the generation of at least some aspects of this specialized actin system and 2) several actin-associated proteins are substrates for tyrosine phosphorylation suggest that protein tyrosine phosphorylation is important in regulating the assembly or activity of the peripheral actin system. We have investegated this possibility in *Aplysia* neurons.

Axotomized *Aplysia* neurons placed in culture form growth cones with well developed peripheral actin systems even in the absence of growth factor. Immunofluorescence microscopy with a monoclonal antibody to phosphotyrosine reveals staining throughout the growth cone but especially bright staining at the tips of most filopodia. Treatment with an inhibitor of protein-tyrosine kinase (PTK), genistein, eliminates the tip staining from most filopodia. VEC-DIC microscopy reveals that genistein and another PTK inhibitor, lavendustin A, rapidly cause a temporary elongation of filopodia as well as a disappearance of ribs (bundles of actin filaments) in lamellipodia. In addition, either inhibitor completely prevents the formation of filopodia and veils that normally occurs massively along an *Aplysia* axon when it is transected in culture. These results suggest that protein tyrosine phosphorylation, even in the absence of growth factors, is important in regulating the peripheral actin network of the growth cone. One important site of interaction of tyrosine phosphorylated proteins and the actin network may be the tips of filopodia.

618.8

DEVELOPMENTAL EXPRESSION OF G PROTEINS IN MIGRATORY NEURONS OF THE INSECT ENTERIC NERVOUS SYSTEM. P.F. Copenhaver*, M. LaGrange, and A. Horgan, Cell Biol. & Anat. L215, Oregon Health Sci. Univ., Portland, OR 97201.

The formation of the enteric nervous system (ENS) in the moth, *Manduca sexta*, involves the migration of about 400 neurons, the EP cells, along a series of pre-formed pathways on the gut musculature. We are using this system to investigate the mechanisms regulating the onset, duration, and directionality of the cell migratory process. Specifically, we have examined the developmental expression and possible function of the heterotrimeric guanyl nucleotide binding proteins (G proteins) in the course of neuronal migration. Antibodies against the α-subunits of G proteins cloned from *Drosophila* (Wolfgang et al., Devt. **113**, 527) were used to map the patterns of G protein expression during embryonic development. While none of the G proteins could be detected in the premigratory EP cells, all of the cells began to express detectable levels of Goα as they began to migrate. The intensity of staining gradually increased during the migratory period, with immunoreactive material distributed throughout the axonal processes of the EP cells as well as their somata. When preparations were exposed to 10 uM aluminum fluoride for 30 min just prior to the onset of migration, we found that cell migration was completely blocked, although subsequent axonal outgrowth still occurred. To test the possible role of Goα in regulating the extent of neuronal migration, we are using intracellular injections of GTP analogues and purified fragments of the G protein-specific antibodies to characterize the sensitive period and specificity of this putative novel function for G proteins in development. Supported by NSF # BNS 9010538 and by the American Heart Association.

618.9

ACTIN DISPOSITION IN, AND SUBSTRATE ADHESION BY, FILOPODIA EXTENDED FROM PIONEER GROWTH CONES *IN SITU*. T.P. O'Connor* and D. Bentley, Dept. Molecular and Cell Biology, University of California, Berkeley, CA 94720

Filopodia previously have been shown to be essential for correct steering by the Ti1 peripheral pioneer neuron growth cones migrating *in situ* in embryonic grasshopper limb buds. Contact between an extending filopodium and a guidepost neuron can result in selective extension of microtubules into the branch forming from that filopodium. F-actin disposition may be important in controlling microtubule access to filopodia, and in regulating the stability of filopodia. Here, we examined actin disposition in filopodia and the consequences of F-actin depolymerization by cytochalasin.

In an embryonic limb bud fillet preparation, the spatial arrangement of F-actin in pioneer growth cones was observed by injecting rhodamine-labeled phalloidin (2.5U/ul) into Ti 1 cell bodies and allowing it to diffuse throughout the cell. Dense F-actin labeling was observed in filopodia and small branches that extended from the growth cone, and in the peripheral cortical actin network of the axon; little staining was observed in the central region of the growth cone or the axon. Within approximately 2-4 minutes of the addition of cytochalasin D (0.01 - 10 ug/ml), phalloidin labeled F-actin collapsed. If cytochalasin was washed out within 30 min. of initial application, the original pattern of phalloidin labeling was rapidly reconstituted.

Following application of cytochalasin to DiO labeled growth cones, some (predominently short) filopodia retract into the growth cone. However, in every growth cone tested many lengthy filopodia remained extended for periods up to two hours (after 10-20 hr they eventually were withdrawn). Video imaging of DiO labeled growth cones double-labeled with phalloidin confirmed that F-actin did depolymerize following cytochalasin application.

These results suggest that individual filopodia form periodic substrate adhesions; these adhesions may maintain filopodia for several hours in the absence of F-actin. If this is the case, it may account for both the unusual length of filopodia *in situ*, and the ability of individual filopodia to steer along favored guidance substrates.

618.10

VIDEO MICROSCOPY OF VACUOLES WHICH FORM FOLLOWING OSMOTIC PERTURBATION OF MOLLUSCAN GROWTH CONES. J.A. Harris, K.E. Smith* and C.E. Morris, Loeb Institute (and Biology Dept.) University of Ottawa, OCH, Ottawa, Ontario, Canada K1Y 4E9

Molluscan neurons regenerating in culture (usually unidentified *Lymnaea* neurons, occasionally *Aplysia* bag cells) were subjected to a variety of acute (minutes) and longer term (hours) osmotic shocks. Cells were monitored on video. Within minutes of return to isosmotic medium, vacuoles (post hyposmotic vacuoles: pHOSvac) formed in the growth cones and at adhesion sites; surprisingly, they disappeared within minutes when neurons were re-shocked. The process was repeatable and the pHOSvacs characteristically reappeared in their previous locations. Isolated growth cones were competent to form pHOSvacs.

pHOSvac formation did not represent irreversible damage to the neurons. Time lapse video of neurons exposed to a distilled water pulse (<2 min) followed by return to isomotic solution (inducing pHOSvacs) demonstrated that over 24 h most of the pHOSvacs disappeared and neuronal arborization processes (eg. lamellipodial searching, axonal transport, retrograde ruffling) continued.

Both pHOSvac formation and their rapid disappearance during a second HOS seem counterintuitive; by pursuing the phenomenon we hope to learn something about processing of cytoplasmic lipid pools. Supported by NSERC, Canada.

618.11

IDENTIFICATION OF AN OCTOPUS PROTEIN RELATED TO A HELISOMA ECM NEURITE OUTGROWTH-PROMOTING PROTEIN. J. D. Robertson*, R. D. Hadley and C. E. Hammond, Dept. Neurobiol., Duke Univ., and Dept. Cell Biol. and Anat., Med. Univ. of SC.

We have evidence that tactile learning in *Octopus* is mediated in part by extension of filopodia within neuropils; it can be blocked by treatments that block actin-based filopodial extension (cytochalasin D) and is stimulated by nerve growth factor. To block filopodial extension independently, we plan to interfere with neurite outgrowth-promoting molecules in *Octopus* brain using Fabs and examine the effect on learning.

A ~300 kD *Helisoma* ECM protein has neurite outgrowth-promoting activity for cultured *Helisoma* neurons (i.e., anti~300 kDa Fabs block growth cone initiation, or cause collapse and retraction of active growth cones). We have identified an Mr ~300 kD protein in *Octopus* brain that comigrates with the *Helisoma* protein, and shows a similar Mr shift under reducing vs. nonreducing conditions on SDS PAGE. Antibodies against the *Helisoma* ~300 kD protein also recognize the octopus protein on immunoblots. Antibody Fab fragments against the octopus protein will provide an important tool to address the question of whether neurite outgrowth (i.e. filopodial extension) is crucial to octopus learning. Supp. by NIH 1RO1-NS-26853-01A2.

618.12

INTERACTIONS BETWEEN DIFFERENT BRANCHES IN A SINGLE LEECH NEURON W.B. Gan, E.R. Macagno* Dept. of Biol. Sciences, Columbia Univ., New York, N.Y. 10027

In a developing nervous system, a neuron usually sends many axonal branches to interact with different environments. The outgrowth of one branch may only depend on its own environment or, alternatively, different branches may influence each other's growth. Here we choose AP neurons in *Hirudo medicinalis* as our model system to test these two possibilities. During embryogenesis (before embryonic day 10), AP neurons extend lateral projections through two roots to peripheral targets and longitudinal projections through the connective towards neighboring ganglia. Only the lateral projections are found in the adult; the longitudinal projections are eliminated through the inhibition by adjacent segmental homologues during development. Previous studies have shown that either killing the adjacent AP homologues or cutting both roots with scissors at E11-E15 induces the longitudinal projections to grow into the adjacent ganglia and out of their roots to the periphery. (W.Q. Gao & E.R. Macagno, 1987, 1988).

In this report, we studied the relationship between lateral and longitudinal branch outgrowth. DiI staining revealed that there is an enormous amount of AP process outgrowth in the periphery during the time that the longitudinal projections stop growing (E11-E15). After killing its adjacent homologues at E9-E10 and examining the cell three days later, we found that the lateral projections slowed down their outgrowth in the periphery when the longitudinal projections extended ectopically into the periphery through the adjacent ganglia.

Since in the pervious experiments all the nerves in the roots were severed, we tested whether cutting only the AP's lateral projections can induce the longitudinal projection to grow and to overcome the inhibition. To do this, an individual AP cell was stained at E12-E13 with DiI and cut its two lateral projections with a laser microbeam. It was found that the longitudinal projections grew into the periphery within two days via the adjacent ganglia. Cutting only one projection within the root induced the longitudinal projections to grow close to or into the adjacent ganglion but not to the periphery.

Taken together, these results suggest that in the intact nervous system, in which a neuron has many axonal branches, outgrowth state of one branch can influence the outgrowth of the others.

618.13

IN VITRO GROWTH OF CRAYFISH AXONS FROM TONIC AND PHASIC MOTONEURONS. K. Egid* and G. A. Lnenicka Depart. of Biol. Sci., State Univ. of New York, Albany, N.Y. 12222

Tonically and phasically active crayfish axons and motor terminals have well-characterized differences in physiology and morphology (Lnenicka, *N.Y. Aca. Sci. 627*:197-211, 1991). To investigate the ontogeny of these differences, we have developed a culture system in which we can observe the regenerative growth of crayfish tonic and phasic motor axons. Abdominal nerve cords, including ganglia one through five, were dissected from juvenile crayfish and plated onto coverslips in culture medium. Within three days, growth was observed from the cut ends of the phasic and tonic branches of the third root. Using standard electrophysiological techniques, we determined that tonic and phasic neurons in culture for one week, retain their different electrical activity patterns. Many tonic cells were spontaneously active, while phasic cells showed no spontaneous impulse activity but responded to stimulation of their cell bodies.

In our initial studies, we have examined the growth patterns of the tonic and phasic motor axons. Cultures were photographed, and the negatives projected and traced. Either length or area of growth was calculated and branching patterns described. Axonal growth was observed as early as 24 hrs after plating and continued for an additional 7 to 10 days. Three patterns of growth repeatedly occurred within a single culture dish: 1) slender with little or no branching, 2) bush-like with extensive branching, 3) extremely wide and flat with little branching. Growth rates and branching pattern of axons differed between the two populations of cells. Growth from tonic axons was almost exclusively type 1, while the growth of the phasic cells consisted of all three types. (Supported by NSF grant BNS-9121757)

618.14

COLCHICINE BLOCKS AXONAL REGENERATION IN AN IDENTIFIED NEURON OF *HELISOMA*. **P. J. Kruk* and A. G. M. Bulloch. Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.**

Colchicine is known to bind to microtubules and block axonal transport in many animal classes, including mammals and gastropods, it also blocks neurite elongation in mouse neuroblastoma and PC12 cells. We tested the effect of colchicine on adult *Helisoma* buccal neuron 4 (B4) regenerating after axotomy. This neuron has two axons, one in the ipsilateral and one in the contralateral esophageal nerve trunks, which together innervate the paired salivary glands. The experiments were performed on semi-intact preparations that consisted of the buccal mass, the salivary glands, and all the central ganglia, including the buccal ganglia. Axotomy was performed by crushing both esophageal nerve trunks at a distance of 200-400 μm from the buccal ganglia (20-40% of the nerve length). These semi-intact preparations were cultured separately in medium with various concentrations of colchicine. After culturing for 6-7 days, one of the two neurons B4 in each semi-intact preparation was injected with Lucifer yellow to test its morphology. In control experiments (no colchicine in medium), both axons of the neurons B4 showed extensive regenerative neurite outgrowth. However, neurite outgrowth was blocked in the presence of micromolar or millimolar concentrations of colchicine. It is concluded that colchicine prevents axonal regeneration in *Helisoma* neuron B4, presumably by blocking axonal transport.

Supported by AHFMR and MRC (Canada).

618.15

SEROTONIN INHIBITS LESION INDUCED SPROUTING IN THE SNAIL CNS: AN *IN VIVO* STUDY. M.W.Baker* and R.P.Croll. Dept. of Physiol. & Biophys., Dalhousie Univ. Halifax, N.S. Canada.

The bilaterally symmetric, serotonergic metacerebral giant (MCG) cell projects to the buccal ganglia of *Achatina fulica* via the ipsilateral cerebrobuccal connective (CBC). This innervation is predominately unilateral providing the only 5-HT in the buccal ganglia. Serotonin-like immunoreactivity (SLIR) in the buccal ganglia 3-7 days after a cut to the CBC, reveals a unilateral loss of 5-HT immunoreactivity in the lesioned ganglion, suggesting the degeneration of severed fibers from the MCG. While ipsilateral regeneration is prevented by a cut to the CBC, SLIR nevertheless returns to the lesioned ganglion 3-4 wks after the lesion. Dye fills and electrophysiological measures reveal that this serotonergic innervation is the result of neuritic sprouting from the contralateral, uninjured MCG (cMCG) into the denervated ganglion. We have previously shown that pharmacological depletion of 5-HT in *Achatina* results in the supernumerary labeling of neuronal elements in the CNS (Baker & Croll, Soc. Neurosci. Abstr: 17,295), thus suggesting that lesion induced depletion might also act to trigger neuronal sprouting. This possibility was tested by administering exogenous 5-HT in conjunction with a cut to the CBC. Sprouting by the cMCG after injection of 5-HT (0.5 mg every second day), was considerably retarded when compared with the normal sprouting response seen 3-4 wks following lesion with vehicle control, DA and 5-HTP injections. These results support a previously suggested role for 5-HT as a neuritogenic modulator in the central nervous system of the snail.
Supported by NSERC Canada to R.P.C.

DEVELOPMENT OF NEUROTRANSMITTER SYSTEMS: ACETYLCHOLINE AND SEROTONIN

619.1

NICOTINIC ACETYLCHOLINE RECEPTORS ARE DECREASED IN RAT DENTATE GYRUS FOLLOWING ENTORHINAL CORTEX LESIONS. J. Aubert*, J. Poirier, A. Baccichet, S.Gauthier and R. Quirion. Douglas Hospital Research Center, Departments of Psychiatry and Neurology & Neurosurgery, Center for Studies in Aging, McGill University, Montreal, Quebec, Canada, H4H 1R3.

It has been shown that lesions of the entorhinal cortex (EC) in rat promote the sprouting of acetylcholinesterase (AChE) positive fibers in the dentate gyrus (DG) of the hippocampus. It was suggested that these fibers were cholinergic in nature and originate from the septum nucleus. In our model, lesions of the EC in adult Fisher-344 rats were performed and the status of various cholinergic markers was assessed using receptor autoradiography at various days post-lesions (DPL) (2, 4, 8, 14, and 30). As reported by others, (AChE) staining was significantly *increased* in the ipsilateral DG from 8 to 30 DPL. In contrast, [^{3}H]AF-DX 384 (2nM) and [^{3}H]acetylcholine (20nM)/muscarinic-M2, [^{3}H]pirenzepine (15nM)/muscarinic-M1, [^{3}H]hemicholinium-3 (10nM)/high affinity choline uptake blocker, [^{3}H]AH-5183 (20nM)/blocker of acetylcholine vesicular storage binding sites and choline acetyltransferase (ChAT) activity remained *unchanged* throughout the whole time course. Interestingly, [^{3}H]*N*-methylcarbamylcholine (20nM)/nicotinic binding sites are significantly *decreased* in the ipsilateral molecular and granular layers of the DG from 8 to 30 DPL. Taken together these findings reveal that sprouting AChE-positive fibers of the DG in the EC lesions model are unlikely to be of functional cholinergic nature. The *decrease* in nicotinic binding sites further support this contention on the basis of their likely presynaptic localization in the septo-hippocampal pathway. Supported by the Alzheimer Society of Canada, the American Health Assistance Foundation and MRCC.

619.2

NICOTINIC ACETYLCHOLINE RECEPTOR ALPHA-3 SUBUNIT mRNA IN RAT VISUAL CORTEX: ONTOGENY AND EFFECTS OF NEONATAL ENUCLEATION. T.A. Austin*, S.M. Grady and J.L. Fuchs. Dept. Biological Sciences, University of North Texas, Denton, TX 76203.

The present study examines the role of the alpha-3 nicotinic AChR subunits in developing rat visual cortex. Brain sections from hooded rats were used for in situ hybridization. Slide-mounted sections hybridized with antisense or sense (control) mRNA were exposed to film and were later dipped in emulsion. In autoradiographs from P0-P2 rats, alpha-3 mRNA was not yet evident in the visual cortex, but by P5, dense label in layer IV distinguished area 17 from other neocortical areas. During the second postnatal week, alpha-3 levels increased in layer IV throughout the neocortex, but remained highest in area 17. The developmental time course resembles that of geniculocortical innervation, suggesting that nicotinic receptors may be associated with postsynaptic targets for ingrowing LGN axons. We therefore tested whether normal expression of alpha-3 depends upon LGN input. In rats monocularly enucleated at birth and examined on P10-11, alpha-3 mRNA declined in deprived cortex, particularly in layer IV of the monocular region of area 17. In other rats, excitotoxic lesions of LGN made on P9-10 resulted in decreased labeling in ipsilateral area 17. Excitotoxic cortical lesions appeared to eliminate hybridization within the lesioned area, supporting the hypothesis that the alpha-3 mRNA was primarily in neurons, rather than in glia. The observation that neonatal enucleation affected levels of alpha-3 mRNA in area 17 suggests that neural input may influence the differentiation of postsynaptic neurons by altering gene expression governing neurotransmission.
Supported by MH41865. We thank J. Patrick for the riboprobes.

619.3

ACETYLCHOLINE RECEPTOR EXPRESSION IN LONG TERM PRIMARY CULTURES OF MAMMALIAN MYOTUBES. C.G. Carlson*, A.M. Bode, M.J. Blake, Y. Feng, J. Faber, B.I. Milavetz. Depts. of Physiology; Pharmacology and Toxicology; Biochemistry and Molecular Biology; Univ. N. Dak., School of Medicine, Grand Forks, ND 58202.

In order to examine synapse-independent mechanisms for regulating the expression of mammalian embryonic (E-) and adult acetylcholine receptors (A-AChRs), a protocol has been developed for preparing and maintaining long term primary cultures of mammalian myotubes. Single channel studies of AChR activity from 9 culture runs revealed both E-AChR (γ_E = 43.4 ± 1.05 pS, SEM, N=55) and A-AChR (γ_A = 65.8 ± 3.2 pS, N=14) activity with a common pipette reversal potential of -67.7 ± 1.8 mV (N=59). The ratio of arithmetic mean burst durations (5 μsec minimum gap duration) for the two event classes (A-AChR/E-AChR) was 0.53 ± 0.03 (N=21). E-AChRs accumulated to maximum levels (minimum channels/patch) between culture days (CD) 10 and 19 and then declined (by about 90%) to a minimum between CD 25 and 29. Northern blot determinations show a corresponding reduction in α, γ, and δ subunit mRNA levels between early (CD13) and late (CD21) culture periods. Evidence for synapse-independent A-AChR expression is based on patch clamp data showing developmental increases in A-AChR activity. Although this expression was lower and more variable than that at intact endplates, up to 40% A-AChR events were observed in individual patches over a 3 day period of expression. The relationship between this expression and that of the subunit mRNAs is under investigation (We thank J.P. Merlie and P. Gardner for kindly providing AChR subunit cDNAs; NSF-EPSCOR 90).

619.4

SEGMENT-SPECIFIC ACETYLCHOLINE RESPONSES OF RETZIUS NEURONS IN THE MEDICINAL LEECH. Lidia Szczupak, Sheryl Jordan, Kathleen A. French*, and William. B. Kristan Jr. Department of Biology., University of California, San Diego, La Jolla, CA, 92093-0322.

Acquisition of the mature phenotype of neurons depends on both intrinsic and extrinsic signals. As part of a study of how these two signal sources interact during neuronal maturation in the medicinal leech, we have studied how Retzius neurons from segments containing the male and female reproductive ducts [Rz(5,6)] and from standard segments [Rz(X)] respond to acetylcholine (ACh) applied to the soma. Single ganglia were isolated and desheathed, Rz neurons were voltage clamped with a single microelectrode and the ionic currents elicited by pressure pulses of ACh were studied. In addition, both types of Rz cells were studied in isolation in a cell culture system.

All Rz cells responded to ACh with an anionic outward current, and Rz(X) in addition developed a rapidly desensitizing cationic inward current. This inward current had a physiological and pharmacological profile typical of neuronal nicotinic receptors. In contrast, the outward current possessed features some of which are typical of nicotinic cholinergic receptors and others of which are typical of muscarinic receptors.

These distinctive responses to the application of ACh mimic how each type of Rz cell responds when pressure-sensitive mechanoreceptor neurons (P cells) are stimulated. Therefore, we will determine whether the P cell to Rz neuron synaptic interaction is mediated by a cholinergic synapse, as a model for the development of postsynaptic responses in segmentally homologous, but physiologically distinct, neurons that derive from the same cell lineage.

This work was supported by an NIH research grant (# NS 25916 to WBK) and the International Human Frontier Science Program Organization (LS).

619.5

AN ULTRASTRUCTURAL STUDY OF SEROTONERGIC CELLS IN THE CNS OF EMBRYONIC AND LARVAL *APLYSIA*. R. Marois*, G. M. Kelly, S. Hockfield & T. J. Carew. Interdepart. Neurosci. Prog., Section of Neurobiol., Depts of Biol. and Psychol., Yale University, New Haven, CT 06520

In adult *Aplysia*, identified serotonergic (5HT) neurons in the cerebral ganglion (CG) contribute importantly to synaptic plasticity (Mackey et al., 1989). Some of these 5HT cells have been identified in embryonic and larval *Aplysia* using whole-mount immunocytochemistry (Marois & Carew, 1990). As an initial analysis of the roles that these cells may exert during the development of the nervous system, we have examined the fine structure and projections of the 5HT neurons of the CG using immunocytochemistry at the LM and EM level.

Our results confirmed the presence and fate of 5 serotonin-like immunoreactive (SLIR) cells in the CG at the onset of the larval period: two bilateral pairs of cells apparently belonging to the ACC cluster of adult *Aplysia* (Nolen & Carew, 1986) and a median unpaired cell that is lost at metamorphosis. These cells have four main projection trajectories. 1) All project centrally to a dense neuritic plexus in the CG, where large SLIR fibers associate with non-serotonergic neurites. 2) One of the bilateral pairs of cells and the unpaired median cell send forward projections to the velum, where they appear to contact both myocytes and velar ciliated cells, supporting a role for 5HT in ciliary beating. 3) SLIR fibers also seem to interact with myocytes of the body wall and internal muscle bundles. 4) Another SLIR fiber, apparently from the second bilateral pair of cells, appears to follow the pleuroabdominal tract of the CNS. This fiber extends posteriorly and ventrally from the CG through an ensheathed nerve bundle to a cluster of cells located dorsal to the statocysts. There it joins a neuropil, and then continues more caudally at the latero-ventral margin of the esophagus. The course of this fiber, together with the known ontogenetic position of the other central ganglia, suggests that it travels through the anlage of the pleural and possibly the abdominal ganglia. Thus it will be of interest to determine the anatomical connections and possible functional roles of this identified serotonergic projection.

619.6

TRYPTOPHAN HYDROXYLASE-IMMUNOREACTIVE NEURONS OCCUR IN THE HYPOTHALAMUS OF THE CHICK EMBRYO. A.M. Gabaldon, J.K. Lobner, S.L. Saavedra and J.A. Wallace*. Dept. of Anatomy, Univ. of New Mexico Sch. of Med., Albuquerque, NM 87131.

Numerous tyrosine hydroxylase-immunoreactive (TH+) cells occur in the chick hypothalamus in patterns observed for dopamine (DA)-containing neurons in mammals. However, the majority of these cells cannot be demonstrated to synthesize DA. In chick embryos near hatching, we have now examined the hypothalamus for the presence of cells immunoreactive for tryptophan hydroxylase (TPOH+), the first enzyme in the synthesis of serotonin (5-HT). Large numbers of intensely stained TPOH+ neurons are found in locations contiguous with sites containing TH+ cells. However, TPOH+ cells occur in the paraventricular organ (PVO) where no TH+ neurons are seen. While 5-HT-immunoreactive cells are detected in the PVO, no other 5-HT containing perikarya are observed in the hypothalamus corresponding to sites of TPOH+ neurons. This is in contrast to the lower brainstem where TPOH+ and 5-HT-immunostained cells co-localize in all nuclei examined. Therefore, most hypothalamic TPOH+ neurons may lack amino acid decarboxylase or the isoform of the TPOH enzyme in these cells is inactive. Supported by MBRS grant GM-08139-18.

619.7

THE DEGREE OF NEUROTOXIC INSULT ELICITED BY METHYLENEDIOXY-METHAMPHETAMINE (MDMA) IS MODIFIED BY POSTNATAL AGE. H.W. Broening*, G.D. Newport, S.F. Ali, W. Slikker, Jr. Division of Neurotoxicology, NCTR, Jefferson, AR 72079.

MDMA has been demonstrated to be a potent and selective neurotoxicant to the serotonergic neurotransmitter system. Persistent depletions in serotonin (5-HT) and 5-hydroxy-indoleacetic acid (5-HIAA) levels as well as long-term loss of 5-HT uptake sites and tryptophan hydroxylase activity are observed following administration of MDMA to experimental animals. Sprague Dawley rats, males and females, were given a single p.o. dose of MDMA at various postnatal ages and 5-HT and 5-HIAA levels were measured by HPLC/EC one week after MDMA exposure. MDMA administration at or prior to postnatal day (PND) 10 did not result in deficits in 5-HT or 5-HIAA levels one week after MDMA exposure. Administration of 40 mg/kg of MDMA on PND 15 or PND 20 resulted in an 11 or 13 % depletion respectively, in 5-HT levels in the hippocampus, but had no significant effect on levels of 5-HIAA one week after MDMA exposure. This is in contrast to administration of 40 mg/kg of MDMA to 150 day old rats, wherein 5-HT and 5-HIAA levels where decreased by 50 and 49 % respectively, in the hippocampus one week after MDMA exposure. These results indicate that sensitivity to the long-term 5-HT depleting effects of MDMA develops as the rat CNS begins to mature. Further study of this phenomenon may provide a greater understanding of the underlying mechanisms responsible for the neurotoxic effects of MDMA.

619.8

THE ROLE OF SEROTONERGIC REUPTAKE BLOCKADE IN PRODUCING COCAINE'S EFFECTS ON CEREBRAL FUNCTION IN PERIWEANLING RATS. G.S. Frick, H.E. Hughes, E.A. Grose, L.A. Freed*, D.L. Dow-Edwards Laboratory of Cerebral Metabolism, Department of Pharmacology, SUNY-Health Science Center at Brooklyn, Bklyn, NY 11203.

Previous studies from our laboratory have shown permanent neurochemical changes in rats administered cocaine during postnatal days 11-20 (Dow-Edwards, Annals NY Acad Sci 562:280-289, 1989). By comparing the effects of fluoxetine, a serotonin reuptake inhibitor, to cocaine, the contribution of this pharmacologic property of cocaine to the long-term neurochemical effects can be determined. Sprague-Dawley rats were mated in our animal facility and on day of parturition, pups were assigned to receive sc injections of cocaine or fluoxetine at 25mg/kg or equivalent volume of vehicle each day during days 11-20. Local rates of glucose utilization were determined when the animals were 21 days old using the deoxyglucose method of Sokoloff. The final dose of drug or vehicle was administered 20 min prior to deoxyglucose administration. ANOVA revealed a significant main effect of treatment in each of the 18 brain structures selected for analysis. Post hoc analysis indicated that cocaine but not fluoxetine administration resulted in altered patterns of brain glucose utilization. We therefore suggest that the effects of developmental cocaine exposure on adult brain function are not due primarily to inhibition of serotonin reuptake.

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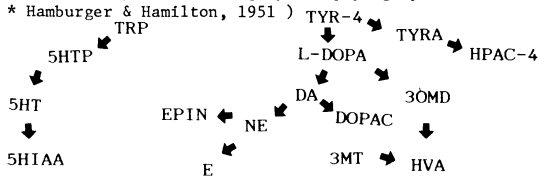
619.9

DEVELOPMENT OF NEUROTRANSMITTER METABOLISM IN THE BRAIN OF THE CHICK, GALLUS DOMESTICUS. Naokuni Takeda *. Dept. of Biotechnology, COSMO Research Institute, Satte, Saitama, 340 - 01, JAPAN.

The dynamics of neurotransmitters including precursor amino acids,biogenic monoamines and their metabolites were examined with the progress of embryonic development. These 30 compounds were analysed simultaneously by coulometric three dimension HPLC system (CEAS: ESA Inc., USA).

In the early stege (St. 5*), primitive pathways were TYR-4 ➡ TYRA ➡ HPAC-4 and TYR-4 ➡ L-DOPA ➡ 3OMD. In the middle stege (St. 10*), TRP ➡ 5HT ➡ 5HIAA pathway appeared. In the late stage (St. 25*), NE was detected, in particular in the hindbrain. Just before hatching (St.44), DA ➡ DOPAC pathway appeared. Levels of 5HT, NE and DA increased with the progress of development. These main pathways are shown below. These results will be discussed from the view point of ontogeny and phylogeny.

(* Hamburger & Hamilton, 1951)



619.10

IMMUNOHISTOCHEMICAL EVIDENCE OF INDOLAMINE AND CATECHOLAMINE CONTAINING CELLS IN CULTURES OF HUMAN CENTRAL NERVOUS SYSTEM. M.C.Calvet* and C.Levallois. INSERM U336, USTL, 34095 Montpellier, France.

Embryonic neurons from monoaminergic nuclei of the brainstem are used for neural transplantation; the knowledge of the localization and of the development of those embryonic cells has to be investigated. As previously described, serotonergic cells are mainly located in the rhombencephalon; catecholamine containing cells are located in the rhombencephalon and an extensive system is described in the mesencephalon. The living dissociated cells isolated from 6-10 week old human fetuses were immunostained for serotonin (5HT) and dopamine (DA) at different ages in vitro. Numerous 5HT-stained neurons in the rhombencephalon and DA-stained neurons in the mesencephalon were observed which developed numerous processes with well individualized growth cones. These results show that monoaminergic cells are characterized in the CNS at the early stages of the human development.

Acknowlegements: Pr. J.L.Viala CHR Montpellier and Dr. M.Geffard.

OTHER FACTORS AND TROPHIC AGENTS: GENERAL II

620.1

RESULTS FROM A RAPID SCREENING TECHNIQUE FOR ASSAY OF NEURONAL DIFFERENTIATION FACTORS. M.-J. Fann*, and P. H. Patterson. Division of Biology, 216-76, Caltech, Pasadena, CA91125.

We have developed a relatively rapid assay for simultaneously screening multiple neuronal differentiation activities. Sympathetic superior cervical ganglia are dissociated from neonatal rats and grown in 96-well plates in the presence of candidate factors. After 7 days, total RNA is extracted and expression of mRNAs for 14 different neuropeptides and neurotransmitter synthetic enzymes is analyzed in parallel by the RT-PCR method. Among the 21 cytokines and growth factors tested so far (IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, INF-γ, TNF-α, GM-CSF, G-CSF, TGF-α, TGF-β, IGF-I, EGF, SCF, oncostatin M (ONC), cholinergic neuronal differentiation factor (CDF), ciliary neurotrophic factor (CNTF), PDGF, aFGF, bFGF), only CDF and CNTF alter neuronal gene expression in this assay. A new finding is that both of these factors induce cholecystokinin and preproenkephalin mRNA. Interestingly, although CDF, IL-6, and ONC are structurally related, and IL-6, CDF and ONC have receptor subunits in common, we are thus far unable to observe an induction of neuropeptide or neurotransmitter synthetic enzyme mRNAs by IL-6 or ONC in the sympathetic neurons.

620.2

QUANTIFICATION OF mRNA LEVELS ENCODING NEUROTROPHIC FACTORS DURING POSTNATAL DEVELOPMENT OF MOUSE NIGROSTRIATAL DOPAMINE NEURONS Cynthia L. Shannon and Mariann Blum * Fishberg Research Center for Neurobiology , Mount Sinai School of Medicine, New York, NY 10029

Neurotrophic factors in the EGF, FGF and neurotrophin family have been shown to increase the survival of midbrain dopamine neurons in vitro. Conventionally, trophic molecules are thought to be provided by targets to support projection neurons. Not only is there evidence for the expression of growth factors in the striatum, a target for dopamine neurons, but growth factor mRNA is also found in the substantia nigra and even in the dopamine neurons themselves. These trophic substances may be acting as target derived factors and/or serve as paracrine or autocrine factors to promote growth and support survival. In order to substantiate the role of neurotrophic factors in the development and maintenance of dopaminergic neurons, we have begun a postnatal developmental study on the expression of EGF, TGF-α, BDNF, bFGF and aFGF mRNA in the dorsal striatum and ventral mesencephalon. Brain tissue from mice was collected at fourteen postnatal time points ranging from two days to twenty weeks. The quantitative nuclease protection assay was employed to determine the levels of growth factor mRNA in total cytoplasmic RNA. The specific mRNA for aFGF, bFGF, TGF-α , and BDNF were detected at every time point assayed and were compared after normalization to total RNA. During postnatal development TGF-α mRNA levels were consistently higher in the dorsal striatum than in the ventral mesencephalon; whereas BDNF mRNA levels were always higher in the ventral mesencephalon. The expression level of aFGF increases in both areas as the animal matures. The advantage to this approach is that within a defined region, at a given time in development, growth factor levels can be compared and relationships between growth factor family members can be identified. Correlating the changes in expression of these neurotrophic factors and known developmental events will give clues to the function of these important molecules in normal brain development.

620.3

MODEL NEURONAL CULTURES TO STUDY THE EFFECTS OF DRUGS ON NEURONAL FUNCTION. W. Chen, P. Coates and S.E. Poduslo*. Depts. of Neurology, Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Model primary cultures of neurons were prepared from midbrain and striatal areas of embryonic rats. The specific brain areas were dissected and subjected to a brief trypsinization step in isolation medium. The softened tissue was passed through nylon screens and the cell suspension plated onto polyethyleneamine coated flasks. After one hour the medium (Dulbecco's high glucose with insulin, 1% BSA and 10% fetal calf serum) was changed to remove cell fragments. Over the next 24-48 hours the cultures consisted primarily of neurons which extended long processes. Choline acetyltransferase and tyrosine hydroxylase enzyme activities were measured in the cultures after 2 days in vitro. The choline acetyltransferase assay measured the production of ^{14}C acetylcholine while the tyrosine hydroxylase assay measured the nonenzymatic decarboxylation of DOPA. The tyrosine hydroxylase assay was modified in that neurons in T_{25} flasks were scraped and homogenized in a buffer containing 0.1% Triton and then concentrated in an Amicon Centricon 10 filter. The pH of the substrate mixture was adjusted to obtain the optimal pH of 6.1 for this assay. The assay was conducted in microfuge tubes in a final volume of 20μl. After the reaction the samples were transferred to glass vials for decarboxylation and subsequent $^{14}CO_2$ absorption. After 2 days in culture, neurons from midbrain had an activity of 20pmol/min/mg soluble protein for tyrosine hydroxylase and 313pmol/min/mg protein for choline acetyltransferase. In addition, mRNA levels for the dopamine receptors and c-fos and c-jun will be assessed. This neuronal culture system will be used to measure the effects of cocaine and methamphetamine on neuronal function. Supported by NIH grant DA07366.

620.4

ANALYSIS OF SERUM ANTI-GM1 ANTIBODY TITER IN PATIENTS RECEIVING GM1 THERAPY. R.K.Yu*, M.Saito, Y.Zhang, R.Fiorentini, F.Khin-Maung-Gyi, Gary H. Friday. Med. Coll. VA, VCU, Richmond, VA 23298 and Fidia Pharmac. Corp., Washington DC 20006

It is well known that gangliosides, particularly GM1, possess neuritogenic and neuronotrophic properties in vitro and in vivo. Gangliosides have also been shown to exert protective effects in certain neurodegenerative conditions, including strokes, spinal cord injury, and neuropathies. Thus, ganglioside GM1 has been used as a therapeutic agent for a variety of neurological diseases. To examine the safety of GM1 therapy, we carried out double blind, placebo-controlled studies in normal subjects and patients with acute ischemic cerebral infarction. Serum samples removed at different time intervals (day 0 to 84) were assayed for anti-GM1 titers using a solid-phase ELISA. Of the 418 samples assayed, only 7 were judged to have a marginal anti-GM1 response at 1:800 dilution. Among the 7 positive samples, 1 was from a subject who received a single dose (1,200 mg) of GM1, 2 were from a subject before and after receiving 400 mg of GM1, and the other 4 were from 3 subjects receiving no GM1. We conclude that there is no clear association between GM1 therapy and the development of anti-GM1 titers.

620.5

COMBINED BUT NOT INDIVIDUAL GROWTH SUBSTANCES MIMIC THE REPARATIVE EFFECT OF GM1 GANGLIOSIDE ON CULTURED DOPAMINE NEURONS DAMAGED BY MPP+. N. Stull* and L. Iacovitti, Dept. of Neurology, Hahnemann Univ. Phila. PA 19102

We have previously demonstrated that, in culture, GM1 ganglioside can increase the number of dopamine neurons surviving MPP+ toxin treatment as well as enhance their biochemical function (Stull, et al. 1991). Cytokines and neuronal growth factors are also thought to play a role in the survival and biochemical development of these neurons. Therefore, we sought to examine whether growth substances either individually or together could mimic and/or enhance the reparative effects of GM1 on MPP+ damaged dopamine neurons. The ventral mesencephalon was dissected, plated, and maintained on standard media. On day 4, some cultures were fed media containing 2.5 μM MPP+. One day later, cultures were refed media supplemented with one or all of the following: NGF, bFGF, aFGF, EGF, IGF, CNTF, TGFβ, IL1, LIF, and Insulin (1-100ng/ml). To test whether growth substances could further enhance the GM1 effect, in some cases, media was simultaneously supplemented with 100μM GM1. After 7 days in vitro, cultures were processed for assay of tyrosine hydroxylase (TH) activity and TH-immmunostaining. Exposure to MPP+ for 24hrs produced a 23-30% decline in the number of surviving TH+ neurons. The addition of individual growth substances had no effect on cell loss due to MPP+. However, the addition of a mixture of all growth substances to MPP+-treated cultures restored the number of TH+ neurons to nearly control levels (93%), and simultaneously induced the level of TH activity/TH+ neuron (33%). Incubation with TGFβ alone resulted in a comparable degree of biochemical induction(35%) but had no effect on dopamine cell survival. Addition of GM1 to individual growth substances or the mixture did not further enhance survival or dopamine biochemistry. These data suggest that while individual factors like TGFβ may influence transmitter biochemistry, it is the combination of growth substances which may be important for improving dopamine cell survival after MPP+ damage. Since GM1 does not act synergistically with these substances, it is possible that growth substances and GM1 both work through a common pathway to achieve their beneficial response in injured dopamine neurons.

620.6

GM1 REGULATES THE MORPHOLOGY OF NEURO-2a NEUROBLASTOMA CELLS BY ALTERING THE DISTRIBUTION OF MAP-2. L-J. Wang, G. Yorke* and F. Roisen. Department of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, KY 40292.

Our laboratory has demonstrated that exposure of either primary sensory neurons or established neuroblastoma lines to the ganglioside GM1 enhances microtubule (MT)-dependent neurite formation. Taxol (MT stabilization) potentiates GM1-mediated neuritogenesis, whereas Colcemid (MT disassembly) blocks ganglioside-induced neurite elongation. Several reports indicate that GM1 increases tubulin mRNA in neuroblastoma cells. Therefore, the mechanism underlying GM1's neuritogenic action appears MT-related. Since developing neurons contain microtubule associated proteins (MAPs) and Tau proteins, which have the capacity to regulate neuronal morphology, this study examined immunocytochemically the effect of GM1 exposure on the level and distribution of MAP-2, tubulin, MAPs and Tau proteins in Neuro-2a murine neuroblastoma cells. Cells were plated in the presence and absence of GM1 (150 μg/ml) for 24 hr prior to immunolocalization with monoclonal antibody (mAb) specific for MAP-2 and a polyclonal antibody (pAb) against MAPs and Tau protein. Further studies employed mAb and pAb against tubulin. GM1 increased MAP-2 reactivity of the distal neuritic regions but did not alter the distribution or reactivity of MAPs or Tau-positive material. Simultaneous exposure of Neuro-2a to GM1 and taxol (2 hr) resulted in intense label of MAP-2 over all distal neuritic regions. Western blot analysis of cells exposed to GM1 for 24 hr revealed levels of MAP-2 equivalent to that found in the untreated controls. Further studies employing immunoelectron microscopy are in progress. Our results are consistent with the belief that gangliosides facilitate neuronal development by producing a redistribution of existing intracellular structural and regulatory protein pools. Furthermore, they suggest that GM1 changes neuronal morphology by altering the distribution of MAP-2 which regulates MT assembly. Supported by a grant from the Alliant Community Trust Fund.

620.7

DEVELOPMENTAL EXPRESSION OF G41, A NOVEL LAMININ-LIKE CDNA CLONE IN NORMAL AND MUTANT MOUSE BRAIN T.T.Quach*, A.M.Duchemin, B.K.Schrier[1]. Dept of Pharmacology,OSU,Col.of Med.,Columbus,OH,[1] Technology Unlimited,Wooster,OH.

We have designed a 30 mer oligonucleotide representing a common and conservative amino-acid sequence of NGF and BDNF to screen a cDNA library constructed in plasmid pGEM, from poly(A+)RNA extracted from lesioned rat brain. More than 30 clones with a positive signal were sequenced with T7DNA polymerase from both ends. One of the above clones appears to be potentially interesting in light of the trophic and/or adhesive activity of the gene product(s). Sequence comparison of partially sequenced insert (1000bp on 1.3Kb) revealed homology from both ends, of 53% and 55% with B2 laminin chain, and of 67% with IGF over 170 bp in the middle of the insert. In addition, G41 has a motif representing 100% homology to N-CAM. We have previously shown that in the rat, the unique hybridizing band was detected at a position corresponding to approximately 3.8Kb and that G41 gene is regulated during brain injury.

A unique band with an apparent similar size was observed in mouse brain. In the present study, we examined the developmental pattern of G41 expression in normal and mutant mouse brain -staggerer, weaver, and purkinje deleted mutant- to identify whether this gene is similarly regulated or whether there is some abnormal expression in mutants during development. Experiments designed for this purpose are currently underway.

620.8

EFFECT OF CONCANAVALIN A (Con A) ON GROWTH OF CULTURED NEURONS FROM MAMMALIAN CENTRAL NERVOUS SYSTEM (CNS). P.W. Coates* and T. L. McKee. Cell Biology & Anatomy, Texas Tech Univ. HSC - School of Medicine, Lubbock, TX 79430.

The lectin Con A is a useful tool for inducing alterations in properties of some cultured neurons. Besides changing membrane properties (ion channel activity, neurotransmitter responses and synaptic connectivity), Con A promotes nerve fiber outgrowth in *Aplysia*, leech and chick dorsal root ganglia. To determine whether neurons from mammalian CNS grow more in response to Con A, neurons from fetal rat brain (striatum) were cultured at low cell density in three-dimensional (3D) collagen lattices or polyethylenimine (PE) coated dishes in the presence or absence of Con A (0.5 or 5.0 μg/ml). Nerve fiber length of single neurons was measured using image analysis. Morphological indicators of complex growth were also assessed. Data from three experiments were pooled and analyzed using analysis of variance. Total nerve fiber length per neuron increased significantly for neurons cultured at low dose Con A on 3D. Fiber outgrowth of neurons on PE showed a similar pattern. At higher Con A concentration, there was an increase in mean number of primary processes for neurons on both substrates, and in average number of terminals of neurons on PE. Observations suggest that neurons from mammalian CNS can grow longer nerve fibers and express somewhat more complex growth patterns under the influence of Con A. The precise molecular mechanism by which Con A induces alterations in neuronal growth (or membrane properties) is not known, but it has been suggested that different mechanisms might be involved rather than a single receptor and transduction mechanism. It is also conceivable that under these culture conditions Con A could affect mechanisms for fiber elongation differently than mechanisms influencing complex growth. Supported by SITRF.

620.9

TROPHIC ACTIONS OF MIDKINE (MK), A HEPARIN-BINDING PROTEIN, ON MOUSE SPINAL CORD AND SENSORY GANGLION NEURONS IN CULTURE. M. Michikawa*, H. Muramatsu, T. Muramatsu and S.U. Kim. Dept. of Neurology, Univ. of British Columbia, Vancouver, Canada; and Dept. of Biochemistry, Kagoshima Univ., Kagoshima, Japan.

Midkine (MK) is a 14 kDa protein product of a retinoic acid responsive gene, MK, and is a member of a new family of heparin-binding growth factors structurally unrelated to FGF (J. Cell Biol, 110, 607, 1990). It has a 50% sequence homology with pleiotrophin and has been found to promote neurite extension in embryonic rat CNS neurons. Trophic effects of MK were studied in dissociated cell cultures of fetal mouse spinal cord and dorsal root ganglion (DRG). There was a 3-4 fold increase in the number of MAP-2 immunoreactive neurons and a 2-4 fold increase in choline acetyltransferase activity (ChAT) in MK-treated spinal cord (13-14 day fetal) cultures. The number of surviving neurons following MK treatment was 3-4 fold over controls in 13-14 day fetal and 17 day fetal DRG cultures, while there was no apparent difference in neuronal survival in neonatal DRG cultures. These results suggest that MK has definite trophic effects on mouse spinal cord and DRG neurons such as neuronal survival and ChAT induction activities.

620.10

MENINGES RELEASE A DIFFUSIBLE ACTIVITY THAT PROMOTES CORTICAL VIABILITY AND NEURITE EXTENSION.

Makoto Sato* and D.D.M. O'Leary. The Salk Institute, La Jolla, CA 92037.

At very early stages of development, the influence of the mesoderm on the ectoderm is crucial for the establishment of the nervous system. However, mesodermal influences on the later development of the central nervous system have received little attention. Here we demonstrate that the meninges, which are of mesodermal origin and cover the brain and spinal cord, substantially influence cortical development in vitro. The viability of dissociated cortical neurons cultured in the presence of meninges, but at a distance, is enhanced 3-fold when grown in a collagen matrix and more than 10-fold when grown on polylysine. Meninges co-cultured at a distance with cortical explants or dissociated cortical cells in 3-D collagen matrices also greatly enhance neurite outgrowth and the rate of axon extension. Meninges-conditioned medium (MCM) shows the same effects. Axons cultured with meninges or MCM have a more deviated growth behavior and are much less fasciculated than those grown in control medium. In addition, the effect of MCM is farily acute. Use of time-lapse video imaging shows that the growth rate of cortical axons is dramatically enhanced about 40 min. after exposure to MCM. These facts imply that the meninges-derived factor(s) may have an adhesive-nature and act directly on the tips of growing neurites (i.e. growth cones). The extracllelular matrix components laminin and fibronection, which have adhesive properties and increase the rate of axon growth of the peripheral neurons, do not mimic the effects of MCM in these cortical cultures. When we treat MCM with centricon (Amicon, molecular weight cut off device), we observe the neurite extension activity in the fraction under 30kD. These findings show that meninges release a diffusible factor(s) that promotes in vitro cortical viability and neurite extension. The meninges may play a crucial role in promoting multiple aspects of cortical development. We are pursuing the precise nature and identity of the responsible molecule(s).

620.11

AMNIOTIC BASEMENT MEMBRANE REGULATES SMOOTH MUSCLE RESPONSES TO SUBSTANCE P. C.W. Bowers* and L.M. Dahm. Beckman Res. Inst., Div. Neurosci., Duarte, CA 91010.

Using a novel smooth muscle (SM) preparation, the avian amnion, we have examined the extracellular factors that determine the responsiveness of SM cells to specific neurotransmitters. The avian amnion is an anatomically simple tissue, neither innervated nor vascularized, containing only a single layer of epithelium, and 1-2 layers of SM. While responsive to many types of neurotransmitter, the amniotic muscle is normally unresponsive to the neuropeptide substance P. This is unusual, because most non-vascular SMs contract vigorously in response to nanomolar substance P. Culturing the intact amnion in a defined medium for 1 wk did not induce the appearance of substance P responses, as assessed by contractility measurements. However, when the amniotic SM was dissociated from the rest of the tissue and cultured in the same defined medium as used for the intact cultures, SM cells became responsive to substance P within 24 hr, with 90% of the SM cells contracting in response to nanomolar substance P by 4 days in culture. These results were not associated with proliferation and were specific for substance P. When amniotic SM cells were dissociated and plated onto basement membrane purified from amnion, the induction of substance P responsiveness was completely suppressed. Smooth muscle cells plated onto the same coverslip, but not in direct contact with the purified basement membrane, showed the usual induction of substance P responsiveness. The data suggest that molecules in the basement membrane normally suppress the amnion's response to substance P. We hypothesize that other tissues expressing diverse pharmacological phenotypes may also be regulated by molecules in the extracellular matrix.

620.12

CHEMICAL LESION INDUCED 5-HT SPROUTING CO-OCCURS WITH INCREASES IN LAMININ, S100, AND 30-45KD PROTEINS, BUT NOT 5-HT1 RECEPTOR IN THE STRIATUM. D. Beversdorf●*, C. F. Pu●, B. A. Al-Seikhan●, E. C. Azmitia○, and F. C. Zhou•. Indiana Univ. Sch. Med.●, Dept. Anatomy, Indianapolis, IN 46202 and Dept. Biology, New York Univ.○, NYC, NY 10003.

Excitotoxic lesion in striatum induced intense sprouting of 5-HT fibers (Zhou et al., 1991). We report here that lesions altered the trophic state of brain profoundly. Ibotenic acid (IB) injected into striatum of Sprague-Dawley rats induced four levels of changes post-lesion: (a) removal of intrinsic neurons at injection site at 3 days; (b) accumulation of reactive astrocytes at 3, 8 and 21 days; (c) hyperexpression of laminin and S-100; (d) stimulation of expression of 45KD protein and increases in levels of 49, 35, 30 KD proteins. Area of 5-HT innervation coincides with that of hyperexpression of trophic substance S100 and laminin, which is at injection site, but not reactive GFAP-positive astrocytes. 5-HT receptor binding with [3H]-5-HT did not show an increase in autoradiograph density within area of lesion. The hyperexpressed S100 and laminin marked a subpopulation of astrocytes which is smaller than population of GFAP-positive reactive astrocytes. These reactive, hyperexpressed astrocytes may be responsible for hyperinnervation. Similar 5-HT hyperinnervation was seen in hippocampus after IB/ kainic acid lesioning. (supported by NIH grant NS23027 to FCZ)

620.13

REGULATION OF VASOACTIVE INTESTINAL PEPTIDE (VIP) AND SUBSTANCE P (SP) EXPRESSION IN SUPERIOR CERVICAL GANGLION (SCG) AFTER AXOTOMY AND IN CULTURE. M. S. Rao *, Y. Sun, U. Vaidyanathan, S. C. Landis, and R. E. Zigmond. Dept. of Neuroscience, Case Western Reserve University, School of Medicine, Cleveland, OH 44106

The expression of VIP- and SP- immunoreactivity (IR) increase in sympathetic neurons of the neonatal and adult SCG when the ganglion is maintained in organ culture for 48 hrs. In addition, VIP-IR increases in SCG in situ within 48 hr of postganglionic axotomy (Soc. Neurosci. Abstr. 17:400,1991). We have examined the time course of VIP induction after axotomy and found that levels of VIP-IR increased significantly by 24 hr, reached a peak by 6 days, and remained elevated for at least 2 weeks. To determine whether SP is also increased by axotomy, we measured SP-IR 48hr and 2 weeks after surgery, using a radioimmunoassay. Consistent with a previous report (Brain Res. 234:182,1982), there was no difference in the level of SP-IR between axotomized and sham-operated ganglia 2 weeks after surgery; however, 48 hr after axotomy, SP-IR was increased 12-fold. Immunohistochemical studies revealed an increase in SP-IR in principal neurons in the SCG 48 hr after axotomy. Previous studies have indicated that the increase in substance P that occurs in organ-cultured SCG can be reduced by the synthetic glucocorticoid dexamethasone. We have compared the effects of dexamethasone (0.1 μM) on SP and VIP expression in organ culture and in cell culture. Dexamethasone blocked the increases in SP-IR normally seen in both types of culture almost completely and reduced the increases in VIP-IR by about half. Thus, qualitatively there are marked similarities in the regulation of VIP and SP in the SCG, though there appear to be quantitative differences. (Supported by NS12651, MH00162, HD25681 and a AHA fellowship to MSR.)

620.14

A SOLUBLE FACTOR SECRETED BY GANGLIONIC NON-NEURONAL CELLS INCREASES VIP LEVELS IN DISSOCIATED CELL CULTURE AND EXPLANTS OF RAT SUPERIOR CERVICAL GANGLION (SCG) . Y. Sun*, M. S. Rao, R. E. Zigmond and S. C. Landis. Department of Neurosciences, Case Western Reserve University, Cleveland, OH 44106.

Explantation causes an increase in VIP levels in both adult and neonatal rat SCG. A similar increase is seen in dissociated cultures of SCG, in both defined and serum-containing medium. To investigate whether ganglionic non-neuronal cells play a role in VIP induction, cultures were preplated to reduce the non-neuronal cell population by an estimated 80-90%. Compared to control cultures, cultures that had been preplated showed a 60% reduction in VIP per neuron after 2 days. To determine if the effect of the non-neuronal cells is mediated by cell contact or the release of a soluble factor, we examined the ability of non-neuronal cell conditioned medium (NNCM) to alter VIP levels. The NNCM increased VIP levels in dissociated cell cultures by 2-3 fold. Medium conditioned by neuronal cultures that had been preplated had no effect. The NNCM also caused a 2-3 fold increase in VIP content in SCG explants. Two previously described factors, CDF/LIF and CNTF, have an effect in culture similar to that of NNCM and thus might be responsible for the alterations in VIP levels produced by NNCM. To determine whether the NNCM contains CNTF, we performed ciliary neuron survival assays. The NNCM did not have any detectable trophic activity, suggesting that the factor in NNCM is not CNTF. Whether the NNCM contains CDF/LIF remains to be determined. (Supported by NS12651, MH00162, HD25681 and a AHA fellowship to MSR)

620.15

REGULATION OF VASOACTIVE INTESTINAL PEPTIDE (VIP) EXPRESSION IN RAT SUPERIOR CERVICAL GANGLION (SCG) IN ORGAN CULTURE BY AGENTS THAT ELEVATE cAMP. R. P. Mohney and R. E. Zigmond*. Dept. of Neuroscience., Case Western Reserve University, School of Medicine, Cleveland, OH 44106 ,

VIP expression increases in sympathetic neurons in adult rat SCG in organ culture. The VIP gene contains a cAMP response element that is required for cAMP-regulated transcription of the gene. VIP itself and secretin, another member of the same peptide family, have been shown to act in many tissues via stimulation of adenylate cyclase. Therefore, we examined whether these peptides can increase VIP expression in the SCG. Adult rat SCG explants were cultured for 30 min in medium containing 500 μM IBMX alone or IBMX together with 10 μM forskolin or 3 μM secretin. Both forskolin and secretin significantly increased cAMP levels in the ganglia. After 24 or 48 hr in culture, explants treated with forskolin or secretin also showed a significant (1.5-fold) increase in VIP-like immunoreactivity (IR). In contrast, 10 μM isoproterenol increased cAMP levels without altering VIP-IR. This apparent discrepancy may be due to isoproterenol causing changes in cAMP levels in non-neuronal cells, but not in neurons, in the ganglion. Peptide histidine isoleucine amide (PHI) is coded for by the same mRNA that codes for VIP. Preliminary data indicate that 10 μM VIP increases the level of PHI-IR. The data raise the possibility of a positive feedback loop in which VIP could stimulate its own synthesis.

620.16

VASOACTIVE INTESTINAL PEPTIDE (VIP) REGULATES DEVELOPMENT OF MULTIPLE NEURONAL POPULATIONS: SENSORY AND CEREBELLAR GRANULE CELLS E. DiCicco-Bloom,* K. Emsbo and I.B. Black, Dep't. Neurosci. & Cell Biol., UMDNJ/Robert Wood Johnson Medical School, Piscataway, NJ, 08854

Previous studies indicated that VIP is a multifunctional regulator of sympathetic development, stimulating neuroblast mitosis, neuritogenesis, and survival during a critical embryonic period in the rat (Pincus *et al*, Nature 343:564). Moreover, the local production of VIP in sympathetic ganglia in vivo suggested that the peptide acts via autocrine mechanisms (Pincus *et al*, Soc. Neurosci. XVII:908; A. Davidson *et al*, this vol.). To define the spectrum of populations responsive to VIP mitogenic and trophic activity, we examined effects in developing sensory and cerebellar granule neurons in culture.

Dissociated embryonic day 14.5 dorsal root ganglion cells were preplated to reduce non-neurons and cultured in serum-free media with NGF for 48hrs. The major neuronal population (>95%) consisted of small round or oval cells exhibiting mono- or bipolar neurites. VIP elicited a two-fold increase in neuron number. Neurons cultured in the presence of VIP, however, did not incorporate [3H]thymidine, a marker for DNA synthesis, suggesting that the peptide was not mitogenic. To the contrary, cell counting indicated that VIP elicited increased neuronal survival. VIP trophic activity was specific, since several related peptides were without effect. While NGF was permissive for VIP effects, peptide trophic activity did not depend on the presence of the neurotrophin or non-neurons.

In contrast to sensory neurons, VIP exhibited only mitogenic activity in cultures of postnatal day 7 cerebellar granule neurons and precursors. VIP stimulated a two-fold rise in the percentage of neuroblasts entering the mitotic cycle without affecting cell survival. Our observations suggest that the multifunctional peptide, VIP, exerts different ontogenetic actions in different neuronal populations. (Dysautonomia Fdn, NIH:HD23315, UMDNJ Fdn)

620.17

THE NEUROTROPHIC ACTIONS OF PACAP27 ON PC12 CELLS. C.L. Weill*, M. Treuil and A. Arimura. Depts. of Neurol. & Anat., Louisiana State Univ. Med. Ctr, New Orleans, LA 70112 and U.S.-Japan Biomed. Res. Labs., Tulane Univ. Hebert Ctr., Belle Chasse, LA 70037.

Pituitary Adenylate Cyclase-Activating Peptide (PACAP) is a new member of the vasoactive intestinal peptide/glucagon/secretin peptide family. PACAP binds with high affinity to cells of the pheochromocytoma cell line PC12h and stimulates adenylate cyclase (Watanabe et al., BBRC 173:252, 1990). In the present study we examined some of the neurotrophic properties of PACAP27 on PC12 cells including: metabolism, cell survival and neurite outgrowth. PC12 cells were maintained in RPMI medium plus heat inactivated serum, 10% horse and 5% fetal bovine. At 10 nM PACAP caused a 1.5-fold and 1.3-fold increase in cell diameter and cell protein respectively. Cell survival was determined in serum free medium in the absence and presence of PACAP27 from 0.01 to 100 nM and NGF at 2 nM. After 5 days the following levels of survival were determined relative to the initial number of cells plated: serum free control, <20%; NGF, 88%; PACAP 0.3 to 3 nM, 60% and 10 to 300 nM 100%. In the presence of 1% horse serum, 10 nM PACAP27 supported neurite outgrowth from 70% of the cells at a nearly linear rate of 7.3 µm/day. Neurites possessed varicosities and terminal growth cone-like structures and were stable for up to two weeks. Thus, the hypothesis that PACAP is a new neurotrophic factor is supported by virtue of the localization of PACAP and its receptor to both the central and peripheral nervous systems and the data presented here demonstrating PACAP's capacity to regulate the metabolism, survival, and morphological differentiation of PC12 cells.

620.18

PACAP27 INDUCES TRANSCRIPTION OF IMMEDIATE EARLY GENES IN PC12 CELLS. J.M. Lyles,* C.L. Weill and A. Arimura. Depts of Neurol. & Anat., Louisiana State Univ. Med. Ctr, New Orleans, LA 70112 & U.S.-Japan Biomed. Res. Labs., Tulane Univ., Belle Chasse, LA 70037.

Pituitary Adenylate Cyclase-Activating Peptide (PACAP) is a new member of the vasoactive intestinal peptide/glucagon/secretin family of peptides which binds with high affinity to pheochromocytoma cells (PC12h) and stimulates adenylate cyclase (Watanabe et al., BBRC 173:252, 1990). PACAP27 was recently shown to act as a neurotrophic factor: it increased PC12 cell diameter and total protein, enhanced cell survival in low serum medium and promoted neurite outgrowth (see Weill et al., 1992 Soc for Neurosci Abstr.). We have examined the induction of several immediate early genes in cultured PC12 cells treated with PACAP27 and with nerve growth factor (NGF) by employing a modified nuclear run-on procedure involving the rapid extraction and column purification of hnRNA. PACAP induced a different pattern of gene expression than did NGF: whereas 2nM NGF enhanced the level of expression of several genes (c-fos>b-actin>c-jun>c-myc), 10nM PACAP most noticeably enhanced c-fos, but had little effect on c-myc, c-jun or b-actin expression at 30 min. A dose response study over 0.1-100nM showed that PACAP effectively stimulated gene induction at physiological (nM) concentrations. A time response study showed maximal c-fos and c-jun induction at 10 min, while b-actin was slightly induced later at 1-2h. Thus, PACAP was shown to regulate the transcription of immediate early genes in PC12 cells which further supports the hypothesis that PACAP is a new neurotrophic factor.

620.19

INNERVATION DEPENDENT PRODUCTION OF CHOLINERGIC DIFFERENTIATION FACTOR BY SWEAT GLAND CELLS S. J. Tresser, M. S. Rao, and S. C. Landis*. Depts. of Neurological Surgery and Neurosciences, Case Western Reserve University, Cleveland, OH 44106.

During normal development the sympathetic innervation of rat sweat glands undergoes a switch from a noradrenergic to cholinergic phenotype. Transplantation experiments indicate that the switch is induced by the target tissue. Previous studies have shown that cholinergic inducing activity that could be responsible for directing the switch is present in extracts of developing and adult rat footpads. In the present studies we have examined whether this activity is due to sweat gland cells and begun to study its regulation. First, homogenates of footpads of tabby mutant mice which lack sweat glands were tested for their ability to induce choline acetyltransferase (ChAT) activity in cultured sympathetic neurons. Levels were significantly reduced, suggesting that most of the activity in normal footpads is associated with the glands. Second, primary cultures of rat sweat gland cells were established. Like sweat gland cells *in situ*, the cultured cells possessed immunoreactivity (IR) for G-protein and cytokeratin but lacked IR for vimentin. In contrast, primary cultures of fibroblasts were IR for vimentin but not G-protein or cytokeratin. Low levels of differentiation factor, measured by induction of ChAT activity in cultured sympathetic neurons, were released by sweat gland cells grown in the absence of neurons. Co-culture with neurons, however, resulted in a twelve-fold increase in the amount of sympathetic cholinergic activity. In addition, vasoactive intestinal (VIP) levels increased significantly, similar to the increase in VIP which accompanies the transition from noradrenergic to cholinergic phenotype *in vivo*. Our results suggest that cell-cell interactions between neurons and sweat gland cells are necessary to induce the latter to produce cholinergic differentiation factor.

OTHER FACTORS AND TROPHIC AGENTS: GENERAL III

621.1

CHANGES IN TAURINE-LIKE IMMUNOREACTIVITY DURING DEVELOPMENT OF THE HIPPOCAMPUS AND CEREBELLUM OF THE RAT. Kathy R. Magnusson.* Dept. of Anatomy & Neurobiology, Colorado State University, Fort Collins, CO 80523

Taurine is believed to play an important role in the development of the central nervous system, but the function(s) are not yet clear. The goal of this study was to determine whether there are changes in anatomical localization of taurine during development of the cerebellum and/or hippocampus of the rat, which could indicate the function(s) of taurine during the developmental process. Rats were perfused transcardially with 4% paraformaldehyde - 2% glutaraldehyde and vibratome sections were labeled with a monoclonal antibody raised against fixative-modified taurine. Taurine-like immunoreactivity (Tau-LI) appeared to be homogeneous in the layers of the hippocampus and dentate gyrus (DG) in 7 and 14 day old animals and differentiation of stained structures in the molecular layers was not possible. By 28 days of age, the labeled dendrites of pyramidal cells were identifiable in CA1 and CA3. By 56 days of age, pyramidal dendrites and cell bodies and granule cells all stained more intensely than the surrounding neuropil. In the cerebellum of 7 day old rats, there is a narrow band of Tau-LI in the molecular layer adjacent to the Purkinje cell layer. This band broadens toward the surface by post-natal day 14 and, by day 28, the entire molecular layer is stained with Tau-LI. Purkinje cell and dendrite staining increases relative to neuropil staining from 14 to 56 days of age. These results show that the anatomical localization of taurine does change during development to young adulthood in the rat in two different brain regions. The identification of these changes should help elucidate the role(s) that taurine plays in development.

621.2

CHANGES IN TAURINE- AND GLUTAMATE-LIKE IMMUNOREACTIVITY DURING REGENERATION IN THE DENTATE GYRUS OF THE RAT. Catherine Tannert, Tim D. Hassinger* and Kathy R. Magnusson. Dept. of Anatomy & Neurobiology, Colorado State University, Fort Collins, CO 80523

Glutamate has been suggested to act as a stop signal for axons, prior to synapse formation. Taurine, which is known to be involved in development of the nervous system, is protective against glutamate toxicity. Our goal was to determine whether these neuroactive amino acids are linked during regeneration by examining changes in immunohistochemical staining in the dentate gyrus following unilateral electrolytic lesions of the entorhinal cortex. With the use of monoclonal antibodies, changes in immunoreactivity were determined by comparing lesioned to sham-operated animals. We have observed an increase in taurine-like immunoreactivity (Tau-LI) in the outer molecular layer of the dentate gyrus, ipsilateral to the lesioned entorhinal cortex in 2 month-old rats. These changes were present at 3, 7, 14, 28 and 56 days post lesion. The increase in Tau-LI within the molecular layer appeared to be associated with the region of increased ACHE staining. Increases in Tau-LI were also seen in the ipsilateral stratum lacunosum/moleculare of CA1, which is another terminal zone for the perforant pathway. Increases in glutamate-like immunoreactivity (Glu-LI) were observed in the outer molecular layer of the dentate gyrus ipsilateral to the lesion in some animals. No changes were observed in Glu-LI in the stratum lacunosum/moleculare. These results suggest that taurine may play a role(s) in the process of regeneration in the young adult rat brain, but these roles are not always linked with changes in glutamate.

621.3

SEROTONIN, TAURINE AND REGENERATION OF GOLDFISH RETINA. L Lima*, P. Matus, M. Urbina. Lab. Neuroquímica, Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas, Apartado 1827, Caracas, Venezuela.

Post-crush goldfish retinal explants have been used to study the regulation of the outgrowth by neuroactive agents Taurine has a trophic effect on this retina in culture and in vivo, partially mediated by the entrance of calcium (Lima et al., 1988,1991). The serotonergic receptors, the transporter and the modulation of the serotonergic system by light stimulus has been also studied in this retina (Lima et al., 1992; Schmeer et al., 1991). Serotonin (5HT) has a selective effect on the development of neurons of invertebrates and vertebrates (Murrain et al., 1990; Sikich et al. 1990). The nerve growth index of retinal explants was evaluated as the product of fiber length and density. Monoamines and metabolites were determined by HPLC with EC. Serotonin (5HT) at nM concentrations completely blocked the regeneration and antagonized the stimulatory effect of taurine. 8-OH-Dipropyl-aminotretalin DPAT, a $5HT_{1A}$ receptor agonist had a similar effect. The $5HT_2$ receptor agonist, (+) -1-2,5dimethoxy-4-iodophenil-2-aminopropane DOI, and serotonin uptake blockers, such as imipramine and fluoxetine were less potent in the impairment of outgrowth. The concentration of 5HT decreased 3 and 5 days after the crush of the optic nerve, 5HT and taurine play a role in the regeneration of the post-crush retina.

Grant S1-2228 - CONICIT

621.4

PRELIMINARY DATA ON NEUROCHEMICAL AND BEHAVIOURAL CONSEQUENCES OF NEONATAL CHRONIC BLOCKADE OF NMDA RECEPTOR IN THE RAT. A. Contestabile,F. Facchinetti,E. Ciani,M. Virgili and R. Dall'Olio. Depts. of Biology and Pharmacology,University of Bologna,Italy. (Spon: ENA)

Recent evidence suggests that NMDA receptor may be involved in survival and differentiation of neurons and synapses. We report data on chronic blockade of this receptor during the critical period of postnatal brain maturation. Neonatal rats were injected dayly with increasing s.c. doses of a non competitive (MK-801) or a competitive (CGP 39551) antagonist of the NMDA receptor from postnatal day 1 to 22. At 24 days of age,body weight was decreased by more than 50% and brain weight by about 25% in treated rats. D-(3H) aspartate uptake and choline acetyltransferase were significantly increased in the cerebellum of treated rats,while markers for GABAergic neurons,astrocytes and myelination were at the same levels of controls. No significant neurochemical differences were noticed in the cortex and hippocampus,except for delayed maturation of the myelination marker. The treated rats,tested with actometric cages,showed a marked hypermotility which lasted for at least 2 weeks after the end of the treatment. Data on adult rats treated in the same way,will be presented.

621.5

Chronic neonatal NMDA receptor blockade with MK-801 alters monoamine metabolism and affects spatial learning in the adult rat. J.A. Gorter*, G.J. Boer, M.G.P. Feenstra, M.H.A. Botterblom and J.P.C. de Bruin. Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands (Spon: ENA)

We examined the question whether chronic MK-801 treatment in neonatal rats (0.25 mg/kg injected s.c.; twice per day, from postnatal day 8 through 19), which previously had been shown to alter NMDA receptor function, would also affect spatial learning in the adult rat. MK-801 treated rats were able to learn the spatial (watermaze) task as well as control rats but at a significantly slower rate. Visual cue learning was not affected by the neonatal treatment, indicating that the slower spatial learning is not caused by either locomotor or sensory deficits. Although MK-801 injections induced increased motor activity throughout the treatment period, in adulthood there were no longer differences apparent. At the same time, however, monoamine metabolism of the frontal cortex and striatum as detected by high pressure liquid chromotography with electrochemical detection, was higher in the MK-801 treated group than in the control group. Dihydroxyphenylacetic acid (DOPAC) concentrations were elevated (>40%) in both regions tested, while 5-hydroxyindol-acetic acid (5-HIAA) was significantly elevated only in the cortex (19%), and 3-methoxy-4-hydroxyphenylglycol (MHPG) was elevated only in the striatum (47%). The increase in the concentration of monoamine metabolites along with the previous demonstrated alterations in NMDA receptor function, could contribute to the impairment in spatial learning in adult rats neonatally treated with MK-801.

621.6

STIMULATION OF SOMATOSTATIN EXPRESSION IN CULTURED CILIARY GANGLION NEURONS BY ACTIVIN IN CHOROID CELL CONDITIONED MEDIUM. J. N. Coulombe[1], R. Schwall[2], A.S. Parent[3], F.P. Eckenstein[3], and R. Nishi*[3]. [1]Dept. of Anatomy, Uniformed Services University of the Health Sciences, Bethesda MD 20814; [2]Dept. of Endocrinology, Genentech Inc., So. San Francisco CA 94080; [3]Dept. of Cell Biology & Anatomy, Oregon Health Sciences University, Portland OR 97201.

We are interested in testing the hypothesis that neurotransmitter phenotype can be regulated by interactions between neurons and the target tissues that they innervate. The chicken ciliary ganglion (CG) contains two populations of neurons: ciliary neurons that innervate striated muscle in the iris and ciliary body and choroid neurons that innervate vascular smooth muscle in the choroid layer of the eye. Ciliary and choroid neurons differ in their transmitter phenotype in that choroid neurons use the neuropeptide somatostatin as a co-modulator with acetylcholine. We have previously shown that CG neurons are induced to express somatostatin-like immunoreactivity (SOM-IR) by co-culture with choroid cells. This interaction is mediated by a macromolecule in choroid conditioned medium *(Coulombe & Nishi, J. Neurosci. 11: 553).* Here we present evidence that this somatostatin stimulating activity is likely to be activin A, a molecule that has been implicated in a variety of other developmentally important phenomena. Our results are the following: 1) human recombinant activin A induces SOM-IR in cultured CG neurons; 2) ChCM mimics activin by inducing hemoglobin synthesis in K562 cells; 3) hemoglobin synthesis induced in K562 cells by ChCM is inhibited by inhibin; 4) western blot analysis of ChCM with activin A specific antibodies reveals one band that co-migrates with human activin A; 5) northern blot analysis of poly-A+ RNA from cultured choroid cells reveals a band that hybridizes at high stringency with an activin A (inhibin) β subunit riboprobe; 6) follistatin, an activin-binding and inactivating molecule, abolishes the ability of ChCM to induce hemoglobin in K562 as well as its ability to induce SOM-IR in CG neurons. These results suggest that activin may serve as a target-derived factor controlling somatostatin in vivo. Supported by NS25767 (RN), AG07424 (FPE), EY06178 (JNC), and EY06352 (ASP).

621.7

SOMATOSTATIN ENHANCES NEURITE OUTGROWTH IN PC 12 CELLS

Donna Ferriero*, R. Ann Sheldon, Robert Messing

Somatostatin (SS) is present in selective regions of the developing central nervous system in very high quantities suggesting a role for the peptide in neuronal differentiation. SS is in growth cones of the human cat retina and can stimulate neurite outgrowth in molluscan neurons. We have studied the effect of SS on neurite outgrowth in a well defined model of neuronal differentiation, PC12 cells after Nerve Growth Factor (NGF) induction. Using this model we have found that SS can enhance the NGF induced neurite outgrowth. We plated PC12 cells at a density of 10×10^3 cells/well and examined cells every 24 hours for neurite formation after incubation with SS (100nM-10uM) alone and in combination with NGF. A neurite was defined as a process greater than one cell body in diameter in length possessing a terminal growth cone. The percentage cells bearing neurites were calculated by counting 100 cells/well in quadriplicate wells. Cells plated on polyomithine coated dishes grown in defined medium containing 50ng/ml NGF began to develop distinct neurites on day 4. Simultaneous treatment with 1uM SS increased the percentage of neurite-bearing cells significantly (NGF 70± 10% v. SS ± NGF 84±6%; $p \leq 0.03$). On day 2 using variable concentrations of NGF (1-4nM), a marked increase in the number of cells bearing neurites was seen in the presence of 1uM SS (70% v. 7%). On inspection of the cells, those treated with SS + NGF had significantly longer neurites than the NGF treated cells (23.4 ± 12.9 v. 18.6 ± 9.3um; $p<0.01$). In addition, the complexity of neuritic networks was greater in the SS + NGF treated cultures. These preliminary data suggest that SS may function as a trophic factor in early neuronal differentiation, promoting neurite outgrowth.

621.8

THYROID HORMONE ALTERS CHOLINERGIC SOMAL ENLARGEMENT AND RECOVERY OF CHAT+ NEURON NUMBER AFTER AXOTOMY OF PROJECTIONS FROM BASAL FOREBRAIN TO MEDIAL CORTEX. T.W. Farris* and L.L. Butcher. Laboratory of Chemical Neuroanatomy and Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563.

To examine the regenerative morphologic effects of putative growth-promoting factors on cholinergic neurons, we administered thyroxine (T4, 2.5 mg/kg, ip) or saline daily for 7, 15, 30 or 60 days to 8 week-old female rats following unilateral knife-cut axotomies of the cholinergic medial pathway which projects from the cholinergic basal nuclear complex (CBNC) to cingulate and occipital medial cortices. Brain tissue was processed immunohistochemically for choline acetyltransferase (ChAT) and for Nissl substance. In the saline groups, computerized morphometry using light microscopy showed surprisingly that ChAT+ medial septum (MS) somata—which do not project appreciably to medial cortex and, thus, were not axotomized—underwent mild transient enlargement at 30 days compared to 7 and 60 days ($p < .05$). Yet, no effect was seen in somata of the vertical limb of the diagonal band (VDB) which do project to cortex and were axotomized. Concommittantly, maximal loss of ChAT+ somal number on postlesion days 7 (for MS) and 15 (VDB) recovered by day 60 ($p < .05$ each). No change in somal number was seen in Nissl-stained sections possibly indicating only altered ChAT expression. T4 advanced the peak MS somal enlargement from day 30 to 15, and peak VDB ChAT+ neuronal number from day 60 to 15. These data imply that adult CBNC neurons, taxed metabolically by a sublethal axotomy, appear to undergo augmented recovery in response to thyroid hormones. [Support: NIH NS 10928 to L.L.B.]

621.9

K252a POTENTIATES EPIDERMAL GROWTH FACTOR-INDUCED DIFFERENTIATION OF PC12 CELLS. C-F. Wu, J. Umbach * & B.D. Howard. Dept. of Biol. Chem. UCLA Sch. of Med. Los Angeles, CA 90024

Epidermal growth factor(EGF) induces the neuronal differentiation of PC12 cells in that it causes the extension of short neurites. The protein kinase inhibitor k252a blocks NGF-induced neurite outgrowth, but it potentiates the EGF effect, resulting in long branched neurites comparable to those induced by NGF. The related compound, K252b which cannot permeate cell membranes, inhibits NGF-induced differentiation, but it neither inhibits nor potentiates EGF-induced neurite outgrowth suggesting that k252a produces its potentiating effect on EGF by acting at an intracellular site. RNA synthesis and activity of the ras proto-oncogene product are required for neurite outgrowth induced by EGF or by a combination of EGF and k252a. EGF increases the mRNA levels of two late response genes (SCG10 and 63) that had previously been found to be induced in PC12 cells only by NGF and FGF. K252a increases the EGF-induced expression of these two genes. These results suggest that EGF and NGF trigger similar but not identical signal transduction pathways.

621.10

EGF INCREASES THE NUMBER OF PROLACTIN CELLS IN NEONATAL RAT PITUITARY CULTURES. R. Felix, A. Navarrete, A. Marin and G. Cota*. Department of Neurosciences, Cinvestav, Mexico, DF 07000.

It is known that chronic treatment of rat pituitary tumor cells with EGF stimulates prolactin production. We investigate here the action of EGF on the prolactin cell population of neonatal (10-day old) male rats. Anterior pituitary cells were cultured for 2 days, in the absence or presence of 5 nM EGF in the culture medium. The relative number and basal secretory activity (prolactin plaque area) of the lactotropes was then determined by using the reverse hemolytic plaque assay. In control conditions, 8.0 ± 0.2% (mean ± SE, n = 3) of all pituitary cells induced plaque formation and plaque area was 1980 ± 230 μm^2. EGF induced a 50-60% increase in both the proportion of plaque-forming cells and mean plaque area. Frequency distributions of prolactin plaque sizes indicated the existence of two lactotropes subpopulations in both experimental conditions: small- and large-plaque formers. The additional prolactin cells induced by EGF all formed large plaques. Thus, EGF seems to stimulate prolactin secretion in pituitary cultures by promoting the differentiation of a lactotrope subtype.

621.11

SEROTONIN AND ALTERED BEHAVIOR IN THE MODELS OF DEPRESSION AND AGGRESSION IN MALE TRANSGENIC TGFα MICE. Hilakivi-Clarke L.A.*, T. Taira, Goldberg, R. and Clarke R. LCRC, and Dept. Psychiatry, Georgetown Univ., Washington, DC; and Dept. Physiol., Univ. Helsinki, Helsinki, Finland

Transgenic MT42 male mice overexpress the human transforming growth factor α (TGFα) in multiple tissues, including the brain. These animals exhibit increased depressive tendencies in the swim test and increased aggressive behavior in the resident-intruder paradigm, when compared with appropriate CD-1 control mice. Brain monoamine analyses revealed that the levels of norepinephrine, dopamine and serotonin (5-HT) were not significantly altered in the hypothalamus, frontal cortex, or brain stem in the male TGFα mice. However, these animals showed reduced 5-HT turnover in the brain stem ($p<.05$). To elevate the 5-HT turnover, the male TGFα and control mice were treated intraperitoneally with 100 mg/kg tryptophan, a precursor of 5-HT, or with 5-HT uptake inhibitors zimeldine (12.5 mg/kg) or clomipramine (10 mg/kg) 30 min prior to the behavioral tests. Tryptophan did not influence the immobility of the CD-1 mice, but significantly reduced immobility in the swim test in the male TGFα mice ($p<.01$) ($F(2,22)=7.4$, $p<.006$). Similarly, zimeldine reversed the elevated immobility in the male transgenics ($p<.05$), while it increased the immobility time in the controls ($p<.05$) ($F(2,31)=6.9$, $p<.003$). In the resident-intruder paradigm, the TGFα exhibited highly elevated levels of aggression. Tryptophan did not alter the time spent in aggression, but clomipramine significantly reduced aggressive behavior both in the controls and TGFα mice ($F(1,24)=15.3$, $p<.004$).

These results indicate that the increased depressive and aggressive tendencies noted in the male TGFα mice appear to be reversed by 5-HT uptake inhibitors. Thus, TGFα may interact with 5-HTergic systems in the brain.

621.12

PRENATAL HALOPERIDOL (HAL) OR RESERPINE (RES) STUNTING OF RAT BODY AND BRAIN WEIGHT: IN VIVO AND IN VITRO EXAMINATION OF SENSITIVE PERIODS AND DOPAMINERGIC MECHANISMS. P.J. Webb, R.R. Holson*, S.F. Ali, T.F. Grafton, and D.K. Hansen, NCTR/FDA, Jefferson, AR 72079-9502.

Pregnant albino rats were exposed to vehicle, HAL (5 mg/kg twice daily) or RES (0.2 mg/kg twice daily) over mid gestation (gestation days [GD] 12-16) or late gestation (GD 16-20). Another control group was pair-fed both to HAL and RES dams. Offspring body weight, regional brain weight, DNA and protein were evaluated in youth (28-30 days age) or adulthood (90-112 days age). Mid-gestational HAL or RES permanently reduced offspring body weight, regional and whole brain weight and regional brain DNA and protein content at both ages. HAL and RES had comparable effects on whole brain weights (reduction to 90% of control) while RES exposure produced a greater stunting of body weight than did HAL. Comparisons to pair-fed controls revealed that this growth stunting was not due to reduced maternal food intake. Late gestational RES exposure was highly feto-lethal, unlike late HAL exposure. Late gestational exposure to either drug had no effects on offspring body or brain weight that were different from pair-fed controls, with the exception of a permanent and specific HAL not RES stunting of caudate weight, DNA and protein content. In a second experiment, cultured GD 9 embryos were grown for 48 hr in medium containing a range of concentrations of HAL, RES, sulpiride (a specific D_2 antagonist) or SCH23390 (a specific D_1 antagonist). HAL substantially reduced embryonic growth at much lower concentrations than did any of the other compounds. We conclude that prenatal HAL reduces offspring body and brain weight after mid- but not late- gestational exposure, through a mechanism which is probably not dopaminergic.

621.13

MECHANISM OF A HEPATOCYTE SECRETED FACTOR AS AN ACTIVATOR OF NERVE REGENERATION. H.HORIE[1]*, T.KADOYA [2] AND M.TAKANO [3]. Dept. of Physiol.[1] and Ophthalmol.[3],Sch. of Med., Yokohama City Univ., Yokohama 236 and Pharmaceut.,Lab., Kirin Brewery Co.[2], Maebashi 371, JAPAN.

Hepatocyte-conditioned media enhanced neurite regeneration and their survival from nerve-transected terminals of adult dorsal root ganglia with nerve fibers(Horie et al.,NeuroReport, 1991). But this media did not promote neurite regeneration of dissociated adult DRG neurons. Application of a hepatocyte secreted factor to adult rat retina explants showed that this factor enhanced neural regeneration not only in matured peripheral nervous tissues but also in adult central nervous tissues. In comparison with other known neurotrophic factors, NGF, IGF-I and II, EGF, aFGF, bFGF, Insulin, Interleukins(1 to 8) were applied to the peripheral explants. NGF, IGF-I and II, Interleukin 3 and 6 enhanced neurite regeneration, but not survival. NGF did not affect neurite regeneration in adult retina explants. These results indicate that the factor secreted from hepatocytes was different from the other known neurotrophic factors. To analyze mechanism of the factor anti-NGF was administered to the peripheral tissues. This antibody inhibited neurite regeneration from nerve-transected terminals under treatment with hepatocyte conditioned medium. After 5 days in culture the addition of this antibody did not inhibit neurite survival. This result suggests that the factor secreted from hepatocytes may stimulate Schwann cells to release NGF and a survival factor.

621.14

DO NIGRAL NEURONS PRODUCE A TROPHIC FACTOR THAT SUPPORTS THE GROWTH OF OTHER NIGRAL NEURONS? C. M. Buhrfiend*, L.R Ptak, D.K. Sierens, S.J. Yu, and P.M. Carvey. Rush-Presbyterian St. Lukes MC, Chicago IL, 60612

The growth of mesencephalic cultures is density dependent, i.e. with a linear increase in cell number, the number of viable cells with processes increases geometrically. This is often referred to as the "autoconditioning" effect. We performed two series of experiments to determine whether or not this autoconditioning effect was due to a soluble factor. Primary, dissociated, E15.5 rostral mesencephalic tegmentum (RMT) cultures were plated out at a density of 250,000 cells/well and were grown in defined media. Media from these cultures was collected at weekly intervals (1-4 weeks) and stored at -70C. 50 uL of this conditioned media was added to freshly plated low cell density (3,500 cells/well) RMT cultures growing in defined media. 40 hours later, the cultures were scored for the number of viable cells with processes. Cultures incubated with conditioned media from 3 and 4 week old RMT cultures had more viable neurons with processes than cultures incubated with conditoned media from 1-2 week old cultures ($p < 0.01$). In a second experiment extracts of the substantia nigra from adult rats stimulated the growth of low cell density RMT cultures relative to the effects of extracts of the cerebellum ($F = 7.15$). Moreover, the growth promoting effect of extracts of the substantia nigra decreased linearly with age (2-24 months, $r = -0.663$). These data suggest that cells of the substantia nigra produce a soluble factor capable of stimulating the growth of other cells within the nigra. It is possible that the nigral cell loss that accompanies aging and Parkinson's disease may facilitate further neuron loss due to the reduction of this nigral-derived "autoconditioning" factor.

621.15

DIFFERENCE IN THE ELECTROPHORETIC MOBILITY OF RAS PROTEIN FROM DIFFERENTIATING AND PROLIFERATING PC12 SUBCLONES. Y. Y. Rozenberg, A. Grinnell* and B.D. Howard. Dept. of Biol Chem. UCLA Sch. of Med. Los Angeles, CA 90024

Oncogenic ras causes differentiation of PC12 and proliferation of other cells. A PC12 flat cell variant was transfected with inducible oncogenic ras and subclones were selected. Ras expression caused differentiation of one subclone, 6B3, but it caused proliferation of another subclone, 3A4. Ras protein (p-ras) from 6B3 and 3A4 migrated as 22 kd and 21 kd forms, respectively. After phosphatase treatment, p-ras from 6B3 cells migrated as a 21 kd protein, but the mobility of p-ras from 3A4 cells did not change. This suggests that phosphorylated ras may be involved in differentiation. After treatment of the cells with mevenolin, which inhibits the synthesis of cholesterol precursors and thus protein farnesylation, p-ras from either cell migrates as a 23 kd protein indicating that each is farnesylated. Mevinolin causes PC12 to extend precess that look like neurites. This morphological change is inhibited by introduction into the cells of anti-ras antibody suggesting that ras is involved in this morphological change.

621.16

INFUSION OF MONOCLONAL ANTIBODIES AGAINST AN ENDOGENOUS DA-RELEASING PROTEIN (DARP) INTO THE SUBSTANTIA NIGRA (SN) OF FREELY MOVING MALE RATS DIMINISHES STRIATAL DA AND DOPAC CONCENTRATIONS. H. Cardenas, V. D. Ramirez*. Department of Physiology & Biophysics, University of Illinois at Urbana-Champaign, IL 61801.

The presence of an endogenous DARP has been reported in extracts from adrenal, striatum and cerebellum. DARP has a potent DA-releasing activity and neonatal administration of monoclonal anti-DARP antibodies arrest fetal development and decrease brain catecholamines (Moll. Cell. Neurosci. 2:410-417, 1991). To disclose a possible role for DARP during adulthood we administered monoclonal anti-DARP antibodies (IgM) into the SN of male Sprague-Dawley rats (300-400g B.W.). The antibody was infused at a dose of 10µg/10µl/10 min during 5 consecutive days through a stereotaxically implanted cannula made with a 23 g syringe fixed to the skull. Controls were infused with an IgM anti-fluorescein antibody. On the sixth day, the rats were submitted to an amphetamine test (1 mg/kg i.p.) and their behavior quantified by a computerized activity monitor (OMNITECH) during 30 min. Striatal DA and DOPAC levels were determined by HPLC with electrochemical detection after the amphetamine test. The antibody treatment did not change the ipsilateral DA (controls: 11.6 ± 1; Exps = 12.2 ± 0.6, n=7) nor DOPAC* (Controls: 5.9 ± 0.3; Exps = 6.2 ± 0.5) levels. But decreased ($p<0.01$, n=7) DA (from 13.7 ± 0.4 to 8.5 ± 0.8) and DOPAC (from 6.1 ± 0.4 to 4.7 ± 0.3) levels in the contralateral striatum. There was no difference in the behavioral response to amphetamine. Our results suggest that the blockade of endogenous DARP interfered with the uptake of DARP by terminals in the SN arising from the contralateral SN. This would then lead to a decrease in the catecholamine levels in the contralateral striatum.

*values are in ng/mg of wet tissue.

621.17

A NEUROTROPHIC FACTOR OPERATIVE IN THE GENICULOCORTICAL PATHWAY OF RATS: ISOLATION, PURIFICATION AND *IN VITRO* TESTING. K.L. Eagleson*, P. Levitt, I. Fischer and T.J. Cunningham. Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, Pa. 19129.

We have previously identified neuron survival promoting activity present in medium (CM) conditioned by explants of the embryonic primordia of the geniculocortical pathway. The active fraction of the CM promotes the survival of drosal lateral geniculate nucleus (dLGN) neurons following visual cortex lesions in both the neonate and the adult rat. To identify the molecule(s) responsible for this activity, we raised monoclonal antibodies to proteins present in the active fraction. One antibody, 8G6, recognises a 55kD molecule on Western blots. Following immunoaffinity purification and silver staining of gels, we have detected an additional minor band at 110kD which is diminished upon incubation with DTT, indicating the native protein may be present as a dimer. To assess the neurotrophic effects of this molecule *in vitro*, dissociated embryonic day 17 lateral thalamic neurons (which include those of the dLGN) were grown with different concentrations of the afinity-pure 8G6 antigen in defined, serum-free medium. Neurons and dendrites were identified immunocytochemically using an antibody against microtubule-associated protein 2 (MAP 2). Neurite outgrowth is enhanced at 0.05-5ug/ml of the 8G6 antigen and inhibited at 50ug/ml. The same effect is seen at 2 and 4 days in culture. Interestingly, the higher concentration of 8G6 antigen also caused decreased neuron survival. We suggest that the 8G6 antigen is a neurotrophic component of the CM fraction, which we have shown previously to act in a similar concentration-dependent manner *in vivo* (see Eagleson *et al*, 1990). Supported by NS16487 from NINCDS and TRG-89-014 from Alzheimer's Association.

621.18

A NEUROTROPHIC FACTOR OPERATIVE IN THE GENICULOCORTICAL PATHWAY OF RATS: LOCALIZATION AND NEURON SURVIVAL *IN VIVO*. T.J. Cunningham*, F.A. Haun, S.E. Kennedy, P. Levitt and K.L. Eagleson. Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, Pa. 19129.

Previous studies have shown that a fraction of culture medium(CM) conditioned by the embryonic primordia of the geniculocortical pathway will prolong survival of dorsal lateral geniculate nucleus (dLGN) neurons when delivered to the cavity of a large posterior cortex lesion in newborn rats. A molecule that provides neurotrophic support *in vitro* to posterior thalamic neurons (including those of the dLGN) has been isolated and purified from this CM fraction using a monoclonal antibody (8G6). In this study, we tested the neuron survival-promoting activity of this molecule *in vivo*, and examined its distribution in the cerebral cortex and thalamus of neonatal rats. The entire posterior neocortex was ablated from newborn rats and calcium-alginate gel beads, loaded with the affinity-pure 8G6 protein, were placed in the lesion cavity. Five days later, when the dLGN in untreated animals has virtually disappeared, operates with implants of the 8G6 antigen show a significant increase in dLGN neuron survival. Immunostaining of normal neonatal rats reveals patchy 8G6 labeling in several cortical regions with dense staining in parietal and occipital areas. Within these patches, stained cells can be found in all cortical layers but the most consistent labeling is in the soma and dendrites of pyramidal cells in layers III and V. In addition, labeled cells are particularly prominent in neurons of layer VIB. The cortical white matter and resident glial cells are not stained. Surprisingly, immunopositive cells are rare in thalamo-cortical projection nuclei (including the dLGN) which may reflect rapid processing of the factor by responsive neurons. The results suggest that the 8G6 antigen is an endogenous neurotrophic factor in the neonatal rat. Supported by NS16487 from NINCDS and TRG-89-014 from Alzheimer's Association.

621.19

NEUROTROPHIC ACTIVITY OF OTOCYST-DERIVED FACTOR: COMPARISON WITH OTHER GROWTH FACTORS AND GANGLIA IN CHICK. L.M. Bianchi* and C.S. Cohan Dept. of Anatomical Sciences, SUNY at Buffalo, Buffalo, N.Y. 14214

During early stages of auditory development, the otocyst releases a factor which promotes outgrowth from the associated statoacoustic ganglia (SAG). Although the release of this factor decreases at later stages of development, the SAG maintain an ability to respond to the otocyst-dervied factor (ODF). We compared the neurotrophic activity of the ODF to other growth factors by 1.) placing ODF on ganglia known to respond to specific growth factors and 2.) testing the ability of specific growth factors to influence SAG. Application of ODF promoted outgrowth from explants and disscoiated cell cultures of chick DRG and sympathetic ganlglia, but not from ciliary or trigeminal ganglia (DM or VL). Outgrowth from DRG and sympathetic ganglia may indicate an NGF-like molecule is released by the otocyst. However, addition of mouse anti NGF antibodies did not block the ability of ODF to promote outgrowth from DRG, sympathetic ganglia or SAG. Experiments were then done to compare whether other growth factors mimic the response of the ODF. At early stages of development (E4-E6), both NGF and CNTF produced outgrowth from explant and dissociated cell cultures of SAG. However, compared to ODF, the length of outgrowth and the number of surviving cells was less in the presence of these growth factors. Additionally, NGF and CNTF were unable to promote outgrowth from older stage SAG (E7-E13). Thus, NGF and CNTF did not mimic the neurotrophic effects of the ODF. These experiments suggest that the ODF has neurotrophic activity which is different from NGF and CNTF.

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621.20

EXPRESSION OF THE PLEIOTROPHIN GENE IN DEVELOPING MOUSE CENTRAL NERVOUS SYSTEM. H.-J. Yeh, I. Silos-Santiago, R.P. Guillerman, Y.-S. Li, W.D. Snider, and T.F. Deuel*. Dept. of Medicine, Biochemistry, and Neurology, Washington Univ. and the Jewish Hospital, St. Louis, MO 63110.

Expression of the pleiotrophin (ptn) gene was analyzed within the developing mouse central nervous system (CNS) by in situ hybridization. Ptn mRNA was first detected at embryonic day 12 (E12). At E15 and E18 in spinal cord, ptn transcripts were primarily localized in neurons of the dorsal horn of the intermediate gray and of the region surrounding the central canal. Levels of the ptn transcripts decreased progressively postnatally and were absent in adults. In the brain at E15 and E18, ptn mRNA was highly expressed in cells of the ventricular zone and ependymal layer bordering the ventricles. Levels increased progressively in cortical neurons during the postnatal period and were also detected in the dienceph-alon, midbrain, and brainstem. In adults, prominant expression of ptn mRNA was seen among Purkinje cells and Golgi-epithelial cells of the cerebellum, pyramidal cells of hippocampal field CA1, and neurons of cortical layers II and III. Cortical glial cells and cells in the hippocampal white matter also expressed ptn during adulthood. These results indicate that the ptn gene is expressed in a highly spacially and temporally restricted manner in the developing CNS and support a unique role of the ptn gene during embryogenesis and in maturity.

622.1

Cells Genetically Modified to Secrete NGF Induce Dense Growth of Sensory Fibers after Transplantation to the Spinal Cord.

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Dept. of Neurosciences, Univ. of California-San Diego, La Jolla, CA. 92093-0627.

In the brain, grafts of cells genetically modified to produce trophic factors or other substances improve neuronal survival and promote morphological and functional recovery after injury in the adult. We sought to determine whether neurites of the adult rat spinal cord are also responsive to trophic factors, and if grafts of nerve growth factor (NGF)-secreting genetically modified cells to the spinal cord could promote neuronal recovery. Primary fibroblasts of adult Fischer 344 rats were genetically modified to produce NGF or ß-galactosidase (as controls), and grafted to the intact spinal cord. Fibroblasts naturally secrete collagen and fibronectin, which could produce a "bridge" over which neurites might grow.

32 subjects (16 NGF, 16 ß-gal) underwent T7 laminectomy and received suspension grafts of $6x10^5$cells in 5 ul into the center of the cord. The large graft volumes caused local cord damage and transient motor deficits in the legs. Two weeks later, graft survival was evident in all subjects examined; however, NGF-secreting grafts showed a very dense ingrowth of fibers, indicated by NGF receptor and neurofilament immunocytochemistry. These fibers appeared to be of sensory origin, since they labeled robustly for calcitonin gene-related peptide (CGRP), but not choline acetyltransferase, acetylcholinesterase, or tyrosine hydroxylase. After two months, control grafts were substantially smaller than NGF-secreting grafts; again, fibers grew only into the NGF-secreting grafts. Thus, sensory neurites respond vigorously to intraspinal grafts of NGF-secreting cells. Grafts of fibroblasts genetically modified to secrete NGF or other trophic factors may provide an environment that promotes neuronal recovery after spinal cord injury.

622.2

AXONAL PROJECTIONS BETWEEN THE ADULT RAT SPINAL CORD AND EMBRYONIC NEURAL TISSUE TRANSPLANTS: ORIGIN and DISTRIBUTION Prashant Rai*, Jane Brasko and Gopal D. Das Purdue University, Dept. of Biological Sciences, W. Lafayette, IN 47907 and Univ. of Pennsylvania School of Medicine, Dvn. of Neurosurgery, Philadelphia, PA 19104.

The pattern of afferent connectivity achieved by transplantation of 15-days old embryonic neocortex (n=12), spinal cord (n=12) & brainstem (n=12) into a central hemorrhagic (CH) lesion in the spinal cord of the adult rat was examined in this study. The animals were allowed to survive for 2-3 months post-operatively, and towards the end of this period, a second lesion (right hemisection or CH) was made 2-3 segments rostral or caudal to the site of transplantation to induce degeneration in any long descending or ascending fibers that might have sprouted & given off afferents to the transplants. The animals were perfused & the spinal cords were processed for the Fink-Heimer technique, to permit visualization of degenerating afferents, and for Cresyl violet staining, to permit accurate assessment of host/graft interface regions. Consistent with other transplantation paradigms, afferents were observed to cross the interface and enter the transplants from the host parenchyma. These were collaterals derived from host fibers in the immediate vicinity of the interface, most often from the gray matter. The afferents did not demonstrate any specificity or pattern of distribution. In general, the distribution of these collaterals was somewhat random within the transplant neuropil, & was restricted to the borders of the transplants. There was no evidence to indicate that these afferents extended into the middle of the transplant or that they traversed the length of the transplant to end in the spinal cord on the opposite side. Their distribution was also quite independent of the location of the second lesion in the host spinal cord. The amount of afferent ingrowth in general could be classified as low to moderate in all the transplants, regardless of tissue type. These results indicate that axons of the traumatized adult spinal cord are capable of sprouting into different kinds of embryonic neural tissue transplants, & this capacity is rather limited & restricted to axons near the lesion site only.

622.3

INFLUENCE OF FETAL NEURAL TISSUE TRANSPLANTS ON HISTOPATHOLOGICAL REACTIONS OBSERVED IN THE TRAUMATIZED ADULT SPINAL CORD Jane Brasko*, Prashant Rai and Gopal D. Das Univ. of Pennsylvania School of Medicine, Dvn. of Neurosurgery, Philadelphia, PA 19104 & Purdue Univ., Dept. of Biological Sciences, W. Lafayette, IN 47907.

Embryonic neural tissues transplanted into the traumatized spinal cord of the adult rat have been shown to influence the expression of various histopathological reactions, e.g., glial cell & fibroblast proliferation, spinal cord necrosis & cyst/cavity formation (Das 1986). In the present study, we analyzed the extent of these host histopathological reactions following the transplantation of 1 of 3 different donor tissue types-- 15-days old embryonic neocortex (n=12), brainstem (n=12) or spinal cord (n=12), into a central hemorrhagic (CH) cavity at T-10/T-11 or L-2/L-3 of the adult rat. Lesioned animals that did not receive transplants served as controls (n=7). Histological observations of the spinal cords of these animals after 2-3 months post-operative survival revealed the following: glial cell proliferation and host spinal cord necrosis was very pronounced in the spinal cords of the control animals. However, cyst/cavity formation in these animals was negligible, observed in only 2 of the 7 cases. The expression of these histopathological entities was likewise highly exaggerated in spinal cords that received brainstem or spinal cord transplants. Due to the limited growth of these transplants, there was often space left between them and the walls of the cavity, which permitted excessive proliferation of glial & connective tissue cells, as well as the degeneration of adjacent host tissue. Conversely, the expression of such a response was very limited in spinal cords receiving neocortical transplants. This was due, in general to the great volumetric growth of these transplants, which partially suppressed the development of the host pathological response. These results indicate that the presence of a neural transplant may ameliorate the histopathological sequelae of a CH lesion in the adult spinal cord. However, this effect is not uniform but directly related to the type of tissue transplanted into the site of injury.

622.4

AXONAL GROWTH INTO SCHWANN CELL-SEEDED GUIDANCE CHANNELS GRAFTED INTO TRANSECTED ADULT RAT SPINAL CORD.

X.M.Xu, V. Guénard, N. Kleitman, and M.B. Bunge*. The Miami Project, Univ. of Miami Sch. of Med., Miami, FL 33136.

Schwann cells (SCs) have been shown to effectively promote axonal regeneration in both PNS and CNS. We are testing the ability of SCs to enhance regeneration in adult rat spinal cord when they are present in guidance channels grafted into transected cords. SCs were purified in culture from adult rat sciatic nerves, suspended in a 30:70 solution of Matrigel:DMEM (M/D), and seeded into PAN/PVC permselective channels (2.6 mm ID x 10 mm long; MWCO, 50K Da) at a final density of 80 or 120 x 10^6 cells/ml. Some SCs were labeled with Hoechst 33342 nuclear dye before implantation. Empty and M/D channels served as controls. The spinal cord was transected at T-8, and 3 segments caudal to this level were removed. The rostral cut end was inserted approximately 1 mm into the channel and the distal end of the channel was capped. One month after grafting, a blood vessel-rich cable had formed within the channel in all animals studied. Numerous Hoechst labeled SCs were observed in the cable, many in close association with ingrowing axons, indicating that cultured SCs had survived in the channel. In SC-seeded channels, numerous myelinated axons existed within the cables and some of them extended the whole length of the channels (9-10 mm). Electron microscopy revealed that many unmyelinated axons had grown into the cables as well. A subset of axons entering the graft stained for CGRP. More axons were observed in SC-seeded channels than in the empty channel. In conclusion, when SCs are placed in permselective channels grafted into transected adult rat spinal cord, they stimulate the ingrowth of both myelinated and unmyelinated axons. (Supported by NIH grants NS 09923 and NS 28059 and The Miami Project)

622.5

REDUCTION IN ADJUVANT ARTHRITIS-INDUCED HYPERVENTILATION IN RATS WITH ADRENAL MEDULLARY TRANSPLANTS IN THE SPINAL SUBARACHNOID SPACE. H. Wang* and J. Sagen. Department of Anatomy & Cell Biology, University of Illinois at Chicago, Chicago, IL 60612

Our previous studies have shown that transplanted adrenal chromaffin cells into the CNS pain modulatory regions can reduce acute pain sensitivity. We have extended this work to the reduction of chronic pain by the implants. The adjuvant-induced arthritis model in rats was chosen in the present study since behavioral features of this model, such as a retardation of weight gain and hyperventilation are similar to those seen in human chronic pain conditions. The purpose of these studies was to assess whether adrenal medullary transplants could reduce chronic arthritis pain. To assess this, two groups of adult rats were implanted with either rat adrenal medullary tissue or striated muscle tissue into the spinal cord subarachnoid space, and were injected with Mycobacterium butyricum intradermally into the their tail bases. Body weight, tibio-tarsal joint circumferences and ventilatory responses were measured at weekly intervals. Results showed that spinal adrenal medullary implants in arthritic rats could attenuate the severe retardation of body weight gain seen in control implanted animals. In addition, chronic hyperventilation, associated with chronic pain, was markedly reduced in arthritic rats with adrenal medullary implants, but not control implants. These effects of adrenal medullary implants were attenuated by opiate antagonist naloxone and alpha-adrenergic antagonist phentolamine. Together, these results indicate that transplanted adrenal medullary tissue reduces chronic arthritic pain by releasing catecholamine and opioid peptides into the spinal subarachnoid space. (Supported by NIH grant NS25054)

622.6

PAIN-RELATED BEHAVIOR IN RATS WITH PERIPHERAL NEUROPATHY IS REDUCED WITH BOVINE CHROMAFFIN CELLS TRANSPLANTED INTO THE SPINAL SUBARACHNOID SPACE. A. T. Hama* and J. Sagen. Dept. of Anat. and Cell Biol., Univ. of IL at Chicago, Chicago, IL 60612.

Adrenal medullary tissue allografts transplanted into subarachnoid space, by releasing catacholamines and opioid peptides, may be a clinically useful procedure in reducing pain. Previous work has shown that adrenal medullary tissue transplanted into animals with unilaterally-induced peripheral neuropathy reduced allodynia and hyperalgesia for up to two months. Bovine adrenal chromaffin cells have also been shown to be effective in reducing nociception and may be used as an alternative to human adrenal tissue. The long-term effectiveness of bovine chromaffin cells in reducing pain was evaluated in an animal model of painful peripheral neuropathy, induced by loosely ligating the right sciatic nerve. Various pain syndromes are observed including thermal allodynia and thermal and mechanical hyperalgesia. To quantify thermal allodynia, animals were placed on an innocuous cold (5° C) metal plate for 20 min. and the number of hindpaw lifts and duration of lifting were recorded. To assess mechanical hyperalgesia, hindpaw withdrawal thresholds were measured using the Randall-Selitto test. Finally, to quantify thermal hyperalgesia, hindpaw withdrawal latencies to a noxious radiant heat source were measured. After baseline behavioral evaluation, isolated bovine chromaffin cells were transplanted in subarachnoid space at the level of the lumbar enlargement. Animals were tested weekly for 9 weeks. Animals that received chromaffin cell transplants showed increased withdrawal latencies and thresholds to noxious heat and pressure, respectively, compared to control animals. Also, transplanted animals showed decreased reaction to an innocuous thermal stimulus compared to control animals. Chromaffin cells revealed immunostaining for tyrosine hydroxylase two months after transplantation. These findings indicate that bovine chromaffin cell transplants may be effective therapy in reducing neuropathic pain. Aided by a grant from the Paralyzed Veterans of America Spinal Cord Research Foundation.

622.7

SODIUM BUTYRATE-TREATED PC12 CELLS AS AN ALTERNATIVE GRAFT SOURCE FOR PAIN REDUCTION. P.H. Kim and J. Sagen*. Dept. of Anat. and Cell Biol., Univ. of Il at Chicago, Chicago, Il 60612.

Our lab has previously demonstrated that chromaffin cells transplanted into the spinal cord and periaqueductal gray can reduce pain by providing a basal source of opioid peptides and catecholamines. However, large scale application of this approach is limited by the availability of these postmitotic cells. PC12 cells have the added advantages of ready availability, reproducibility, and uniformity. PC12 cells, unlike mature adrenomedullary chromaffin cells, contain very low levels of opioid peptides. The goal of these studies was to produce PC12 cells with increased opioid peptide production for pain reduction. Preliminary *in vitro* studies are in agreement with previous studies showing that sodium butyrate treatment can increase Met-enkephalin levels 2 to 3 fold in PC 12 cells (Byrd et al., 1987). However, the Met-enkephalin levels return to baseline after butyrate treatment is stopped. In order to permanently increase Met-enkephalin production, butyrate stimulated PC12 cells were treated with antimitotics Mitomycin C and Bromo-2'-deoxyuridine (BrDU), either immediately after butyrate stimulation or four days following withdrawal of butyrate. Both groups were treated with the antimitotics for two days, and were then prepared for neurochemical analysis on the eleventh day of the experiment. Preliminary data revealed a 15 to 25 fold increase in Met-enkephalin levels in the first group compared to controls, while the second group only showed a 2 to 3 fold increase compared to controls. In addition, the antimitotic treatments were success- ful in inhibiting PC12 cell proliferation. The data suggests both synergistic and perhaps independent action of antimitotic agents with butyrate on increasing Met-enkephalin production. Preliminary findings have revealed that treated PC12 cells transplanted into the PAG showed inhibited tumor growth and good survivability. These results suggest that butyrate-treated PC12 cells may provide an alternative donor source for pain reduction (Supported by NIH grant NS25054).

622.8

THE PRESENCE OF FETAL SPINAL CORD TRANSPLANTS ALTERS THE RESPONSE OF MACROPHAGES AND MICROGLIA AFTER SPINAL CORD INJURY. E. KUNKEL-BAGDEN*. Dept. of Anatomy and Cell Biology, Georgetown Univ., Washington, DC 20007.

Although fetal spinal cord transplants support the growth of axons and promote the recovery of function in both the neonate and the adult after spinal cord injury, the growth and recovery is more limited in the adult than in the neonate. The influence of macrophages and microglia on axonal growth is unclear; both inhibitory and supportive roles have been suggested for these cells. In order to begin to understand the role of macrophages and microglia in regeneration and plasticity, we sought to determine whether 1) the response of these cells to injury is similar in the neonate and the adult and 2) the presence of fetal transplants alters the response of the macrophages and microglia after injury. Newborn (25) and adult (20) rats received a mid-thoracic hemisection and spinal cord (E14) or hippocampus (E18) transplants were placed into the lesion site. At one week (acute response) or 4-8 weeks (chronic response) after surgery the presence of macrophages and microglia in the lesion/transplant area was examined immunocytochemically with the monoclonal antibodies ED1 and OX-42. After hemisection, at chronic survival times, only a few scattered cells were labeled with the ED1 antibody in the neonatal operates, but ED1 labeled cells were present throughout the lesion area in the adult operates. The adult operates with transplants, however, had fewer ED1 labeled cells present in the host, and no labeled cells were present in the transplants. ED1 labeled cells also were not present in the transplants of the neonatal operates. The macrophage/microglia response after injury is very different in the neonate and the adult, and the presence of fetal spinal cord transplants alters this response. (Supported by NS19259 to B.S. Bregman)

622.9

NIMODIPINE ENHANCES VASCULARITY OF INTRASPINAL FETAL GRAFTS AND ALTERS INJURY INDUCED BEHAVIORAL OUTCOMES. P.J. Horner,B.T. Stokes*, Dept. Physiol., The Ohio State Univ., Columbus, Ohio and P.J. Reier, Dept. Neurosci., Univ. Florida., Gainesville, FL.

Fetal transplants have been reported to promote at least partial recovery of a variety of motor behaviors after intraspinal grafting procedures in injured adult animals (Stokes et.al.,1992). In spite of these lasting improvements, certain spontaneous behavioral paradigms (open-field, inclined plane and grid-walking analysis) often reveal early deficits in transplanted animals when compared to injured-controls. We have also previously associated graft integration and potential behavioral effects with neovascularization of the graft segment. In the present experiments, we have investigated whether suspension transplants placed concomitantly with a potent angiogenic agent (nimodipine) can improve these early alterations and diminish the differences in final outcome scores.

Rats were pretested behaviorally, anesthetized and injured with an electro-mechanical impactor device. Ten days later they received an intraspinal transplant of dissociated E_{14} spinal cord cells. In addition, 2 (10 mg) slow-release tablets impregnated with nimodipine were placed (S.Q.) in order to enhance the angiogenic process. Behavioral testing was conducted weekly for 2 months and the success of angiogenic procedures estimated from stereological analysis of semi-thin plastic sections of areas in and around the graft site. Graft capillarity (33% increase in surface fraction), mean vascular diameter and graft size were all enhanced by nimodipine. Integration of graft tissue with the adjacent host was excellent and grafts often included large fascicles of preserved myelinated fibers. Previously described differences in behavioral outcomes after grafting were not seen in the nimodipine treated animals. Angiogenesis may therefore be an important factor in graft development and behavioral outcomes. Supported by NS-10165 and NS-27511.

622.10

STRATEGIES IN TRANSPLANT THERAPY FOR SPINAL CORD INJURY. Claire E. Hulsebosch* and John Dorman. Dept. Anatomy and Neurosciences, University of Texas Medical Branch, Galveston, TX 77055

Spinal cord injury results in a devastating loss of behavior below the level of the lesion. In a typical lesion, several tracts are severed, grey matter is destroyed and nearby mildly injured and uninjured neurons may be able to grow neurites a few mm. but no further. In the presence of exogenous therapy, the abortive growth may be encouraged. Examples of therapy to encourage growth include the use of one or more of the following: peripheral nerve transplants, antibodies against inhibitory substances, antibodies to neurotrophins, neurotrophins, and the use of transplanted cells (both embryonic and adult tissue and cultured cell lines). These therapies are used to provide a permissive environment which may encourage neuronal growth. We will present data which coorborates and extends previous work on the ability of an autologous transplant of a peripheral nerve to support neurite growth within the spinal cord after lesions which include the cortical spinal tract. Technical issues will be presented to insure success. In addition, progress in our laboratory on other strategies for spinal cord repair will be presented. Supported by NIH Grants NS 11255, NS 01217, Bristol-Squibb Myers.

AGING I

623.1

SEX DIFFERENCES AND LIFE-SPAN CHANGES IN THE VISUAL ACUITY OF QUAIL. **W. Hodos*, A. L. Holden, J.-Y. Lee, M. A. B. Diamgoz and V. Porciatti. Dept. of Psychology, Univ. of Maryland, College Park, MD 20742, USA; Dept. of Biology, Imperial College, London, SW& 2BB, UK; Instituto di Neurofisiologia del CNR, 56217 Pisa, Italy.**

Male (n=52) and female (n=60) quail were maintained on long days (16L/8D) from 0.5 - 19 months. Their visual acuity was determined by recording spatial-frequency tuning curves of the pattern electroretinogram that was generated in response to grating stimuli. The long-day lengths resulted in continuous ovulation by the females from sexual maturity until death. Both males and females showed systematic changes in acuity throughout the life span. Male acuity is highest at 0.5-3.0 month; thereafter it declined slowly. Female acuity was not significantly different from that of the males at the youngest ages. In contrast to the slow, steady decline of the males, female acuity rapidly rose to a peak at 9-12 months; thereafter it declined rapidly and reached the male level at 18-19 months. Preliminary findings with female quail maintained under short day length (8L/16D), which inhibits ovulation, indicated an improvement in acuity. The biphasic age-acuity curve of female quail is similar to the human age-acuity curve, which also is biphasic. Both of these curves reach a peak at approximately 20% of life span. These results suggest that in females, the endocrine system plays a role in visual aging and that female quail are a useful model for some aspects of human visual aging.

623.2

PERIPHERAL RETINAL DEGENERATION IN THE FISCHER 344 RAT: GENDER, REGIONAL, AND AGE EFFECTS. J. Ison, D. DiLoreto, Jr., D. Grover, E. Lazar, C. del Cerro*, P. Bowen, and M. del Cerro. Departments of Psychology, Ophthalmology, and Neurobiology, University of Rochester, Rochester, NY, 14642.

Fischer 344 (F344) rats are reported to suffer from an age-related retinal degeneration, but little data are available on the exact gender, regional, and age effects on peripheral degeneration. Given the importance of this strain for visual and aging studies, we undertook a longitudinal analysis of its retina from age 1 to 24 months. Outer retinal thickness was measured from sagittal sections at six positions in each eye: superior peripheral (SP), equatorial (SE), and central (SC), and inferior central (IC), equatorial (IE), and peripheral (IP). At least 8 eyes, per sex, per age were analyzed. Histological studies, fundus examinations, ERG recordings, and behavioral tests were performed. Morphometric analysis revealed a significantly thicker female outer retina from 1 to 12 months of age. A significantly increased rate of peripheral degeneration was found in males after 12 months in the SP which was total and complete by 18 months. The IP did not show an increased rate of degeneration until after 18 months. Paving-stone degeneration was seen in both sexes in various loci as early as 3 months. Significant decline in ERG activity was noted as early as 8 months. Functionally, changes in visual kinetics were seen after 18 months that are characteristic of both light-blinded and RCS rats. We found that the pattern of retinal degeneration in the F344 rat is affected by the combination of gender, regional, and age-related factors. (Supported by NEI-05262, and the Rochester Eye Bank)

623.3

EFFECTS OF SIZE OF ATTENTIONAL FOCUS ON VISUAL SEARCH IN AGED ADULTS. P.M. Greenwood, Raja Parasuraman*, Sangeeta Panicker and J.V. Haxby, Catholic University of America, Washington,DC and NIA, Bethesda, MD, 20064.

Feature-integration theory (Treisman & Gelade, 1980) claims that conjunction search requires focal attention directed serially over the display, while feature search depends on parallel processing. Plude & Doussard-Roosevelt (1989) reported that older adults are disproportionately slowed on conjunction but not feature search. Greenwood, Parasuraman and May (1992) reported that engaging visuospatial attention in response to valid cues is unimpaired in normal aging, while disengaging attention from invalidly cued locations is slowed with age. Now we ask whether (a) age alters the ability to adjust the size of the attentional focus and (b) varying the precision of location cues counteracts the effects of age on conjunction search. Forty old and young Ss searched for both features and conjunctions of features over 10 or 15 display elements comprised of three colors and three letters. Cues preceded targets by 500 msec and were rectangles drawn around either the target letter, column containing the target or side of the screen containing the target (valid) or another letter, column or side (invalid). Increasing display size slowed the old more than the young in conjunction search but not in feature search. Cue size also had little effect on feature search. In conjunction search RT decreased linearly with decreases in cue size but similarly in young and old. Cue validity effects were largest in conjunction search at the smallest cue size with old Ss showing greatest effects. Results indicate (a) old Ss are as adept as young in manipulating size of attentional focus, (b) old Ss are slower than young to shift attention during serial search even with valid cues, and (c) serial but not parallel search is dependent on attentional focus.

623.4

Effects of age on motor and cognitive performance in three rat strains. E. L. Spangler*, B. Hess, J. Hengemihle, D. M. Roberts, and D. K. Ingram. Gerontology Res. Ctr., (NIA), Baltimore, MD 21224; Essex Comm. College Essex, MD 21237; Towson State Univ. Towson, MD 21204

Representative groups of the adult life span of male Fischer 344 (F344; 7,13,23 mo), Brown Norway (BN; 7,13,23 mo) and F344/BN F1 (F1; 7,13,23,30 mo) rats were tested in a battery of behavioral tests including 15-min and 24-hr spontaneous activity (SA), inclined screen (IS), wire hang (WH), rotorod (RR), shock-motivated one-way active avoidance (AA) and shock-motivated 14-unit T-maze (TM) performance. ANOVA's and subsequent Bonferroni (BON) post-hoc tests ($p's < 0.05$) revealed age-related declines in all strains for tests of spontaneous activity (24-hr SA), agility and coordination (RR), strength and mobility (TR, IS) and to some extent in learning ability (AA,TM), although the 30-mo old F1 rats made significantly fewer errors than their 23-mo old counterparts in the TM. Evaluation of strain effects with BON indicated that BN and F1 compared to F344 rats were less active in 24-hr SA, were more agile (RR), and were more efficient at learning (AA,TM). Thus, these tests appear to be sensitive to both age-related changes in performance and genetic analysis. Hybrid vigor as well as selection bias may explain the superior performance of the 30-mo old F1 rats in maze learning.

623.5

SEVERITY OF SPATIAL LEARNING IMPAIRMENT IN AGING ASSESSED BY THE DEVELOPMENT OF A LEARNING INDEX Rebecca D. Burwell*, Margaret Burchinal, and Michela Gallagher. Dept. of Psychology, Univ. North Carolina, Chapel Hill, NC 27599.

The Morris spatial learning task has become widely used in neurobiological studies and in the characterization of cognitive decline in the aged rat. An important insight in the study of aging is that individual differences in spatial learning impairment are often good predictors of neurobiological aging. Thus behavioral characterization can provide an important background for studies of brain aging.

This report describes new methods for analyzing performance in the Morris task. For the development of a learning index that reflects the accuracy of spatial learning, the animal's proximity to the target location of the escape platform was used. This proximity measure was integrated over the course of training using a set of weights based on the learning curve for young rats (N=70). Data from aged rats (N=98) indicated that learning index scores differed significantly from young rats, overall. More importantly, the index scores differed for subpopulations of aged rats (impaired and unimpaired) that were formed on the basis of a criterion employed previously in our research. In addition, the index scores for the unimpaired aged subgroup did not differ from those of the young rats. This learning index score now provides a graded measure of learning performance for individual rats that can be used to characterize the severity of age-related cognitive impairment in neurobiological studies. Supported by NIA grant PO1 AG09973 and a NIMH RSDA to MG (KO2-MH00406)

623.6

MEASURING CHANGES IN TRANSCRIPT LEVELS FOR VARIOUS GENES IN THE MEDIAL SEPTUM AND HIPPOCAMPUS OF RATS DUE TO AGE AND MEMORY IMPAIRMENT. Rhonda M. Greene, Michael D. Robbins, Michela Gallagher, and Michael McKinney*. Mayo Clinic, Jacksonville, Jacksonville, FL 32224 and University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

Thirty-six rats were grouped according to age and spatial learning impairment (young, unimpaired aged, and impaired aged) based on performance in a Morris water maze. Total RNA was isolated from the medial septum and hippocampus and reverse transcribed. Specific mRNAs were assessed by quantitative polymerase chain reaction (PCR). In the medial septum, we studied mRNAs for the 695-amino acid ß-amyloid precursor protein (BAPP), growth associated protein (GAP-43), cholineacetyltransferase (ChAT), and the nerve growth factor receptor. BAPP and GAP-43 were also quantitated in the hippocampus, along with the m1 muscarinic acetylcholine receptor. All data was normalized against the level of glyceraldehyde phosphate dehydrogenase mRNA. GAP-43 and ChAT decreased significantly in the medial septum with age. Furthermore, the mRNA for BAPP increased in the hippocampus of impaired aged rats but remained unchanged in the medial septum. Supported by NIA Grant PO1 AG09973 and a NIMH RSDA to MG (KO2-MH00406).

623.7

EFFECTS OF AGING IN BEHAVIORALLY CHARACTERIZED RATS ON MUSCARINIC RECEPTOR SITES USING *IN VITRO* AUTORADIOGRAPHY. T. M. Gill*, M. McKinney†, and M. Gallagher. Department of Psychology, University of North Carolina, Chapel Hill, NC 27599 and †Mayo Clinic, Jacksonville, FL 32224.

Young (4 mo, N=8) and aged (25 mo, N=15) male Long-Evans rats were behaviorally characterized for spatial learning in the Morris water maze. These same subjects were then used to study hippocampal muscarinic binding with quantitative *in vitro* autoradiographic techniques. Tritiated quinuclidinyl benzilate (QNB) alone and [^{3}H] QNB in the presence of the M1 receptor antagonist, pirenzepine, were used to determine total muscarinic, M1, and non-M1 binding.

No age differences in total, non-M1, or M1 binding were found throughout the hippocampus. However, analysis of covariance for M1 sites in ventral dentate gyrus revealed that subgroups differed significantly when binding was assessed in relation to learning: high binding was associated with better learning in aged rats with preserved spatial abilities (r values range from -.70 to -.80), but opposite correlations were found for the young and aged impaired subgroups (r values range from .50 to .60). Further examination of these autoradiograms will indicate whether age-related changes in muscarinic sites are evident in other areas (i.e. frontal cortex and medial septum).

Supported by NIA grant PO1 AG09973 and a NIMH RSDA to MG (KO2-MH00406).

623.8

AGE-RELATED CHANGES IN MUSCARINIC CHOLINERGIC SUBTYPES IN RAT BASAL FOREBRAIN AND FRONTAL CORTEX. Robert P. Yasuda*, Sean A. Satkus, Jason S. Weisstein, and Barry B. Wolfe. Department of Pharmacology, Georgetown University School of Medicine, 3900 Reservoir Road, Washington, D.C. 20007

Muscarinic cholinergic receptor subtypes were examined in young (4-5 months) and aged (24-25 months) Long-Evans rats. The aged rats were further divided into two groups: 1) Aged Unimpaired rats that had similar Morris water maze performance to that of young rats, and 2) Aged Impaired rats that did not perform as well as young rats in the Morris water maze. Antisera selective for the m1-m4 receptor subtypes were used to examine differences in receptor density in these three groups of rats in the basal forebrain and frontal cortex (Mol. Pharmacol., 39: 643-649, 1991; Mol. Pharmacol., 40: 28-35, 1991; Mol. Pharmacol. 40: 783-789, 1991; Soc. Neurosci. Abstr., 17:, 1532, 1991). There were no differences in any receptor subtypes between the aged impaired and the aged unimpaired rats in either brain region. However, there was significant decreases in at least the m4 receptor subtype in aged animals compared to young in the basal forebrain (young, 0.819 ± 0.047; aged unimpaired, 0.599 ± 0.048, $p < 0.01$; aged impaired, 0.583 ± 0.041 pmoles/mg protein, $p < 0.01$; N=8). These decreases in m4 receptor were not observed in the frontal cortex (young, 0.380 ± 0.027; aged unimpaired, 0.417 ± 0.007, $p>0.05$; aged impaired 0.382 ± 0.036 pmoles/mg protein, $p>0.05$; N=5). Supported by AG09973 and AG09884.

623.9

NORTHERN ANALYSIS OF MUSCARINIC RECEPTOR mRNA IN THE BRAIN OF 3, 18, AND 33 MONTH OLD RATS. H.-J. Lee, M. Clagett-Dame, W. Heideman, and M. Weiler*. School of Pharmacy, University of Wisconsin-Madison and Hazleton Wisconsin, Madison, WI 53706.

The purpose of this investigation was to examine the expression of the five muscarinic receptor mRNAs in three rat brain regions and to determine if there are age-related differences in the expression of these transcripts. Poly $(A)^+$ RNAs of neostriatum, hippocampus and cortex from Fischer 344 X Brown Norway hybrid rats (3, 18 and 33 months) were analyzed by Northern blotting with ^{32}P-labeled oligonucleotide probes of m1, m2, m3, m4 and m5 receptors. Messenger RNA levels were quantitated by densitometry. All five muscarinic receptor mRNAs were detected in blots from the neostriatum and hippocampus, and all but m5 mRNA were detected in cortex. This study revealed the presence of a 6.0 kb m5 mRNA in the neostriatum and hippocampus.

The relative regional abundance of each of the five mRNAs was similar in all the age-groups. The m1, m2 and m3 mRNAs were more abundant in cortex and hippocampus than in neostriatum, the difference being more pronounced with m3 mRNA. The m4 mRNA was most abundant in the neostriatum. The m5 mRNA was most prominent in the hippocampus, weakly detected in neostriatum, and not detected in cortex. With age the expression of m3 mRNA in the neostriatum decreased. No significant age-related changes in the expression of other receptor mRNAs were observed. How this finding relates to other well documented changes in neostriatum with aging remains to be determined. (Supported by a grant from American Federation for Aging Research).

623.10

AGING DIFFERENCES IN THE CYCLIC AMP MODULATION OF L-TYPE CALCIUM CURRENTS IN HIPPOCAMPAL SLICE NEURONS. O. Thibault*, L.W. Campbell and P.W. Landfield. Dept. Pharmacol., Univ. Kentucky Col. of Med., Lexington, KY, 40536.

A number of studies have shown that cyclic AMP can modulate calcium (Ca) channels in several preparations. Last year we reported that L-type Ca currents in the rat hippocampal slice neurons were greatly enhanced by the intracellular application of dibutyryl cyclic AMP (dcAMP) (Thibault *et. al.*, Soc. Neurosci. Abstr., 91). Using single electrode voltage clamp, several types of voltage-activated Ca currents are seen in this preparation, including an L-type current that is associated with a prolonged, dihydropyridine-sensitive Ca *aftercurrent*.

Hippocampal CA1 neurons from aged rats also exhibit increased voltage-sensitive Ca spikes and Ca currents. This aging-dependent enhancement of Ca current resembles the dcAMP effect in young neurons. In this study, therefore, we tested the response of aged neurons to intracellular application of dcAMP. We recorded Ca currents from young (3-5 months) and aged (25-27 months) rat neurons treated with TTX and TEA and impaled with pipettes filled with 0.5 M CsCl, 1 mM Mg-ATP, and $\pm$ 1 mM dcAMP.

In young neurons dcAMP again significantly increased the Ca aftercurrent, whereas this treatment did not influence the aftercurrent in aged neurons (which already show an increased aftercurrent). Neither activation/inactivation ranges, nor calcium-dependent inactivation were affected by administration of dcAMP in young or aged neurons.

Since the effects of aging and dcAMP were not additive, the results suggest that these two processes may operate on a common pathway, and that the effects of aging on increased Ca currents may be mediated at least, in part, by increased cAMP-dependent phosphorylation. (Supported by AG 04547 and Miles Inc.)

623.11

ATTENUATION OF TYROSINE HYDROXYLASE ACTIVATION FOLLOWING NEUROTOXIC INSULT TO THE HIPPOCAMPUS OF AGING RATS. J.R. Unnerstall*. Dept. of Anatomy and Cell Biology, Unv. of Ill. at Chicago, Chicago, IL 60680.

The locus coeruleus (LC) loses up to 50% of its neurons during normal aging. However, no differences, or possibly, increases in static measures of synaptic integrity, such as norepinephrine content or tyrosine hydroxylase (TH) activity, have been reported in brain regions innervated by LC neurons in aged vs young animals. Yet, behavioral deficits associated with attenuation of LC function seen in aging animals suggest that this neural system has lost its ability to rapidly respond to stimuli or insult. In these preliminary studies, the neurochemical adaptability of LC neurons in aging rats has been assessed using a paradigm originally reported by Acheson and Zigmond (*J. Neurosci.* **1**:493, 1981). Using this model, TH activity was assessed in the hippocampus of young (2 month) and old (24 month) Fisher-344 male rats 72 hours following the infusion of 200 µg of the neurotoxin 6-hydroxydopamine or vehicle into the lateral ventricle. Interestingly, it was noted that baseline TH activity measured under optimal conditions (pH6.2, 3.0 mM 6-MPH_4, 30 µM tyrosine) was two-fold higher in unlesioned old animals compared to the young animals (~12 vs 6 pmoles/min/mg protein). The lesion resulted in a 55-59% decrease of TH activity measured under optimal conditions. When measured under suboptimal conditions (pH6.6, 0.7 mM 6-MPH_4, 30 µM tyrosine), TH activity in young lesioned animals was 83% of that measured in young vehicle treated animals. However, in the old lesioned animals, TH activity measured under suboptimal conditions was only 40% of that measured in age-matched control animals. These data suggest that the ability of LC neurons to rapidly respond and compensate to this insult is attenuated in the old animals due to a deficit in this system's capacity to activate TH. *(NIA grant AG09587.)*

623.12

AGE-RELATED IMPAIRMENT OF SYNAPTIC CONNECTIVITY IN THE RAT DENTATE GYRUS REVEALED BY THE UNBIASED DISECTOR TECHNIQUE. F. Morrell*, Y. Geinisman, L. deToledo-Morrell, I.S. Persina and M. Rossi. Depts. of Neurol. Sci. and Psychol., Rush Med. Coll. and Dept. of CMS Biol., Northwestern Univ. Med. Sch., Chicago, IL 60612.

Previous attempts to elucidate whether a loss of hippocampal synapses occurs during normal aging provided conflicting results, possibly, due to the unavailability, at the time, of unbiased methods for synapse quantitation. This study was designed to reexamine the issue by means of modern technical approaches which provide unbiased estimates of synaptic numbers. Groups of 14 young adult (5 months old) and 14 aged (28 months old) male F-344 rats were compared. Synapses were analyzed in the middle (MML) and inner (IML) zones of the molecular layer of the hippocampal dentate gyrus where synaptic contacts are mainly formed by the entorhinal or commissural-associational fibers, respectively. The number of synapses per postsynaptic neuron was estimated using the disector method. The results showed that the total number of synaptic contacts per neuron was significantly diminished in the MML (by 23.6%) and IML (by 22.7%) of aged rats relative to young adults ($p < 0.005$ in both cases, two-tailed randomization test for two independent samples). This age-related synaptic loss involved axospinous, but not axodendritic, junctions of the MML (-24.4%) and IML (-24.0%). Both perforated and nonperforated axospinous synapses (distinguished by a discontinuous or continuous postsynaptic density, respectively) exhibited a comparable age-dependent decrease in numbers, though this decrease did not reach statistical significance in the case of perforated junctions of the IML. The observed loss of axospinous synapses, which are formed by two afferent systems on neurons that survive in senescence, may underlie a reduction in the amplitude of excitatory postsynaptic potentials and a decline in functional synaptic plasticity detected in the dentate gyrus of aged rats.

Supported by Grants AG 08794 from NIA and BNS-8819902 from NSF.

623.13

STRUCTURAL SYNAPTIC REMODELLING FOLLOWING THE INDUCTION OF LONG-TERM POTENTIATION (LTP) IN AGED RATS. Y. Geinisman*, L. deToledo-Morrell, F. Morrell, I.S. Persina and M. Rossi. Dept. of CMS Biol., Northwestern Univ. Med. Sch. and Depts. of Neurol. Sci. and Psychol., Rush Med. Coll., Chicago, IL 60611.

Although aged animals are deficient in the retention of LTP, they potentiate to the same extent as young ones (Barnes, *J. Comp. Physiol. Psychol.*, 1979, *93:* 74; deToledo-Morrell et al., *Neurobiol. Aging.*, 1988, *9:* 581). We showed earlier (Geinisman et al., *Brain Res.*, 1991, *566:* 77) that the induction of LTP in young rats is followed by a selective increase in the number of axospinous synapses with a segmented postsynaptic density (PSD). The aim of the present study was to determine if old potentiated animals exhibit the same structural synaptic modification. Aged F344 rats (27 mo. old) were implanted with stimulating electrodes in the medial perforant path and recording electrodes in the hilus of the ipsilateral dentate gyrus. Potentiated animals were stimulated (with fifteen 20 ms bursts of 400 Hz delivered at 0.2 Hz) on each of 4 consecutive days and sacrificed 1 h after the fourth stimulation. The number of synapses per neuron was differentially estimated for various synaptic subtypes in the middle (MML) and inner molecular layer of the dentate gyrus using the disector technique. Only axospinous synapses with segmented PSDs were significantly increased in numbers in the MML of potentiated aged rats relative to their stimulated (at a frequency of 0.2 Hz) or implanted controls. The magnitude of this increase in old rats was comparable to that found in young adults suggesting that such a modification might account for the equal extent of potentiation observed in the two age groups. However, the absolute number of synapses with segmented PSDs was significantly lower in aged potentiated rats than in young ones. The age-related diminution in a synaptic subtype, which may be essential for plasticity associated with LTP, might result in a deficient retention of LTP.

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623.14

DENDRITIC GROWTH IN ADULT GRANULE NEURONS IN THE RAT DENTATE GYRUS. N. Blake, S. Seay-Lowe, and B. Claiborne*. Division of Life Sciences, University of Texas, San Antonio, TX 78249.

During late postnatal development (14 to 60 days of age), granule cells in the dentate gyrus lose dendritic branches while their remaining branches continue to elongate as the molecular layer expands. These simultaneous processes of growth and regression lead to a conservation of total dendritic length (Rihn & Claiborne, 1990, Dev. Br. Res. 54: 115). Because the dentate gyrus continues to enlarge throughout the rat's life, we wanted to determine whether the processes of dendritic growth or regression continue as the animal matures.

Granule neurons in the dorsal blade were injected with horseradish peroxidase in in vitro slices from 1-year-old Sprague-Dawley rats. Their dendritic trees were analyzed in three-dimensions using a computer-microscope system. Strict criteria were applied to ensure that only well-filled neurons with a minimum of cut branches were analyzed. Results showed that granule neurons (n=17) had 29 $\pm$ 1 branches (mean $\pm$ S.E.), with 63 $\pm$ 4% of branch points located in the inner third of the molecular layer, 30 $\pm$ 3% in the middle third and 7 $\pm$ 3% in the outer. Total dendritic length per neuron was 4201 $\pm$ 167 um.

When compared to our previous data on granule neurons (n=19) from 40- to 60-day-old rats, these results show that branch number (29 in young rats) remains constant during the first year, while the percentage of branch points in the outer third of the layer decreases (17% vs. 7%). In contrast, total dendritic length increases (3457 vs. 4201 um) as the animals mature. From these data we conclude that dendritic regression does not occur after 60 days-of-age and that dendrites continue to elongate as the hippocampal formation enlarges. Thus there is an overall increase in the size of the granule cell dendritic tree in rats between the ages of 60 days and 1 year. (Supported by NIA and MARC.)

623.15

AGE-RELATED INCREASE OF ANDROGEN RECEPTOR mRNA IN THE RAT HIPPOCAMPUS. J.E.Kerr*, L.H.Burgess, S.G.Beck and R.J.Handa. Depts. of Pharmacol. and Exp. Ther. and Cell Biol., Neurobiol., and Anat. Loyola Univ. Chicago, Stritch School of Medicine, Maywood, IL 60153.

Previous studies have revealed age-related losses of glucocorticoid receptor and mineralocorticoid receptor in the rat hippocampus. To determine whether androgen receptor (AR) levels, which are normally found to be relatively high in the rat hippocampus, are similarly altered, we examined the AR mRNA content in various brain regions of young (5 mos) and old (22 mos) intact male Fischer 344 rats (Harlan Inc., Indianapolis IN). Animals were sacrificed and their brains were frozen for later dissection and homogenization in GITC to obtain total RNA for analysis by RNase protection assay. The AR mRNA RNase protection assay used a 141 nucleotide long ^{32}P-labeled rat antisense RNA probe. This probe hybridizes to the N-terminal end of the coding region of both the 9.5 Kb and 11 Kb forms of rat brain AR mRNA. These forms seem to be divergent primarily in their 5' untranslated regions. Dilutions of *in vitro* transcribed sense strand RNA ranging from 2.0 to 50 amol were used to generate standard curves which were linear and had correlation coefficients consistently greater than 0.995. The lower limit of sensitivity of this assay is approximately 2 amol/total input RNA. The hippocampal AR mRNA concentration was 539 ± 54 amol (protected probe) mRNA/mg input RNA in the young animals (n=7) as compared to 729 ± 46 amol/mg RNA in the older rats (n=5) which represents a 35% age-related increase ($p<0.05$). No significant differences due to age were found in either the cortex or hypothalamus. These data suggest that there are age-related and tissue-specific changes in AR regulation in the rat hippocampus which is different from other hippocampal steroid hormone receptors previously studied.

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623.16

ENTORHINAL CORTEX LESIONS PRODUCE CHANGES IN GLUCOCORTICOID BUT NOT MINERALOCORTICOID RECEPTOR GENE EXPRESSION IN THE RAT HIPPOCAMPUS. D. O'Donnell*[1], N. Aumont[1], A. Baccichet[1], J.R. Seckl[2], J.Poirier[1,3], and M.J. Meaney[1,3]. [1]Douglas Hosp. Res. Ctr, Depts. of Psychiatry and Neurology & Neurosurgery, McGill Univ., Montreal, Canada H4H 1R3; [2]Dept. of Med., West. Gen. Hosp., Univ. of Edinburgh, Scotland EH4 2XU; and, [3]Center for Studies in Aging, McGill Univ.

Entorhinal cortex lesioning (ECL) destroys a major hippocampal input and leads to axonal sprouting in the dentate gyrus. Glucocorticoids are known to inhibit this reinnervation process. In the present study, we examined hippocampal glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) mRNA expression using *in situ* hybridization following unilateral ECL in the rat. Film autoradiography showed that as early as one day post lesion, a dramatic bilateral *decrease* in GR mRNA was observed in the granular dentate gyrus. By contrast, in the CA1 region, a moderate *increase* in GR mRNA was detected. GR mRNA levels in both regions returned to those of control animals 2 days post lesion indicating that these effects were transient. Adjacent sections hybridized with probes to MR mRNA revealed no apparent changes in expression in any hippocampal sub-regions as a result of ECL. Western blots from total hippocampus homogenates, using the BuGR2 antibody (Affinity Bioreagents), confirmed an overall decrease in GR protein in the hippocampus. This decrease was also transient with maximal effect being observed 2 days post lesion and levels returning to control values 4 days post surgery. These results suggest that the regulation of GR but not of MR is associated with synaptic reorganization. Interestingly, the opposing changes in GR mRNA expression seen in the dentate gyrus and CA1 following ECL may be related to the pronounced vulnerability of the CA1 cell field and relative resistance of the dentate gyrus to a number of conditions that threaten neuron survival.

623.17

INCREASED DENSITY AND SENSITIVITY TO GABA OF HIPPOCAMPAL ω_1 (Bz_1) MODULATORY SITES IN THE AGED RAT. **J.Vitorica#, D.Ruano#, S. Feldblum@, B. Scatton*§ and J.Benavides§. #Department of Biochemistry, School of Pharmacy, Sevilla (Spain). §Department of Biology, Synthélabo Recherche, 31,Av.P.Vaillant-Couturier, 92220 Bagneux. @INSERM U336, Montpellier (France)**

We have recently reported that the enhancement by GABA of (^{3}H)-flunitrazepam binding to hippocampal ω modulatory sites was much larger in 22-24 month-old than in 3 month-old rats (Ruano et al. J.Pharmacol.Exp.Ther 256, 902-908, 1991). Displacement studies with selective ω_1 ligands suggested that this increased sensitivity to GABA was due to changes ocurring at the level of ω_1 modulatory sites. To further characterize these changes we have now compared the properties of hippocampal ω_1 sites of 3 and 22 month-old Fischer 344 rats.

Saturation curves of (^{3}H)-zolpidem binding to hippocampal membranes demonstrated a 60% increase (n=5, P< 0.05) in the B_{max} for this ligand in the aged rats with no change in affinity.Moreover, 100 µM GABA increased the binding of 3 nM (^{3}H)-zolpidem to well washed hippocampal membranes by 107 ± 10% and by 170 ± 25% in 3 and 22 month-old rats, respectively. Similar changes were observed in rats of the Wistar strain. In sections incubated in the presence of 5 nM (^{3}H)-zolpidem and a saturating (100 µM) concentration of GABA, and processed for autoradiography it was observed that at the hippocampal levels the largest increases in ω_1 binding site density occured in the CA_2 (+90%) and CA_3 (+70%) fields whereas smaller changes were quantified in the CA_1 field (+60% in the ventral part and +20% in the dorsal part) and in the dentate gyrus (+55%). No significant increases were observed in other areas. Detection by in situ hybridization of mRNAs encoding for the two isoforms of Glutamic Acid Decarboxylase (GAD_{65} and GAD_{67}) revealed lower levels of GAD_{65} mRNA in individual hippocampal cells of aged rats but no evident neuronal loss.

Taken together these results demonstrate an increase in the density and sensitivity to GABA of ω_1 sites in the aged rat hippocampus. These alterations probably represent the mechanism whereby hippocampal neurons cope with a decreased GABAergic input.

AGING II

624.1

EFFECT OF AGE ON THE ACTIVITY OF MOLECULAR FORMS OF AChE. C.G. Triplett and K.A. Skau*. Div. of Pharmacol/Med Chem, Coll. of Pharmacy, U Cincinnati, Cincinnati, OH 45267-0004.

Changes in cholinergic transmission have been associated with the decline of memory in the aging human brain. We have studied the effect of age on the activity of AChE in rat brain and several peripheral tissues. Activity of the different molecular forms of AChE in 6 areas of the brain and 7 peripheral tissues were compared between 3- and 24-month old F344 rats by standard AChE assay methods. Significant decreases in activity were seen in G4 forms in olfactory bulb (49% decrease), striatum (39% decrease) and medulla/pons areas (43% decrease). Nonsignificant reductions were seen in fore brain, parietal cortex and cerebellum. Nonsignificant reductions were seen in the G1 forms of the brain areas studied. Significant decreases were seen in total AChE of parietal cortex (36% decrease), striatum (54% decrease), cerebellum (36% decrease), and medulla/pons (26% decrease). Nonsignificant reductions in total AChE were seen in olfactory bulb and fore brain. The decrease in activity of the G4 forms and not the G1 forms suggests a change due to the aging process of the synthesis or degradation of the G4 molecular form. (Supported by an Alzheimer's Association/United Airlines Foundation pilot research grant.)

624.2

SPECIES DIFFERENCES IN CALBINDIN-D_{28K} IMMUNOREACTIVITY WITHIN THE BASAL FOREBRAIN. M.L. Smith*, K.G. Baimbridge[1] and R.M. Booze. Dept. of Pharmacology, University of Kentucky Medical Center, Lexington, KY, [1]Dept. of Physiology, University of British Columbia, Vancouver, B.C.

Calbindin-D_{28k} is a calcium binding protein reported to be localized within the cholinergic projection neurons of the human nucleus basalis of Meynert. These neurons malfunction with age and in Alzheimer's disease and may contribute to the cognitive deficits present in both situations. Calbindin-D_{28k}-IR neurons are present within the rat basal forebrain; however, they do not appear to be co-localized with the cholinergic projection neurons. In this study we examined 1) co-localization of calbindin-D_{28k} with GABAergic neurons within the basal forebrain and 2) localization of calbindin-D_{28k} within basal forebrain neurons which project to the cortex .

Fischer-344 male rats were perfused and the brains sectioned on a Vibratome (40µm). Serial sections were collected through the nucleus basalis to be used for double-labeling immunocytochemistry. Sections were incubated in a solution containing primary antibodies against either 1) ChAT and calbindin-D_{28k} or 2) GAD and calbindin-D_{28k}. Primary antibodies were detected using FITC and Rhodamine labeled fluorescent secondary antibodies. Neurons containing both calbindin-D_{28k} and GAD were identified throughout the nucleus basalis of the rat. Numerous neurons were identified in the basal forebrain containing only GAD or calbindin-D_{28k}. As previously reported, no co-localization was detected between ChAT and calbindin-D_{28k}-IR neurons in the basal forebrain of the Fischer-344 male rat. Injection of fluorescent-tagged latex microspheres into the cortex suggests that co-labeled GAD/calbindin-D_{28k}-IR neurons may project cortically. Our results indicate that alternative animal models are needed to more accurately represent the human nucleus basalis of Meynert in which cholinergic neurons contain calbindin-D_{28k}. Supported by NIA grant #AG10747.

624.3

ALTERATIONS IN MUSCARINIC STIMULATED LOW K_M GTPase ACTIVITY IN AGING AND ALZHEIMER'S DISEASE: IMPLICATIONS FOR ALTERED SIGNAL TRANSDUCTION. R. Cutler, J.A. Joseph*, K. Yamagami, R. Villalobos-Molina and G.S. Roth. Gerontology Res. Ctr./NIA, Baltimore, MD.

Previous reports have shown that there are age-related reductions in muscarinic receptor (mAChR) sensitivity to agonist stimulation, and that these alterations are the result of specific changes in mAChR-G protein interactions [expressed as reduced carbachol-stimulated low K_M (C-SLK_M) GTPase activity (an indicator of receptor/G protein coupling/uncoupling) in hippocampus (HIP) and caudate-putamen (CPU)] and subsequent reductions in phosphoinositide (PI)-mediated signal transduction (ST). Present experiments assessed putative age- and disease-related reductions in C-SLK_M GTPase activity in young (Y), Alzheimer's disease (AD), and age-matched control (AMC) HIP and basal ganglia (BG). Results showed that while there were age- and disease- related reductions in C-SLK_M GTPase activity in BG [$F(4,40) = 7.05$ $p < 0.01$), (e.g., 10^3 carb. df = 40 t's =: Y vs AM 2.71, $p < 0.01$; AM vs AD 2.22, $p < 0.05$; Y vs AD 4.93, $p < 0.001$), only age- and not AD-related deficits were observed in HIP (e.g., t (6) Y vs AD = 1.08, $p > 0.05$; t (7) Y vs AM = 2.58 $p < 0.05$). Results suggest that there are age-and disease-related changes in mAChR-G protein interactions that could contribute to reduced PI-mediated ST. Moreover, there could also be some compensatory alterations in AD HIP in C-SLK_M GTPase activity. Additional experiments examining C-SLK_M GTPase activity in HIP and CPU from irradiated rats have shown reductions in this parameter as well as ST deficits, suggesting free radical involvement in these deficits.

624.4

AGE-RELATED CHANGES IN THE RAT STRIATUM: A MICRODIALYSIS STUDY. R.E. Maloney* and G.A. Gerhardt. Depts. of Psychiatry & Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

In vivo electrochemical studies have shown that extracellular dopamine (DA) in the rat striatum following K^+ stimulation is reduced in aged rats as compared to young rats. In this study, the technique of *in vivo* microdialysis was used to further investigate both basal and K^+-evoked overflow of DA in the young and aged rat striatum. Eleven Fischer 344 rats were used(n = 5: 24-26 mos, n = 6: 2-5 mos). Four mm long, 300 μm diameter dialysis probes were stereotaxically implanted into the rat striatum (+1.65 mm A.P., ±1.65 mm Lat. with respect to bregma, Paxinos & Watson). Probes were perfused with artificial CSF at a flow rate of 1.2 μl/min. Samples were taken every 10 mins and analyzed by HPLC with electrochemical detection. When the variability of 5 consecutive DA sample peak heights was < 10%, the perfusion medium was changed to 100 mM K^+ CSF for 10 mins. The K^+ stimulation produced a significant difference in the percent increase for DA, 4119.27% in aged rats and 1509.33% in young rats. Aged rats had significantly lower baseline amounts of DA, DOPAC and HVA. In addition aged animals had significantly lower levels of DOPAC following K^+-evoked DA release. (Supported by USPHS Grants AG06434, AG00441 and NS-09199)

624.5

AGE-RELATED CHANGES IN GLUTAMATE EVOKED DOPAMINE OVERFLOW IN THE STRIATUM OF THE FISCHER 344 RAT. M.N. Friedemann* and G.A. Gerhardt. Depts. of Pharmacology and Psychiatry, University of Colorado Health Sciences Center, Denver, CO 80262.

Glutamate and other excitatory amino acid agonists can elicit dopamine (DA) release from nerve terminals in the striatum. Age-related alterations in this interaction between corticostriatal and nigrostriatal inputs may contribute to motor deficits commonly seen during aging in mammals. The purpose of this study was to examine the effects of aging on glutamate-evoked overflow of DA in the striatum of the rat brain. Male F344 rats that were 6- or 24-months old, were anesthetized with urethane and placed in a stereotaxic apparatus. L-glutamate (0.5 to 3 nmol) was ejected *in situ* from glass micropipettes 300 μm away from a Nafion-coated carbon fiber electrode. DA concentrations were measured in real time using an electrochemical recording system (IVEC-5; Medical Systems, Inc.). The results show that the average amplitude of DA signals was significantly lower in the old rats (0.67 ± 0.09 μM vs. 1.35 ± 0.14 μM; $p < .01$). However, the doses of glutamate applied were significantly greater in the aged rats (1.2 ± 0.1 nmol vs. 0.9 ± 0.1 nmol; $p < .01$). Although this finding may be explained by an increase in uptake of glutamate from the extracellular space, these results suggest that there may be age-related changes in sensitivity to the effects of glutamate on DA nerve endings, in that higher doses of glutamate produced lower amplitude signals in 24-month compared to 6-month old rats. (Supported by USPHS grants AG06434 and AG00441 to GAG and PMAF fellowship to MNF).

624.6

SYNTHESIS RATES OF DOPAMINE D_2 RECEPTOR mRNA IN YOUNG AND OLD WISTAR RAT NEOSTRIATA

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Previous work established significant decreases in receptors for dopamine in the striatum of aged animals, relative to young controls. This phenomena is seen in lower animals, as well as humans. The decrease is more pronounced for the D_2 dopamine receptor subtype (40%). In addition, we have noted a 50% decrease in the levels of mRNA for the D_2 receptor in the old striata. The decrease in mRNA levels in aged striata is greater than the cell loss, and prompts the question of whether the surviving neurons are synthesizing less D_2 receptor mRNA.

Present studies were directed toward determining the relative rates of synthesis for dopamine D_2 receptor synthesis in young and aged striatal nuclei, isolated by sucrose gradients. RNA synthesis was done using alpha-[^{32}P] uridine triphosphate. The isolated RNA was hybridized to cDNA sequences cross-linked to a membrane. The sequence used for the D_2 mRNA was from a double stranded insert in a pBR vector which is homologous to bases 25 to 582. cDNA sequences for actin and tubulin were used as controls for the synthesis. Following hybridization, membranes were analyzed using a Betagen to detect total incorporation.

The D_2 mRNA was synthesized at a decreased rate in the aged nuclei (57%, $p<0.01$). There was no significant difference between young and aged nuclei in tubulin or actin mRNA as detected on the blots. The decrease in D_2 mRNA synthesis is partly attributed to the loss of D_2 cells, but also seems to reflect an age-dependent effect on surviving D_2 neurons.

624.7

AGE-RELATED VARIATIONS IN THE STEADY STATE LEVELS OF ALTERNATIVELY SPLICED D2 RECEPTOR mRNAs IN BRAIN AREAS OF DIFFERENT RAT STRAINS. N. Brunello, F. Fumagalli, F. Della Vedova§ and G. Racagni*, Center of Neuropharmacology, Institute of Pharmacological Sciences, University of Milan, Via Balzaretti 9, 20133 Milan and Farmitalia-Carlo Erba, CNS Division, Nerviano, Italy.

Dopamine D2 receptor gene produces two receptor isoforms by alternative messenger RNA splicing, the so called D2-415 and D2-444 subtypes. Age-related reduction of D2 receptor density in various brain areas, particularly in the striatum, have been reported by means of radioligand binding and autoradiographic techniques. Using the highly sensitive reverse transcription-polymerase chain reaction analysis we have investigated possible changes in the relative abundance of the mRNAs encoding the dopamine receptor subtypes D2-415 and D2-444 in brain areas of aged rats. We have observed age-related decrease in the D2-444/D2-415 mRNA ratio in the striatum and in the hippocampus of aged rats, whereas no significant differences were observed in the nucleus accumbens and in the frontal cortex. Steady state mRNA levels for both D2 receptor isoforms were decreased in striatum of aged Wistar as well as aged Sprague Dawley rats. In both rat strains the greater decrease regarded the long form D2 mRNA. In Wistar rats this mRNA form reached only 30% of the corresponding value in young adult rats. The reduction in D2 mRNA ratio and in particular in the long form might have functional significance since it could play an important role in the responses to dopaminergic receptor activation.

624.8

TYROSINE HYDROXYLASE EXPRESSION IN THE AGING RODENT OLFACTORY BULB. H. Baker*, K. Morel, J.-Y. Cho and D.M. Stone. Cornell Univ. Med. Coll. at Burke Med. Res. Inst., White Plains, NY 10605.

Olfactory function is compromised in humans as a result of normal aging and in neurodegenerative disorders. To begin a biochemical characterization of the aging process in a rodent olfactory model, we tested the hypothesis that age-related alterations occur in the expression of tyrosine hydroxylase (TH), the first enzyme in the dopamine biosynthetic pathway. Previous experiments in young animals demonstrated that TH expression in periglomerular cells of the main olfactory bulb is significantly reduced following either olfactory deprivation or peripheral deafferentation. The current experiments compared TH activity in young (6 month, mo.), middle-aged (18 mo.) and old (>25 mo.) animals. TH activity did not differ between 6 and 18 mo.-old Fischer (F) 344 rats, but was reduced to 77% of 6 mo. levels in 27-29 mo.-old rats. Similarly, TH protein, demonstrated by immunocytochemistry, and mRNA, as indicated by *in situ* hybridization, were reduced in old as compared to 6 and 18 mo.-old rats. The age-related alterations were not uniform, that is, in some F344 rats, TH activity was normal and in others up to 50% decreases occurred. Immunocytochemical and *in situ* hybridization analyses supported the between animal disparity. In contrast, TH activity in adrenal glands from F344 rats exhibited an age-related increase. Surprisingly, TH expression was not reduced in olfactory bulbs of either a second strain of aged (28 mo.) rats, Fischer 344-Brown Norway F1 hybrids, or C57Bl/6 mice (25-26 mo.) as compared to levels in 6 and 18 mo.-old animals. These data demonstrate that during aging, alterations in TH expression are species, strain and region specific. Supported by AG09686.

624.9

ROLE OF CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE-DEPENDENT PROTEIN KINASE IN THE REGULATION OF TYROSINE HYDROXYLASE ACTIVITY IN THE AGED RAT BRAIN. N. Aguila-Mansilla, W. Kedzierski and J.C. Porter*. Dept. OB/Gyn, U.T. Southwestern Med. Ctr., Dallas, TX 75235-9032.

During aging, dopaminergic neurons synthesize and secrete less dopamine (DA) as demonstrated by the observation that the rate of secretion of DA into hypophysial portal blood is significantly less in aged rats than in young rats. The *in situ* activity of tyrosine hydroxylase (TH), the rate-limiting enzyme of catecholamine (CA) synthesis, is regulated by a variety of factors, such as hormones and various protein kinases, including cyclic adenosine 3',5'-monophosphate (cAMP)-dependent protein kinase (PKA). This study was conducted to examine the role of the PKA pathway in the regulation of the *in situ* molar activity of TH in the brains of aged and young castrated female rats. For this purpose, we made use of an *in vitro* incubation system. Hypothalamus and corpus striatum (CS) were incubated for 60 min with various agents that modify the PKA pathway. The incubation mixture contained 10^{-2} M NSD 1015 [dihydroxyphenylalanine (DOPA) decarboxylase inhibitor]. At the end of incubation, the tissue was homogenized and DOPA was measured by HPLC with electrochemical detection and TH by immunoblot electrophoresis analysis. Forskolin, an activator of adenylyl cyclase, at concentrations of 1, 5, and 25 μM significantly ($P<0.01$) stimulated the TH activity in the hypothalamus and CS of young and aged rats. In addition, specific cAMP agonist, (Sp)-cyclic adenosine 3',5'-monophosphothioate, significantly ($P<0.001$) increased the TH activity in tissues from both age groups. Theophylline (1 mM, phosphodiesterase inhibitor) did not affect TH activity in the hypothalamus of aged rats, but did significantly ($P<0.001$) increase TH activity in young rats. In the CS, the TH activity was significantly ($P<0.001$) increased in both groups. These results indicate that the PKA pathway modulates TH activity in the hypothalamus of young and aged rats. Impairment of hypothalamic dopaminergic neurons in aged rats may involve reduced synthesis of cAMP.

624.10

BRAIN SENSITIVITY TO KIANIC ACID EXCITOTOXICITY IS ENHANCED IN AGED RATS. D.L. Yuan*, J.P. Kesslak, and C.W. Cotman. Irvine Research Unit in Brain Aging, University of California, Irvine, CA 92717

It has been reported that kianic acid (KA) injection into the adult rat brain causes a severe neurodegenerative response while the same dose into the infant rat brain produces very little damage. A recent study indicated that subcutaneous injection of KA caused significant behavioral and brain damage to aged rats, less response in middle-aged rats and the least in young rats. The current study was designed to compare the histopathology between the young and old rats after directly administrated of KA into the hippocampus.

Young (110 days old) and old (410 days old) male Sprague-Dawley rats were anesthetized with sodium pentobarbital (50 mg/kg) and placed into a stereotaxic apparatus. KA (0.5 ug in 0.5 ul PBS) was administrated into the CA3 region of the dorsal hippocampus using a 10 ul Hamilton microsyringe. Rats were sacrificed 14 days following the KA administration, perfused, the brains removed, sectioned and stained with cresyl violet. KA injections in aged rats resulted in a much more extensive neuronal damage in dorsal hippocampus and adjacent area, compared to young rats. Quantitative analysis of the lesion size indicated an approximately four-time larger lesion volume in old than in young rats and the t-test revealed a significant difference. The result indicates that aged brain is more vulnerable to the excitotoxicity of KA after an intracerebral injection.

624.11

DECREASED NUMBER OF AMPA RECEPTORS WITH AGE. S. Standley, G. Tocco, J.K. Thompson*, M. Baudry & R.F. Thompson., University of Southern California, HEDCO Neurosciences Bldg., Los Angeles, CA 90089-2520.

Activity-dependent modifications of glutamatergic synapses are likely to be involved in learning processes. Learning deficits are particularly frequent with age and have been postulated to be due to a decrease in NMDA receptor responsiveness. We investigated possible changes in glutamate receptors with ageing using quantitative ligand binding autoradiography. Tritiated AMPA and CNQX on one hand and TCP on the other hand were used to label the AMPA and NMDA receptors respectively.

Two groups of Fisher (F344) rats, 2-3 and 29 months old were sacrificed, their brains rapidly removed and frozen. Ligand binding autoradiography was then performed on 10 um thick sections.

The aged animals exhibited a reduced binding for both AMPA and CNQX throughout the brain while TCP binding remained unchanged. The decreased binding was particularly marked in the hippocampus.

These results suggest that the number of AMPA receptors is decreased with age while the number of NMDA receptors remains unchanged. As AMPA receptor properties have been implicated in learning-induced synaptic plasticity, a decreased number of receptors might account for learning deficits observed with aging.

This work was supported by BNS 911037 from NSF to MB and NIH AG 05142 to RFT.

624.12

REGULATION OF HYPOTHALAMIC NEUROPEPTIDE Y (NPY) mRNA BY FASTING DURING AGING IN MALE RATS. C.V. Mobbs* and S.P. Kleopoulos.

Regulation of glucose metabolism is impaired during aging, elevated glucose can accelerate some age-correlated impairments, and dietary restriction delays some impairments. NPY mRNA in the arcuate nucleus appears to play a role in regulating glucose metabolism and is induced by fasting. We therefore examined if the induction of NPY mRNA by fasting is impaired during aging. 6-month-old, 12-month-old, and 18-month-old male Sprague-Dawley rats (n=6/group) were fasted for 72 hours, then sacrificed at 1900 h, one hour after lights went out. Control rats were fasted for 8 h, allowed to drink milk at 1800 h and sacrificed at 1900 h. Brains were fresh-frozen in dry ice, then cut into 6 micron sections for analysis by in situ hybridization. Single-stranded neuropeptide Y cDNA probes were labelled with tritium by asymetric PCR (using a plasmid generously supplied by S. Sabol). Sections were first exposed to film for 3 weeks, then dipped in emulsion and exposed for another 3 weeks. Quantification of both film and emulsion indicated that in 6-month-old rats NPY mRNA was significantly induced by fasting, but in 12- and 18-month-old rats the induction was not significant. These data suggest that in aging rats regulation of hypothalamic NPY mRNA is impaired. Supported by the American Diabetes Association and a Long Island Heart Foundation Equipment Grant.

624.13

ADVANCING AGE DOES NOT AFFECT α_2 ADRENERGIC RECEPTOR FUNCTION OR BINDING IN F-344 RATS. J. BUCHHOLZ* AND S.P. DUCKLES. Dept. Pharmacology, Coll. of Medicine, Univ. of California, Irvine CA. 92717.

Fractional norepinephrine (NE) release evoked by long stimulation trains significantly increases from 6 to 20 months of age in tail arteries of F-344 rats; however the maximal effect of idazoxan to increase NE release does not change with age. To examine further the effect of age on the overall function of prejunctional α_2-adrenergic receptors tail arteries from 6 and 20 month old rats were stimulated with short stimulation trains in the presence of 3 different concentrations of calcium for 4 sec at 8 Hz (60 V, 1 msec). Perfusate from 5 trains was pooled. Tail arteries were treated with 10^{-5} M deoxycorticosterone and cocaine and NE release measured by HPLC with electrochemical detection. The ability of idazoxan to increase NE release did not change with age regardless of calcium concentration, suggesting that the function of α_2 adrenoceptors in the tail artery remains unchanged with age. Binding studies in kidney and brain homogenates were performed with [^{3}H]-idazoxan. Idazoxan dissociation constant (Kd) and maximal binding (Bmax) did not change with age, suggesting that there are no age-related changes in the affinity or number of α_2-adrenoceptors in the kidney or brain. These data lend further support for the conclusion that advancing age does not result in an overall change in function of α_2-adrenergic receptors.

Increased Fractional Norepinephrine Release by 10^{-6} M Idazoxan pg/pg content/pulse x 10^{-6}

Age in Months	1 mM Calcium	1.6 mM Calcium	5 mM Calcium
6	19.1 ± 5.2	23.0 ± 4.7	18.2 ± 4.1
20	12.4 ± 2.2	21.4 ± 4.8	21.0 ± 6.0

NIH Grants AGO6912 and AGO 5498.

624.14

EFFECT OF GOLD-THIO-GLUCOSE (GTG) ON HYPOTHALAMIC OXYTOCIN RECEPTORS AND NEUROPEPTIDE Y (NPY) mRNA. H. Bergen*, S.P. Kleopoulos, J. Pfaus, P.J. Brooks, and C.V. Mobbs. Rockefeller Univ., New York, NY 10021.

GTG administered systemically to mice causes specific hypothalamic lesions and increased eating. Since infusion of NPY into the periventricular nucleus (PVN) stimulates eating, and fasting increases NPY mRNA in neurons of the arcuate nucleus (AN) which project to the PVN, we hypothesized that the GTG-induced lesions in the ventromedial nucleus (VMN) might lead to increased NPY mRNA in the AN. Therefore oxytocin receptors (as a marker of VMN neurons) and NPY mRNA in the AN were assessed in male CBA mice given GTG (0.5 mg/ gm bw, i.p.) 6 weeks before sacrifice. Oxytocin receptors were assessed by I^{125}-ornithine vasotocin receptor autoradiography. NPY mRNA was assessed by in situ hybridization, entailing single-stranded NPY cDNA probes labelled with tritium by asymetric PCR (using a plasmid supplied by S. Sabol). Six weeks after the GTG injection, cresyl violet stain revealed a loss of cells in the VMN, and a glial bridge connected the VMN on each side of the brain. Oxytocin receptor binding was readily demonstrable in the VMN of control mice, and completely abolished by the GTG injection. NPY mRNA was demonstrable in the AN of GTG-lesioned mice, but not elevated compared with controls. Supported by the American Diabetes Association and the Long Island Heart Foundation.

624.15

REGULATION OF HYPOTHALAMIC OXYTOCIN RECEPTORS AND LORDOSIS REFLEX DURING REPRODUCTIVE SENESCENCE OF FEMALE FISHER RATS. S.P. Kleopoulos*, L. Krey and C.V. Mobbs. Rockefeller Univ., New York, NY 10021.

Reproductive senescence in female rodents is characterized by impairments in estrogen-regulated neuroendocrine functions. Some of these age-correlated impairments appear to be due to persistent deleterious effects of estrogen. We therefore examined if hypothalamic oxytocin receptors and lordosis behavior, both of which are induced by estrogen, exhibit persistent effects of estrogen during aging. 3- and 10-month-old cycling rats, and 15-month-old non-cycling rats were ovariectomized. Ten days after ovariectomy, rats were given silastic capsules containing 5% estradiol, or empty sham implants (n=6/group), and sacrificed four days later. Estradiol (E_2) significantly increased both oxytocin receptors (assessed by in vitro autoradiography using I^{125}-ornithine vasotocin) and lordosis reflex (assessed by daily manual stimulation) in all age groups; conversely, E_2 decreased plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH). In the sham-implanted groups, 2 weeks after ovariectomy, oxytocin receptors and lordosis reflex increased with age, and LH and FSH decreased. These data suggest either that a non-ovarian source of E_2 increases with age, or that there is a persistent effect of E_2 on several neuroendocrine functions during aging. Supported by the American Federation for Aging Research.

624.16

FOOD RESTRICTION SUPPRESSES HYPOTHALAMIC PROOPIOMELANOCORTIN (POMC) MESSENGER RNA THROUGHOUT THE LIFESPAN OF THE MALE FISCHER 344 RAT. J.F. Nelson* and K. Karelus. Dept. of Physiology, University of Texas Health Science Center, San Antonio, TX 78284.

POMC is synthesized in the arcuate nucleus of the hypothalamus (HYPO) and is the precursor of several neuropeptides. HYPO POMC mRNA is suppressed by glucocorticoids. Because the diurnal rise of plasma free corticosterone is higher in food restricted than in ad libitum fed rats, it was of interest to determine if POMC mRNA is suppressed in food restricted rats. We also sought to determine if HYPO POMC mRNA declines with age in the Fischer 344 rat, as reported for other strains of rat and mouse. Rats were food restricted (FR) to 60% of ad libitum (AL) levels beginning at 6 weeks of age or fed AL and were killed between 0800 an 1200 h(lights on: 0400 h) at 6, 12, 18 and 24 months of age. HYPO were dissected, snap frozen in liquid N2 and RNA was extracted by the guanidinium cesium chloride method. POMC mRNA was measured by solution hybridization/RNase protection using a homologous 32P cRNA probe complementary to a portion of rat POMC mRNA. At all ages, POMC mRNA levels were 20-40% lower in FR than in AL rats ($p<0.05$). There was no age-related change in POMC mRNA in either group of rats, but this could be due to surprisingly low levels in the 6 mo AL group. Poly A RNA levels did not differ among treatment or age groups. These results are consistent with the hypothesis that the hyperadrenocorticism of the FR rat has physiological effects. They also indicate that the relatively high levels of POMC mRNA seen in AL rats are not essential for the extended lifespan of food restricted rats. (supported by a grant from the NIA).

624.17

5-HT FAILS TO ENHANCE NMDA DEPOLARIZATION OF CORTICAL NEURONS IN OLD RATS. R.S. Neuman*, S. Rahman, J. McLean and J. Reynolds. Fac. of Med., Memorial University, St. John's, Nfld. Canada, A1B 3V6.

A decrease in cortical 5-HT_2 receptors is found in both aged humans and rats, but little is known of the functional properties of these receptors. In immature rats, NMDA induced depolarization of cortical neurons is enhanced by co-activation of 5-HT_2 receptors (Rahman and Neuman, submitted). We now report this enhancement is dramatically reduced in 25 to 29 month old Fisher rats.

Wedges for "grease" gap recording were prepared from 500 μm thick slices of rat cortex (motor area). In young rats, amplitude of the NMDA (50 μM) depolarization was increased by co-administration of 5-HT (100 and 230% by 10 and 30 μM 5-HT respectively). In old rats 5-HT either had no effect (10 μM) or reduced the NMDA depolarization by 33% (30 μM). DOI (5 μM), a mixed 5-HT_{1C} and 5-HT_2 agonist, enhanced the NMDA response in young rats (175%) but not in old. Phenylephrine (10 μM) and carbachol (10 μM) enhanced the NMDA depolarization in both young (60 and 80% respectively) and old rats (82 and 162%). Functional 5-HT_2 receptors are present in the cortex of old rats since bath application of staurosporine (10nM; 40 min), a protein kinase C inhibitor, led to a 260% facilitation of the NMDA response by 30 μM 5-HT.

In conclusion, comparison of 5-HT_2 with α_1 and muscarinic effector systems demonstrates that the former is selectively reduced in old rats. We are currently using in situ hybridization to correlate 5-HT_2 receptor message with functional changes.

Supported by the Medical Research Council (Canada)

AGING III

625.1

TRANSFORMING GROWTH FACTOR-β1 mRNA INCREASES IN RAT AND HUMAN BRAIN WITH AGING. N.R. Nichols*, S.A. Johnson and C.E. Finch. Andrus Gerontology Center and Dept. of Biological Sciences, Univ. of Southern California, Los Angeles CA 90089-0191.

Transforming growth factor-β1 (TGF-β1) is a cytokine with well-known roles in differentiation and tissue repair. Previously, we cloned TGF-β1 from hippocampus of 3 mo old rats (Mol. Cell. Neurosci. 2:221, 1991). In addition, TGF-β1 mRNA increased both at the cortical wound sites and in the deafferented hippocampus and striatum after lesioning (J. Neurosci. Res. 28:134, 1991). Both temporal and neuroanatomical expression suggested a role for TGF-β1 in synaptic plasticity and tissue repair in adult brain. Since infusion of TGF-β1 into the lateral ventricle resulted in an increase in GFAP mRNA and protein, we compared changes in hippocampal TGF-β1 mRNA during aging in F344 male rats with GFAP mRNA (positive control) by RNA blot hybridization analysis. In a single cohort of F344 male rats, TGF-β1 mRNA increased 50% in the hippocampus of 24 mo compared with 6 and 15 mo old rats. A similar increase at 24 mo was seen in both hippocampus and striatum of a second cohort of F344 male rats. Furthermore, hippocampal GFAP and TGF-β1 mRNA prevalence in individual rats are positively correlated when all age groups are considered together ($r^2 = 0.634$, $P < 0.0001$, $n = 22$). In human brain, GFAP and TGF-β1 mRNA prevalence also increase in total RNA samples from different cortical areas and hippocampus of old (73 ± 7 y) compared with young (41 ± 12 y) individuals. As in the rat, human GFAP and TGF-β1 mRNA prevalence are positively correlated ($r^2 = 0.395$, $P < 0.0001$, $n = 52$). Since an increase in TGF-β1 mRNA may result in a concomitant increase in bioactive peptide, these data suggest a role for this cytokine in gliosis and increased astrocytic reactivity contributing to age-related changes in synaptic plasticity. (Supported by NIH grant AG-07909)

625.2

ULTRASTRUCTURAL PLASTICITY CHANGES OF THE POSTERIOR PITUITARY (PP) DURING AGING. F. Marzban*, C.D. Tweedle, J.Y. Summy-Long,[a] Z. Wang,[b] S. Freeman,[b] M.L. Terrell[b] and M. Kadekaro[b]. Dept. of Anatomy and Neuroscience Program, MSU, E. Lansing, MI; [a]Dept. of Pharmacology, MS Hershey Medical Center, Hershey, PA; [b]Div. of Neurosurgery, UTMB, Galveston, TX.

The hypothalamo-neurohypophysial system (HNS) develops morphological and physiological changes during aging. Here we report the effects of aging and of dehydration on ultrastructural changes in the PP. Male Fischer 344xBrown Norwegian rats (3 and 30 mo. old) were given water or 2% NaCl to drink for 5 days. They were then decapitated and the PP processed for ultrastructural studies. Daily water and saline intake in young rats were higher than in old rats. The intake of saline, however, was higher than water in both age groups. Dehydration in young rats induced a significant increase in axo-capillary apposition and a decrease in the glia-capillary contact. Aging alone induced similar plasticity changes and the presence of large degenerating Herring bodies. Dehydration in aged rats did not induce further ultrastructural changes, probably because of the less intense osmotic stimulus in these animals. The ultrastructural changes in the PP of old rats are suggestive of higher functional activity of the HNS under basal conditions. Vasopressin and oxytocin concentrations in the plasma and glucose utilization in the HNS are being evaluated. Supported by NIH 2RO1NS-23055 (MK) and HD-25498 (J.Y.S.-L.).

625.3

DIMINISHED TRANSLATIONAL REGULATION OF SOMATOSTATIN mRNA CONTRIBUTES TO THE REDUCTION IN GROWTH HORMONE SECRETION WITH AGE W.E.Sonntag*, A. D'Costa, J.E.Lenham and R.L.Ingram. Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157.

With advancing age, there is a decline in the capacity of tissues to synthesize protein. This decline in protein synthetic capacity appears to be closely related to a decline in insulin-like growth factor-1 and growth hormone since these hormones are potent anabolic agents, decrease with age and administration of these hormones increases protein synthetic capacity in tissues. Previous studies have indicated that part of the mechanism for the decline in the amplitude of growth hormone pulses is the result of an increase in somatostatin release from hypothalamic neurons. However, total somatostatin mRNA in the hypothalamus decreases substantially with age. In the present study, we compared polysomal bound and total somatostatin mRNA levels in Brown-Norway rats at 3 ages (6, 15 and 26 months) and in both *ad libitum* fed and dietary restricted animals. Total somatostatin mRNA decreased with age ($p<0.01$) and this decline was prevented by dietary restriction. Polysomal somatostatin mRNA increased approximately 46% with age ($p<0.05$). When data were expressed as polysomal bound somatostatin mRNA/total somatostatin mRNA a substantial increase was observed in *ad libitum* fed animals ($p<0.01$) which was not found in dietary restricted animals. These results indicate that with age there is increased recruitment of somatostatin mRNA onto polysomes suggesting a loss of translational control.

Supported by NIH grant AG07752.

625.4

DIFFERENT AGE-RELATED DECREASES OF ANKYRIN AND SPECTRIN OCCUR IN THE MOUSE TELENCEPHALON. N. Lam, B.A. Bahr, A.C. Godshall, R. Granger*, and G. Lynch. Center for the Neurobio. of Learning & Memory, Univ. of Calif., Irvine, CA 92717

Deteriorating neuropathologic processes are commonly associated with a decrease in neural activity and, eventually, brain function. It has been suggested that such neurodegenerative conditions are often, at least in part, a consequence of normal aging. The stable breakdown products of the membrane cytoskeletal protein spectrin, which act as biochemical markers correlating with the onset of many pathologic processes, have recently been shown to accumulate in the aged mouse telencephalon (Bahr *et al., Neurosci. Lett.* 131:237, 1991). In accord with this study, antibodies to cytoskeletal constituents were used to screen telencephalic immunoblot samples from three (n = 13) and twenty-five (n = 11-13) month old mice. The immunolabelling of neurofilament-200, talin, and Band 3, as measured by densitometric scanning, did not exhibit significant age-related changes, while actin decreased in the aged group as compared to the younger animals by a marginal 9.4 ± 4.5% (*mean* ± S.E.M., $p < 0.06$, two-tailed t-test). On the other hand, labelling of the α and β spectrin subunits decreased significantly with aging by 17 ± 3.5% ($p < 0.001$) and 18 ± 7.8% ($p < 0.05$), respectively. Labelling of the 220 kDa ankyrin had an age-related decrease of 37 ± 5.6% ($p < 0.001$) which is 2.1-fold greater than the decrease in α ($p < 0.01$) and β ($p = 0.05$) spectrins. In another group of mice, the difference in age effects on ankyrin and α spectrin was apparent between 3 (n = 4-5) and 10 (n = 4) months where the immunoreactivity levels decreased 26% and 3%, respectively ($p < 0.01$). Between 10 and 20 (n = 4) months of age, both antigens decreased 8% while between 20 and 25 (n = 4) months ankyrin and α spectrin diminished 1% and 14%, respectively ($p < 0.05$). In conclusion, aging processes appear to act differently on ankyrin and spectrin as far as rate and magnitude of decline (Supported by grant NIA AG00538).

625.5

EFFECT OF ACETYL-L-CARNITINE ON TRAUMA-INDUCED NEUROPATHIES IN THE YOUNG AND AGED RAT: MORPHOLOGICAL, MORPHOMETRIC, FUNCTIONAL EVALUATION. C. De Angelis, C. Scarfò, E. Perna, O. Ghirardi, M. Vertechy, E. Reda, M.T. Ramacci*, L. Angelucci[1]. Institute for Research on Senescence, Sigma Tau, Pomezia, Rome; [1]Institute of Pharmacology II, La Sapienza Univ. of Rome, Italy.

The favorable effect of acetyl-L-carnitine (ALCAR) on behavior and neuromorphological parameters of the CNS in aged rats prompted us to investigate its action on structure and function of intact and lesioned sciatic nerves in young and aged male Sprague Dawley rats. Sciatic nerve sections from animals sacrificed in anesthesia were stained with toluidine blue. In 24-month-old rats treated with ALCAR (150mg/kg/day in drinking water) for 6 months, sciatic nerves contained a lower number of altered myelinated fibers (-47%) and a higher number of normal fibers (+23%) than in age-matched controls. In rats aged 3 and 22 months, crush-induced injury caused 5 days later complete degeneration of the myelinated fibers. In both young and aged rats, treatment with ALCAR (15-60 days and 6-9 months, respectively) revealed an increased density of regenerating axons at 15 days (young) and 30 days (aged) after crush and an increased axonal size at 60 days. Also, the size of the entire myelinated fiber (sheath + axon) was larger in treated aged rats than in controls at 100 days after crush.

In agreement with the morphological features, the functional aspect (walking tracks) in ALCAR-treated rats showed a faster recovery of deambulation. Thus, the neurotrophic action of ALCAR in the CNS is also exerted in the PNS of both young and aged rats, even in the presence of other etiopathogenetic factors, e.g. trauma.

625.6

RATE OF CHANGE IN SIZE OF ABDOMINAL GANGLIA AS A FUNCTION OF WEIGHT IN *APLYSIA C.* J.M. Flinn*, C. Hong, M. Stanley, J. Estes, S. West, R. Holt. George Mason University, Fairfax, VA 22003.

The relationship between abdominal ganglia size and weight was examined for 194 *aplysia C.* whose weight ranged from .03 to 300 gms, by measuring the area of the abdominal ganglion. The area was determined using a compound microscope with calibrated objective. The best fit for the data required three different equations for the three weight ranges .03 - 2.5, 2.5$^+$ - 7.5, and 7.5$^+$ - 300 gms. The equations were:

$.03 < w \leq 2.5$ gms; $g_A = .308w + .08$; $r = .95$, $N = 140$, $p<.001$
$2.5 < w \leq 7.5$ gms; $g_A = .095w + .493$; $r = .85$, $N = 29$, $p<.001$
$7.5 < w \leq 300$ gms; $g_A = .016w + 1.01$; $r = .97$, $N = 23$, $p<.001$

Further analysis using dummy variables and curvilinear transforms will be performed. The transition points may be useful markers in development. dg_A/dw is greatest, .306 mm^2/gm, for the range .03 - 2.5 gms. 2.5 gm animals are approximately 2 - 3 cms, by Cash and Carew's criteria in late stage 12, at which time sensitization has developed. The second change in dg_A/dw at 7.5 gms marks the beginning of a much slower rate of ganglion growth. This is at the beginning of or earlier than other definitions (e.g., length > 10 cms) of early adulthood.

625.7

SYNAPTIC CONNECTIONS BETWEEN IDENTIFIED NEURONS DURING AGING OF A MOLLUSC, C. Janse, W. C. Wildering and M. van der Roest. (SPON: European Neuroscience Association). Department of Biology, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

Although many studies are known on age-related changes in the CNS, hardly any reports are available on the fate of synaptic connections between identified individual neurons during the animal's life span. The pond snail *Lymnaea* has a CNS with identifiable giant nerve cells permitting a life span approach to physiological studies of identified interneuronal synaptic contacts. This neurophysiological study reports on age-related changes in an electrical synaps between two giant peptidergic neurons (VD1 and RPD2) and in chemical synaptic contacts of an identified dopamine containing giant neuron (RPeD1). Experiments on VD1 and RPD2 showed that effectiveness of the electrical synaps between these neurons decreases with age. In addition pacemaker properties changed. The actions of the different changes together can explain the irregularities in the firing characteristics of the VD1/RPD2 system as found in old animals. In the study of properties of chemical synaptic connections attention was focussed on one particular group of follower neurons of RPeD1. These A-group neurons are situated in the right parietal ganglion. In young animals only a few A-neurons were found to receive synaptic input from RPeD1 whereas in animals of 6 - 10 months of age A-neurons receiving synaptic input were commonly found. In old animals (older than 12 months) the number of A-neurons with synaptic input from RPeD1 decreases again. These findings indicate that in *Lymnaea* electrical and chemical connectivity between neurons undergoe a continuous change with age. Future experiments will be directed to the the study of mechanisms governing these age-related changes.

625.8

THE EFFECT OF FIMBRIA-FORNIX LESIONS ON ASTROCYTE RNA EXPRESSION IN THE MOUSE BRAIN. J.R. Goss* & D.G. Morgan. Division of Neurogerontology & Dept. of Biol. Sci., Univ. of Southern California, Los Angeles, CA 90089-0191.

In previous studies we have examined age-related changes in astrocyte gene expression in the mouse brain and have reported a 40-80% increase in the message level for glial fibrillary acidic protein (GFAP) throughout the brain. To more fully understand the dynamics associated with this change, we employed a model of synaptic loss within the hippocampus by unilaterally transecting the afferent fimbria-fornix pathway in 7 month old C57Bl/6Nia mice. Success of the lesions was evidenced by loss of acetylcholinesterase staining in the hippocampus on the side of the lesion. *In situ* hybridization signals for GFAP RNA increased in both ipsilateral and contralateral hippocampi by 1 day post-lesion, peaked between 2 and 4 days post-lesion, was attenuated by 8 days post-lesion, and returned to non-lesioned control levels by 16 days post-lesion. Studies in progress are investigating this same lesion model in 3, 12, and 23 month old mice at 2, 5, and 10 days post-lesion. We will examine RNA for GFAP, glutamine synthetase, apolipoprotein E, sulfated glycoprotein 2, S-100, and amyloid precursor protein; all of which have been shown to have increased levels of RNA in astrocytes in various lesion models and/or neurodegenerative diseases. Supported by AG-07892, AG-00093, AHA-GIA 891079, and AHA-EIA 890173.

625.9

MICROGLIA OF THE AGED RAT BRAIN. M.N. Gordon*, M.A. Myers, L.S. Perlmutter and D.G. Morgan. Div. of Neurogerontology and Dept. of Neurology, Univ. of Southern Calif., Los Angeles, CA 90089-0191.

Increasing evidence suggests that microglia express immune-related antigens, function as antigen presenting cells and produce cytokines. Many aspects of peripheral immune function are reduced during aging, but little is known about microglia of the aged brain. Vibratome sections from F344/Brown-Norway F1 hybrid rats at 9, 18 or 24 mo of age were stained by lectin labeling with *Ricinus Communis* agglutinin I (RCA) or by immunocytochemistry using a monoclonal antibody against the rat MHC class II antigen (OX-6). The number of OX-6+ cellular profiles was counted and the percent area of tissue sections occupied by RCA reaction product was quantified using a video- and computer-based image analysis system.

The number of microglia expressing MHC class II antigen increased with aging in both grey and white matter. In neostriatum, OX-6+ cells increased from 14±3 cells/hemisphere/section (mean±SEM, n=4-5) at 9 mo to 43±4 at 18 mo and 96±25 at 24 mo. The number of microglia expressing OX-6 also increased 3-fold in the hippocampal hilar region (9 mo: 11±3; 18 mo: 28±4; 24 mo: 46±4 cells/0.5 mm^2), and 5-fold in the corpus callosum (9 mo: 24±5; 18 mo: 57±8; 24 mo: 152±16 cells/0.5 mm^2). OX-6+ cells also appeared larger in aged brain. RCA labeled a larger fraction of the microglial population than did OX-6. However, the percent area occupied by RCA reaction product was not increased as a function of age. These findings suggest some microglial markers remain stable during aging, but a larger fraction of microglia appear in an "activated" state, as more cells express MHC class II antigen. Supported by AG07892 & AHA-EIA 890173.

625.10

NEUROFILAMENT GENE EXPRESSION DECLINES IN THE DORSAL ROOT GANGLIA OF AGING RATS. I. M. Parhad*, J.N. Scott, C.A. Krekoski, A.W. Clark. Depts. of Pathology & Clinical Neuroscience, Univ. of Calgary, Calgary, Alberta, Canada.

Neurofilaments (NF)s are intrinsic determinants of axonal caliber. NF gene expression increases during maturation and is associated with an increase in Nf content and caliber of axons. In this study we asked whether a decline in Nf subunit gene expression occurs with aging, and whether this is correlated with axonal shrinkage. Fischer-344 rats were used at 4 ages (3, 5, 12, and 24 months; 3 rats / age group), L4-L6 dorsal root ganglia (DRG) were processed for quantitative Northern and *in situ* hybridization with NF-L (light subunit) cDNA. Morphometric evaluation was done of the L4 DRG and proximal dorsal root. Our results showed a 50% decrease in NF-L by Northern analysis at 24 months as compared to 3-12 months (Mann Whitney U, $p < 0.02$), and a similar decrease in NF-L mRNA grain density by *in situ* hybridization (n= 100 neurons / 1 rat / age group, $p < 0.001$, t test). No changes were seen in GAP-43 mRNA in these same samples with aging, by Northern or *in situ* hybridizations. Morphometric data showed a < 20% decrease in neuronal density in the DRG ($p < 0.05$), but no neuronal shrinkage (n = 100 neurons / age group, $p > 0.5$, F test). There was a 20% decrease in the diameter of large axons in the proximal dorsal roots at 24 months (n = 300 axons / age group; $p < 0.01$). These results indicate that NF-L mRNA declines with age and is associated with axonal shrinkage. Constitutive decline in Nf gene expression can result in axonal shrinkage and may be a substrate for age associated neural degeneration.

625.11

AGE-RELATED CHANGES IN MITOCHONDRIAL ENERGY METABOLISM IN THE SQUIRREL MONKEY. D.A. Di Monte*, M.S. Sandy, S.A. Jewell, I. Irwin, L.E. DeLanney and W.J. Langston. California Parkinson's Foundation, San Jose, CA 95128.

It has recently being suggested that impairment of energy metabolism plays a role in neurodegenerative disorders. In this study, we tested the possibility that age-related changes in mitochondrial activity may predispose to neurodegeneration. Squirrel monkeys were divided into three groups according to their age: young (n=4; age=3 year old), middle age (n=3; age=10 year old) and old (n=4; age= >16 year old). Animals were sacrificed, and brains were removed and dissected. Mitochondria were immediately prepared from the caudate, putamen and cerebellar cortex. Mitochondrial activity was measured as formation of ATP in the presence of different substrates, namely pyruvate and malate or succinate with rotenone. A significant decrease in the rate of ATP synthesis was measured between young and middle age monkeys in all areas of the brain when pyruvate and malate were used as metabolic substrates. In contrast, in the presence of succinate and rotenone, the decline in mitochondrial activity was only evident after middle age. The decreased rate of ATP synthesis could not be accounted for by a decrease in mitochondrial proteins in the tissues; indeed, the activity of other mitochondrial enzymes (*i.e.* citrate synthase and monoamine oxidase A) remained unchanged in all age groups. Thus, impairment of energy metabolism occurs with aging and may be involved in neurodegenerative disorders of the elderly. Our data also suggest that mitochondrial Complex I activity may decline at a relatively earlier age than the activities of other enzyme complexes of the respiratory chain.

625.12

CHARACTERIZATION OF AGE-ASSOCIATED INCLUSIONS IN BRAINS OF C57BL/6 MICE. M. Jucker*, L. C. Walker, L.J. Martin, D.L. Price, and D.K. Ingram. NIA Gerontol. Res. Ctr., NIH, Baltimore, MD 21224 and Neuropath. Lab., Johns Hopkins Sch. of Med., Baltimore, MD 21205.

In brains of C57BL/6 (B6) mice, age-associated inclusions were described (M Jucker *et al., Science,* 255:1443, 1992) that resemble lesions reported in transgenic mice produced with a construct of human amyloid precursor protein β-fragment (D Wirak *et al. Science*, 253: 323, 1991). Inclusions in B6 mice were first identified immunohistochemically with an antibody to a 110 kD laminin binding protein (LBP) and appeared as clusters (30-60 μm) of granules (1-3 μm) located predominantly in hippocampus but also seen in piriform cortex and cerebellum. Distinct inclusions first appeared about 6 mo of age and generally increased in incidence and size thereafter. The apparent immunoreactivity with several polyclonal antibodies, including β-amyloid, was not blocked by antigen preabsorption, indicating nonspecific immunoreactivity. Clusters were negative for hematoxylin and eosin, cresyl violet, thionin, Luxol fast blue, Bodian's Protargol, acetylcholinesterase, and glial fibrillary acidic protein (GFAP) stains. However, the presence of glycosaminoglycans was suggested by positive results using periodic-acid Schiff and Gomori's methenamine silver staining. Ultrastructural analysis revealed intracytoplasmic fibrillar material free of normal organelles; some inclusion materials were located within astrocytic somata. Association with astrocytes was also observed by using combined LBP and GFAP staining to identify inclusions in astrocytic processes and in proximity to capillaries. Etiology of the inclusions remains unidentified, but detection of faintly stained clusters in young mice (<6 mo) might provide clues to their development. Preliminary behavioral and morphological analysis has found little relationship between learning impairments and the density of hippocampal inclusions and glia cells in aged mice.

625.13

HIRANO BODY CONTAINS MOLECULES IMMUNOREACTIVE TO ANTI-HIPPOCAMPAL CHOLINERGIC NEUROSTIMULATING PEPTIDE (HCNP) ANTIBODY. S. Mitake*, K. Ojika*, K. Hayashi and O. Fujimori. 2nd Dept. of Int. Med., and 2nd Dpt. of Anat., Nagoya City Univ. Med. Sch., Nagoya 467, Japan.

A novel peptide (*HCNP: acetyl-Ala-Ala-Asp-ILe-Ser-Gln-Trp-Ala-Gly-Pro-Leu*) from rat hippocampus is involved in the development of specific cholinergic neuron in the *central nervous system in vitro* (*K. Ojika, et al; Brain* Res., 572:164-171, 1992). To investigate immuno-cytochemical distribution of the peptide in the central nervous system of old aged humans, anti-HCNP polyclonal antibody was raised in rabbits and affinity purified. The antibody specifically recognized HCNP and its 21KDa pro-protein. In histochemical studies, HCNP immunoreactivities in ABC method were exclusively associated with Hirano body in Sommer's sector of hippocampus, whereas no immuno-reactivity was observed in other bodies, such as Lewy's body, thalamic inclusion body or Pick's body. Immuno-histochemical mode of reaction of Hirano body were rod-like or granular in light microscope and antigens recognized by the antibody were closely associated with paracrystalline stracuture of the body in immunoelectron microscope.

The results strongly suggested that HCNP and its related molecules are involved in the formation of Hirano body.

625.14

S-ADENOSYLMETHIONINE DECARBOXYLASE IN HUMAN BRAIN. Lesley D. Morrison* and Stephen J. Kish. Clarke Institute of Psychiatry, Toronto, Canada, M5T 1R8.

S-adenosylmethionine decarboxylase (SAMDC) is a key regulatory enzyme in the biosynthesis of polyamines, substances which have been implicated as modulators of brain excitability. Little baseline information is available regarding SAMDC in human brain. We have measured in autopsied brain of neurologically normal subjects, basal SAMDC activity, sensitivity to substrate, activator and inhibitor; regional distribution and influence of aging. In a preliminary experimental animal study, brain enzyme activity declined by 47% after 24 hours postmortem. The specific enzyme activity in autopsied human brain was measured using a CO_2-trapping procedure with a specific inhibitor of SAMDC activity (MDL 73811; IC_{50}=31.5 nM) for blanks. The enzyme was characterised in parietal cortex with regard to substrate affinity (K_m=53 μM, V_{max}=74 pmol$^{14}CO_2$/h/mgP), and enzyme activity was markedly stimulated (+600%) by putrescine (K_a=16.7 μM).

The distribution of SAMDC activity in 12 human brain areas was measured (n=5 brains/area, except white matter n=3). The highest activity was observed in occipital, parietal, frontal and temporal cortices (58.3, 48.1, 44.7 and 36.1 pmol/h/mgP respectively); intermediate activity was observed in cerebellar and insular cortex, pulvinar, caudate and putamen (21.95, 19.46, 12.45, 26.9 and 17.37 pmol/h/mgP respectively) and lowest activity was observed in medial-dorsal thalamus, lateral globus pallidus and white matter (10.98, 6.44 and 2.86 pmol/h/mgP respectively).

The effect of aging on specific SAMDC activity was determined in occipital cortex (n=46, age range 1.3 weeks to 103 years). Enzyme activity increased from negligible levels at 1.3 weeks to ≈25% of adult values at age 2 years ($r=0.96$, $p<0.01$). Enzyme activity reached adult levels by age 14 and remained generally unchanged up to 103 years. (Supported by the Epilepsy Foundation of America).

625.15

Localization of Hemoglobin in Alzheimer's and Control Cerebellum. C.M. Hughes*, D.G. Flood, G.A. Campbell and J.R. Slemmon. Depts. Neurobio. and Anat., Neurol., Biochem., Univ. of Rochester, Rochester, NY 14642

Alzheimer's disease (AD) classically affects the hippocampus and association cortices. However, there has been some speculation that these areas are the first and, thus, most severely affected areas in a disease which proceeds progressively through the brain. Cerebellum, which is generally considered to be less affected in AD, may exhibit early changes if the disease is progressive. RP-HPLC analysis of cerebellum from AD and control cases (73-86 years old) demonstrates elevated levels of fragments of α and β hemoglobin in the AD cases with a percent change ranging from 592% to 164%. Immunocytochemical analysis of sections of cerebellum from most of the same cases shows human anti-hemoglobin immunoreactivity of granule cells and blood vessels. The granule cell staining appears to be membranous. The pattern of staining is similar in the AD and control cases although the staining is generally less intense in the control cases. One young control case (36 years old) shows blood vessel but no granule cell staining. This indicates that the localization of hemoglobin to the brain parenchyma may be an age-related phenomenon which is exaggerated in AD. The presence of hemoglobin in the brain parenchyma suggests the possibility of blood brain barrier malfunction in aging and AD cerebellum. Supported by-R35AG09016 (J.R.S.)

625.16

DISTRIBUTION OF PROTEIN SP40-40-LIKE IMMUNOREACTIVITY IN THE RAT BRAIN: A ROLE IN AGING? M.C. Senut, N.H. Choi (1), F. Jazat, and Y. Lamour*, INSERM U161, 75014 Paris, France; and (1) Showa University, Tokyo, Japan.

The protein SP40-40 is the human counterpart of the rat sulfated glycoprotein 2 (SGP2), whose mRNA has a widespread expression in the developing and the mature rat brain. In the present study, the distribution pattern and the cellular localization of the SP40-40 protein have been studied in various brain and spinal cord regions in young adult (3-4 months) Sprague-Dawley rats, using a well characterized polyclonal antibody. SP40-40-like immunoreactivity was mainly observed in the choroid plexuses and the ependymal cells; a consistent immunolabelling was also observed in the cingulate cortex (mainly layer VI), the retrosplenial cortex (layers II-VI) as well as in the hypothalamus. The SP40-40-like immunoreactivity was mainly observed within cell somata and their processes as a diffuse reaction product. Double-labelling experiments performed with antisera against GFAP demonstrated that SP40-40-like immunoreactivity was mainly localized within neurons. Although the function of protein SP40-40 in the central nervous system still remains to be elucidated, its involvement in neuronal death has been suggested. Therefore, we sought to determine the possible implication of SP40-40 in age-related cell death and atrophy. We performed a similar anatomical study in 12, 20-22 and 30-31 months old rats. With increasing age SP40-40-like immunoreactivity increased or appeared in specific brain areas (cingulate cortex, thalamus, hypothalamus, red nucleus, superior colliculus, olivary nuclei, cerebellum, cranial nerve nuclei). However, these changes did not seem to be associated with obvious signs of neuronal death or degeneration.

NEUROGLIA AND MYELIN V

626.1

$[Ca^{2+}]_i$ TRANSIENTS IN CULTURED SCHWANN CELLS EVOKED BY ACTIVATION OF NICOTINIC AChR's. E. Yoder*, V. Lev-Ram[1], and M. H. Ellisman. San Diego Microscopy and Imaging Resource, Departments of Neurosciences and [1]Pharmacology, University of California, San Diego, La Jolla, CA, 92093-0608.

$[Ca^{2+}]_i$ transients are known to mediate many cellular processes in developing and mature glia of the CNS. In the PNS, transient increases in $[Ca^{2+}]_i$ have been reported to occur in myelinating and terminal Schwann cells following axonal activation (Lev-Ram *et al.*, NS Abst. pg. 1519, 1991; Jahromi *et al.*, NS Abst. pg. 900, 1991); the physiological role of these transients is unknown. In order to gain insight into the nature of these signals, we have examined $[Ca^{2+}]_i$ transients in primary cultures of Schwann cells from neonatal rat sciatic nerves. Experiments were performed using cells loaded with the calcium indicator dye fluo-3 introduced via its AM ester. Carbachol, an AChR agonist, was found to induce a transient increase in $[Ca^{2+}]_i$ in many cells. Some exhibited an oscillation in $[Ca^{2+}]_i$ in response to carbachol. These effects were mimicked by nicotine and blocked by tubocurarine, but were not blocked by atropine. Thus, the observed action of ACh is via nicotinic (nAChR) rather than muscarinic ACh receptors. Nicotine induced $[Ca^{2+}]_i$ transients were observed in the absence of $[Ca^{2+}]_o$ plus EGTA indicating that the Ca^{2+} transient results from the release of Ca^{2+} from intracellular stores. The nature of this Ca^{2+} store was investigated by pharmacological manipulation. Thapsigargin stimulated an increase in $[Ca^{2+}]_i$ whereas caffeine did not. These results suggest that the release of Ca^{2+} from an intracellular store in cultured Schwann cells may be mediated by inositol trisphosphate receptors rather than ryanodine receptors. Mechanisms linking release of Ca^{2+} from intracellular stores in response to nAChR stimulation in Schwann cells, as well as the functional significance of such stimulation remain to be determined.

626.2

SUBPOPULATIONS OF SCHWANN CELLS RESPOND TO NEUROLIGANDS WITH INCREASES IN INTRACELLULAR CALCIUM. S.A.Lyons[1], P.Morell[1]*, and K.D.McCarthy[2]. [1]Dept. of Biophysics and Biochemistry and [2]Dept. of Pharmacology Univ. of North Carolina, Chapel Hill, NC 27599-7365.

Schwann cells interact with neurons during development to ensheathe or myelinate the axons shortly after birth. We hypothesize that one mechanism mediating neuronal/Schwann cell communication is signalling by neurotransmitters. We wish to determine if Schwann cells exhibit neuroligand receptors which are linked to calcium regulation and, if so, to examine the temporal relationship between the expression of receptors for various ligands on Schwann cell differentiation.

Schwann cell cultures were prepared from neonatal rat sciatic nerve and after 6 hours, 1d, 2d, 4d, 6d, and 14 days *in vitro* (DIV) were loaded with the calcium indicator dye, fura 2-AM. The influence of up to eight different neuroligands on Ca_i^{2+} levels was examined using a video-based imaging system. Neuroligands that elicited 2-4 fold increases in Ca_i^{2+} levels above vehicle responses in cultured Schwann cells were bradykinin (BK; 10μM) and adenosine triphosphate (ATP; 10μM). Both ATP and BK increased Schwann cell calcium levels within 15 seconds of drug addition, after which Ca_i^{2+} levels decreased toward baseline over 1-2 minutes. Calcium levels also increased in Schwann cells exposed to histamine (10μM) and glutamate (100μM). However, a smaller and more variable percentage of Schwann cells responded to these ligands. The results of these studies indicate that subpopulations of S100*B* immunopositive Schwann cells respond to one or more neuroligands with a rise in Ca_i^{2+} levels. Furthermore, it appears that developmental processes occurring *in vitro* influence the percentage of Schwann cells responding to different neuroligands with a rise in Ca_i^{2+}.

626.3

ASTROCYTES INHIBIT SCHWANN CELL PROLIFERATION AND MYELINATION OF DORSAL ROOT GANGLION NEURONS IN VITRO. V. Guenard*, P. Wood. The Miami Project to Cure Paralysis & Department of Neurosurgery, University of Miami School of Medicine, Miami, FL.

Schwann cells (SCs) aid central nervous system regeneration. However, little is known about the effect CNS glia may have on SC function. In the present study, the effect of astrocytes (AS) on SC proliferation and myelination was evaluated using pure neuronal cultures [prepared from dissociated embryonic rat dorsal root ganglia (DRG)] which were seeded with pure SC (50 x 10^3 cells/culture) isolated from neonatal rat sciatic nerve. SC function in these DRG-SC cocultures was compared to that in similar cultures to which were added either AS from neonatal rat cortices or fibroblasts (Fbs) from neonatal rat cranial periosteal membrane. To assess proliferation, AS or Fbs (0, 150 or 500 x 10^3 cells) were added to DRG-SC cultures; the cultures were maintained in medium containing 1% FBS. Two days later, the cultures were pulsed with ^{3}H-thymidine and after an additional 24 hours, immunostaining with 217c and GFAP antibodies and autoradiography were performed. AS inhibited SC proliferation in a dose-dependent manner whereas Fbs did not. SC proliferation was decreased by 25% in low astrocyte-density cultures and 82% in high astrocyte-density cultures relative to DRG-SC cultures. To evaluate myelination, DRG-SC cultures were maintained for 2 weeks and then AS or Fbs (0 or 100 x 10^3 cells) were added. For the next 2 weeks, cultures were maintained in medium containing 15% FBS and ascorbate, i.e. conditions which stimulate myelination in DRG-SC cocultures. Cultures were either immunostained (for O_4, GalC, O_1 and GFAP), stained for myelin (using Sudan black) or processed for electron microscopy. AS inhibited SC myelination by 90% relative to DRG-SC cultures. In the presence of AS, the expression of GalC was blocked, but that of O^4 was not. To determine if AS-SC contact played a role in the observed inhibition, AS were seeded on microporous membranes mounted in "tissue inserts" (Falcon) positioned above the DRG-SC cultures. Soluble factors released by AS induced a 27% decrease in SC proliferation and a 90% decrease in SC myelination relative to cultures with empty inserts. The present study demonstrates that AS can inhibit both SC proliferation and myelination in normal conditions in vitro. Our results suggest that AS mediate their effects through soluble factors.

626.4

IN VIVO IDENTIFICATION OF SCHWANN CELL NUCLEI IN MOUSE NEUROMUSCULAR JUNCTIONS. S. Nakashiro* and N. Robbins. Dept. of Neurosciences, Case Western Reserve Univ., Cleveland, OH 44118.

Little is known about the function of the terminal Schwann cell at the neuromuscular junction (NMJ). One strategy is to assess the function of this cell before and after deletion *in vivo*. For this reason, we have developed a technique to identify terminal Schwann cell nuclei in living mouse NMJ's. Superficial endplates in the mouse pectineus muscle were identified by staining the synaptic matrix with fluorescein-conjugated vivia vincia agglutin, and nuclei were stained with Hoechst 33342 in the living animal. By shape and location of the nuclei at the NMJ and the features of their nucleoli, the Schwann cell nuclei were then distinguishable from those of other cells such as muscle, fibroblasts, and capillary endothelium. This was confirmed through EM studies of serial sections of NMJ's. The Schwann cell nuclei, for example, have several small nucleoli which are also seen in Hoechst-stained nuclei above the nerve terminal, whereas muscle nuclei near NMJ's have only one or two prominent nucleoli. This identification was confirmed by S-100 immunostaining for Schwann cells in whole mounts. With this technique, we are now able to destroy the nucleus of the terminal Schwann cell *in vivo* using a focused laser beam. Deletion techniques such as these may make it possible to define the role of the terminal Schwann cell in the maintenance and plasticity of the neuromuscular junction. Supported by NIA grants AG08886 and AG06641.

626.5

UPTAKE OF AXONALLY TRANSPORTED LATEX NANOSPHERES BY PARANODAL AXON-SCHWANN CELL NETWORKS. K.P. Gatzinsky* and H. Persson. Dept. of Anatomy, Univ. of Göteborg, 413 90 Göteborg, Sweden.

The distribution of retrogradely transported red-fluorescent latex nanospheres (Molecular Probes) was studied by light and electron microscopy in lumbosacral ventral root axons of adult rats. The left sciatic nerve was crushed and immediately injected intraneurally with 50 µl of the tracer. After a 48-72 h postinjection survival period, the animals were perfusion-fixed by 4% paraformaldehyde. Vibratome-cut ventral root sections were examined with a Nikon FXA epifluorescence microscope. Spheroid granules exhibiting red fluorescence were distributed within many axons of the injected but not the control side. The granules were often concentrated at nodes of Ranvier, where they were situated in close association with the paranodal myelin sheath. For electron microscopical detection of elements showing this type of fluorescence, photoconversion was performed in epifluorescent light using a solution of 0.1% DAB in Tris buffer. With this procedure a black precipitate, which appeared electron dense at the ultrastructural level, was formed in association with membrane-delimited organelles of various sizes. In the internodal parts of the axons organelles of this appearance were situated in the axoplasm. By contrast, most organelles in association with the paranodal myelin sheath were situated within the so called axon-Schwann cell network, thereby being segregated from the main axoplasm.

Our results show that axonally transported non-neuronal materials can be removed from motor axons via a sequestration within paranodal axon-Schwann cell networks. Most likely, this process represents a local mechanism whereby motor neurons can eliminate retrogradely transported foreign substances before arriving to the neuronal perikaryon in the CNS.

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626.6

ISOLATION OF HUMAN SCHWANN CELLS FROM PEDIATRIC DORSAL NERVE ROOTS FROM DORSAL RHIZOTOMY AND ADULT SCIATIC NERVE FROM AUTOPSY. P.J. Boyer*, L. Rutkowski, G. Tuite, R. Dauser, K. Muraszko, and G.I. Tennekoon. Department of Pathology and Divisions of Pediatric Neurology and Neurosurgery, University of Michigan, Ann Arbor, MI 48109

Human Schwann cells have been isolated, in limited numbers, from adult surgical (sural and sciatic nerves), transplant (phrenic nerve), and autopsy (trigeminal ganglion) specimens and from fetal tissues. We report methods for direct isolation of large numbers of human Schwann cells from two additional sources: (1) nerve roots obtained from pediatric patients undergoing dorsal rhizotomy for spastic diplegia (8 patients, age 4-12 yr, 5 preparations) and (2) sciatic nerves obtained from adults at autopsy (3 patients, age 52-66 yr, 5-12 hr postmortem). Major methodologic challenges included minimizing microbial contamination (for adult specimens) and optimizing enzymatic dissociation, Schwann cell population expansion, and fibroblast growth inhibition. Nerve fascicles were dissected out and cultured in the presence of cholera toxin for 1-2 wk prior to enzymatic dissociation. While less tissue was available from pediatric surgical specimens than from adult autopsy specimens (average 81 mg and 1525 mg of nerve fascicles, respectively), the total cell yield was nearly twice as high from pediatric specimens (average 5.1×10^4 and 2.6×10^4 cells/mg, respectively). Cell viability (over 80%), Schwann cell to fibroblast ratio (20:1), and Schwann cell antigen expression were similar in the two groups. Adult and pediatric Schwann cells responded similarly to mitogens but, while adult Schwann cell populations could be expanded with minimal fibroblast contamination, pediatric fibroblast proliferation was more vigorous and less inhibited by adenylate cyclase activators. In summary, pediatric surgical and adult autopsy peripheral nerve specimens provide additional sources of human Schwann cells. Funded by NIH NS 21700 and National Multiple Sclerosis Society RG 2395.

626.7

DETECTION AND REGULATION OF PMP-22 mRNA AND PROTEIN IN CULTURED SCHWANN CELLS. S. Pareek*, U. Suter#, G. J. Snipes#, A. A. Welcher#+, E. M. Shooter# and R. A. Murphy. Dept. of Anatomy and Cell Biology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2H7, # Dept. of Neurobiology, Stanford Univ. School of Medicine, Stanford, CA 94305-5401, USA.

Peripheral myelin protein-22kDa (PMP-22) is a novel myelin-membrane associated glycoprotein that has been localized within Schwann cells of sciatic nerve (Snipes et al., J. Cell Biol., 117, 225, 1992). The function of PMP-22 is unknown, however recent studies suggest that a point mutation in PMP-22 is a likely candidate for the mouse *trembler* phenotype (Suter et al., Nature, 356, 241, 1992). In this study, we have analyzed PMP-22 in cultured rat sciatic nerve Schwann cells to determine whether these cells express the protein in the absence of neurons and whether the controls that regulate its production are similar to those that regulate production of other myelin-associated proteins. Immunocytochemical studies using polyclonal antibodies raised against synthetic peptides of sequences within the protein show that PMP-22-like immunoreactivity is present within Schwann cells. Northern blot and RNase protection assays reveal that mRNA coding for PMP-22 is present in Schwann cells cultured in serum-containing medium supplemented with glial growth factor and forskolin. In the absence of forskolin, mRNA levels decline but are re-established within 36 hours after forskolin is added to the medium. Increases in PMP-22 protein levels also occurred following forskolin treatment as determined in studies in which Schwann cells were metabolically labelled with ^{35}S-methionine and cellular extracts were immunoprecipitated with anti-PMP-22 antibodies. Taken together, our results suggest that PMP-22 is present in axon-free Schwann cells and its expression is regulated in a manner similar to that of other myelin associated proteins such as MBP and P_0.

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626.8

AXONS MODULATE THE EXPRESSION OF SCIP IN THE PNS. S.S. Scherer*, D.-y. Wang, L. Wrabetz, and J. Kamholz. Dept. Neurol., Univ. Penn. Sch. Med., Philadelphia, PA 19104.

SCIP (also known as tst-1 and Oct-6) is a POU transcription factor that is expressed by Schwann cells and appears to down-regulate the expression of myelin genes. We have analyzed the expression of SCIP mRNA in the peripheral nervous system of the rat. In developing nerves, the steady state level of SCIP mRNA was highest at the day of birth, and fell quickly to a low level that persisted into adulthood. We examined the steady state level of SCIP mRNA in adult sciatic nerves that were either permanently transected (to cause Wallerian degeneration but prevent axonal regeneration) or crushed (to cause Wallerian degeneration but allow axonal regeneration). After nerve-transection, SCIP mRNA increased slightly and transiently at 2 days post-transection, then returned to a low level for at least 58 days. After nerve-crush, SCIP mRNA increased substantially by 8 days post-crush, to much higher levels than in transected nerves, and this high level of expression was maintained for at least 58 days. Since Wallerian degeneration in crushed and transected nerves appears to be similar, the difference in SCIP expression appears to depend on regenerating axons. Thus, we believe that regenerating axons in crushed nerves interact with Schwann cells, leading to the up-regulation of SCIP mRNA.

626.9

THE EFFECTS OF ETHANOL ON TRANSFECTED SCHWANN CELLS. B.M. Labissiere, S. Moore, A. Springer, P.W. Archer, R.M. Knight. Dept. of Life Sciences Virginia State Univ., Petersburg, VA., 23806

Primary Schwann Cells transfected with the SV-40 T-antigen provides a model for obtaining cells that express properties associated with normal Schwann cells in culture. Ethanol has been shown to effect the nervous system, leading to alcohol-related peripheral neuropathy. The effects of various concentrations of ethanol on the morphology, proliferation and protein synthesis of the transfected Schwann cell were investigated. Increases in ethanol treatments (22, 43, 86, and 172 mM) caused a concentration dependent decrease in cell proliferation and protein synthesis. Light microscopic evaluation revealed cellular processes that thickened as the ethanol concentrations increased. These data indicate that transfected Schwann cells may provide an excellent model for studies of ethanol induced neuropathy in Schwann cells.

626.10

PHENOTYPIC FEATURES OF OLFACTORY ENSHEATHING CELLS: AN IMMUNOCYTOCHEMICAL EXAMINATION. R. Doucette* and R. Devon. Department of Anatomy, College of Medicine, and Department of Oral Biology, College of Dentistry, University of Saskatchewan, Saskatoon, Sask., Canada.

Olfactory ensheathing cells possess a mixture of Schwann cell and astrocytic phenotypic features and are the only glia that ensheath the 1^{0}olfactory axons. The objective of this study was to determine what additional phenotypic features ensheathing cells would express when grown under in vitro conditions known to be optimal for the growth-differentiation of Schwann cells or astrocytes. Ensheathing cells were obtained from the nerve fiber layer of the olfactory bulb of E18 rat embryos. For the first experiment, ensheathing cells were grown in either Bottenstein's G5 medium or in DMEM-F12/1%FBS/0.25 mM dBcAMP. Although dBcAMP did induce the appearance of weak GFAP-like immunoreactivity in these cells, they did not differentiate into typical astrocytes even when grown in either media for several weeks. The second experiment examined whether ensheathing cells would assume a myelinating phenotype (like Schwann cells) after being planted onto purified DRG neuronal cultures. By four weeks many of the S100-positive ensheathing cells were Gal-C+ and MBP+ and had begun to myelinate the larger axons, as visualized with the electron microscope. These myelinating glia were not residual Schwann cells that had survived the antimitotic treatment because control pure neuronal cultures contained no glial cells and no evidence of myelination. Thus, ensheathing cells can assume a myelinating phenotype in vitro. (This work was supported by a grant from the MRC of Canada).

626.11

GLIAL CELLS IN THE DEVELOPING OPTIC NERVE OF THE FROG *LITORIA (HYLA) MOOREI*. D.E. Playford* and S.A. Dunlop. Neurobiology Lab., Department of Psychology, University of Western Australia, Nedlands 6009.

We have recently shown that there is a biphasic sequence of myelination in the optic nerve of *Litoria moorei*. The first phase is initiated at the optic foramen in mid-tadpole life, spreads towards the eye and chiasm and is complete at metamorphic climax; the second phase is initiated at the chiasm, spreads towards the eye and results in approximately 2.5% of optic axons being myelinated in the fully mature adult. We have also reported that numbers of glial cells increase throughout life and that the proportion of oligodendrocytes mirrors the patterns of myelination (Playford & Dunlop, 1991, Neurosci. Abs. 17, 157.11). Here, we have examined glial cells ultrastructurally and show that although astrocytes and oligodendrocytes can be distinguished from mid-tadpole life, they continue to mature morphologically until the final adult stage. To determine when and where glial cell division is occurring, animals were injected with tritiated thymidine and killed 4-6 hours later. Dividing cells were seen at all levels in the nerve suggesting that differentiated astrocytes and oligodendrocytes undergo cell division. In addition to differentiated glial cells, there is a distinctive group of undifferentiated glial cells at the chiasmal end of the nerve. Serial reconstruction of wax sections shows that these undifferentiated cells emanate from the pre-optic recess, pass through the chiasm, enter the nerve as a dorsal cell mass and extend along the nerve for up to 50 microns. This cell mass diminishes after metamorphic climax. Dividing cells were also seen within the undifferentiated cell mass.

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626.12

POTASSIUM CURRENTS FROM HEALTHY AND NEUROFIBROMA-DERIVED SCHWANN CELLS IN THE BICOLOR DAMSELFISH, A MODEL OF HUMAN NEUROFIBROMATOSIS. L.A. Fieber* and M.C. Schmale. Univ. of Miami Rosenstiel School of Marine and Atmospheric Science, NIEHS Marine and Freshwater Biomedical Sciences Center, Miami, FL 33149.

Schwann cells are the predominant cell type observed in neurofibromas in Type 1 neurofibromatosis (NF-1) in man. The development of this disease is marked by changes in morphology and growth patterns in Schwann cells which may have physiological correlates. Here we present electrophysiological results on cultured Schwann cells from the only naturally occurring model of NF-1, damselfish neurofibromatosis (DNF), from the bicolor damselfish (*Pomacentrus partitus*). Ionic currents were recorded using the whole cell patch clamp technique from cell bodies of healthy fish Schwann cells from peripheral nerve explant-derived cultures with or without axonal fragments present. These were compared to currents in cultured Schwann-like cells from neurofibromas. Each of these cell types expressed the glial specific antigen, S-100, but not fibronectin, an antigen found in both mammalian and damselfish perineurial cells. Multiple types of potassium currents were observed in both healthy and tumor derived cells, but with different currents predominating. All tumor derived cells had a transient current with a steep, sigmoid activation curve, which was partially blocked by external tetraethylammonium (TEA; 14 mM). This current is similar to A-type potassium current. Tumor derived cells often had only this current. All Schwann cells from healthy fish, regardless of the presence of an axonal fragment, had a delayed rectifier current, and often this current had little or no apparent inactivating component. This current was blocked by external TEA (14 mM).

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626.13

CLONING, SEQUENCING, AND LOCALIZATION OF GLUTAMINE SYNTHETASE FROM THE NERVOUS SYSTEM OF THE SPINY LOBSTER. H.G. Trapido-Rosenthal*, R.A. Gleeson, W.E.S. Carr, R.M. Greenberg, E. Orona, L. Van Ekeris, W. Lesser, and P.J. Linser. Whitney Laboratory, University of Florida, St. Augustine, Florida 32086.

The olfactory organ of the spiny lobster, *Panulirus argus*, consists of a dense array of aesthetasc sensilla on the lateral filament of the antennule. Each sensillum contains the dendrites of several hundred chemosensory cells, and processes of a number of glia-like auxiliary cells. Electrophysiological studies have shown that sensilla include populations of receptor cells that respond to a variety of amino acid odorants, including glycine, glutamate, and alanine. Biochemical studies have shown that sensilla also contain amino acid uptake systems, as well as odorant-metabolizing enzymes. Messenger RNA isolated from the lobster's olfactory organ was used to construct a cDNA library in the vector λZAPII. The library was screened for olfactory enzymes by means of the polymerase chain reaction (PCR). Sequencing of two PCR products revealed an open reading frame of 1083 nucleotides, coding for 361 amino acids showing 63 % identity to the enzyme glutamine synthetase (GS) from *Drosophila melanogaster*. Immunohistochemical and *in situ* hybridization studies showed that, in the brain, GS is localized in glia, and in the olfactory organ, GS is localized in the auxiliary cells. Biochemical studies showed that the olfactory sensilla have 4-fold greater GS activity than does lobster brain. Olfactory GS may play a role in controlling the background levels of amino acids in the environment of olfactory receptors.

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BLOOD-BRAIN BARRIER III

627.1

SUBCELLULAR ANALYSIS OF GLUT-1 IN DEVELOPING BRAIN. S. Hyman, E. M. Cornford*, and W. M. Pardridge. Depts. of Neurology & Medicine, UCLA, and West Los Angeles VA Medical Center, Los Angeles, CA 90073.

Glucose transporter density and localization was defined in the blood-brain barrier of newborn (NB), 14-day-old suckling, 28-day-old weanling and adult rabbits with immunogold electron microscopic methods (PNAS 88:5779, 1991). Newborn capillary profiles were grouped as prepatent, intermediate, or patent by ablumenal & lumenal membrane lengths, and lumenal & endothelial cytoplasmic areas. The number of gold particles/endothelium increased with age. Net cytoplasmic areas decreased, while mean lumenal & ablumenal lengths increased. Recruitment of cytpolasmic transporter to membranes in development is apparent from the reduced cytoplasmic GLUT-1 seen post-natally (Table). The increased (3.5X ablumenal-to-lumenal) transporter confirms previous adult rat studies.

Age	(n)	#Au	LUMEN %	ABLUMEN %	CELL %
NB Pre	12	77	12 ± 6	44 ± 9	44 ± 9
NB Int	53	53	12 ± 6	38 ±10	49 ±10
NB Pat	34	60	12 ± 7	38 ±12	49 ±12
14-DAY	34	68	16 ±10	44 ±11	38 ±12
28-DAY	14	75	13 ± 7	49 ±12	38 ±14
70-DAY	32	142	14 ± 6	46 ± 9	40 ± 9

[Supported by NIH Grant NS 25554.]

627.2

QUANTIFICATION OF mRNA OF BRAIN GLUCOSE TRANSPORTER USING PCR AIDED TRANSCRIPT TITRATION ASSAY (PATTY) METHOD K. C. Wadhwani*, T. Giordano, K. Chandrasekaran, R. Fukuyama and Q. R. Smith. Laboratory of Neurosciences, NIA, Bethesda, MD 20892.

Changes in levels of mRNA of glucose transporters have been observed under various physiological and pathological conditions. In order to quantitate such changes in the glucose transporter, we tested whether the PATTY method (Nucl. Acid. Res., 17:9437, 1989) of quantification of mRNA could be used. A 405bp [^{3}H]-labelled riboprobe containing the coding and 3' noncoding sequences of the glucose transporter was prepared from rat-GLUT1 cDNA plasmid (prGT3). A known amount (400 pg) of this synthetic [^{3}H]-RNA was reverse transcribed using random primers. The cDNA was then amplified using 2 primers corresponding to the noncoding sequences of GLUT1. This resulted in the amplification of a 292 bp fragment specific to the rat GLUT1 gene (target DNA). An oligo nucleotide of 100 bp containing the same sequences as the first and last 50 bases of the 292 bp fragment was also prepared (competitive DNA). Identical amounts of 'target DNA' were then coamplified with different amounts of the 'competitive DNA' (ranging from 24 pg to 24 ng) using the same primers. The 5'- primer was end labelled with ^{32}P. The ratio of radioactivity between the 'target DNA (292 bp fragment)' and 'competitive DNA (100 bp fragment)' was determined quantitatively after separation by gel electrophoresis and radioactivity counting. The results showed that this method gave an accurate determination of the amount of synthetic [^{3}H]-RNA of the GLUT1 transporter and that a 2-fold change in the amounts of the synthetic RNA could be detected. This method is currently being employed to determine changes in the levels of mRNA of glucose transporter using *in vivo* and *in vitro* model systems.

627.3

UPREGULATION OF THE BLOOD-BRAIN BARRIER (BBB) GLUCOSE TRANSPORTER (GLUT1) mRNA BY GLUCOSE DEPRIVATION. L. Wang, R.J. Boado, W.H. Oldendorf*, and W.M. Pardridge. Departments of Medicine and Neurology and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The absence of neuroglucopenia symptoms in chronic hypoglucemia may be due to an upregulation of the BBB-GLUT1 glucose transporter. Therefore, we investigated the effect of glucose deprivation on the abundance of the GLUT1 transcript in bovine brain capillary endothelial cells in culture (ECL). The GLUT1 gene is over-expressed in cell culture. Paradoxically, there is a marked downregulation of GLUT1 gene expression in brain ECL (Boado & Pardridge, Molec. Cellul. Neurosci. 1:224, 1990). Therefore, poly A+ mRNA from ECL was isolated by a single step procedure recently described (Boado & Pardridge, J. Neurochem., 57:2136, 1991). Northern blots preformed under high stringency conditions with 10 μg ECL poly A+ mRNA showed that glucose deprivation (5 mg % glucose) caused a 2.4 ± 0.2 (mean ± SE, n = 3) fold increase in the GLUT1/ actin mRNA ratio vs. control incubations (100 mg % glucose). This rise was time dependent and the maximum effect was observed at 20-24 hours after the hexose deprivation. Nuclear transcription run on assay showed no changes in neither the GLUT1 or actin gene transcription rate 24 hours after glucose deprivation.

Conclusion: i) glucose deprivation increases the abundance of GLUT1 mRNA in brain capillary endothelium, ii) this increase is probably due to enhanced stability of the GLUT1 mRNA without changes in the gene transcriptional rate, and iii) this may represent the initial step in the upregulation of the BBB-GLUT1 glucose transporter in chronic hypoglycemia.

627.4

ENDOTHELIN-1 (ET-1) RECEPTORS AT THE BLOOD-BRAIN BARRIER (BBB). P. Grammas*, T. Botchlet, B. Jacks. Univ. of Oklahoma Health Sciences Center, Okla. City, OK 73190.

ET-1, synthesized by endothelial cells (ECs), is well known role as a powerful constrictor of vascular smooth muscle cells although less is known about other possible ET-1 effects on other cell types. By analogy to another EC product, endothelial derived relaxing factor (NO), ET-1 could play a role as a neuromodulator in the nervous system. The objective of this study is to determine the possible role of ET-1 on the cerebral microcirculation characterizing ET-1 receptors on isolated microvessels (MVs). The results of the radioligand binding experiments show that in MVs from adult rats the binding capacity is 827 fmol/mg and the K_D is 1.31/nM In addition, ET-1 (10-1000 nM) significantly ($p<0.05$) stimulates Na^+- dependent neutral amino acid transport as measured by the uptake of methyl amino isobutyric acid. Furthermore, we have shown that in aged rats (>18 mths) the number of ET-1 receptors decreases approximately 50% with no change in K_D. Our results demonstrate that the cerebral microcirculation i.e.the BBB, possesses receptors for ET-1 and that in response to this peptide amino acid uptake can be modulated. In addition, in aged animals there is a decrease in the ET-1 receptor. These data suggest that BBB functions, such as amino acid transport, could be altered by ET-1 and that ET-1 responsiveness might be diminished in aging. (Supported by AHAF, OCAST and NIH NS 30457).

627.5

CHRONIC NICOTINE TREATMENT INCREASES BLOOD-BRAIN TRANSPORT AND CAPILLARY SEQUESTRATION OF VASOPRESSIN. M.N. Lipovac*, E. Barrón, L.S. Perlmutter, J.G. McComb, M.H. Weiss and B.V. Zlokovic. Depts. Neurol. Surg. and Neurol., and Divn. Neurosurg., CHLA USC Sch. Med., Los Angeles, CA 90033

Plasma vasopressin (AVP) may be responsible for reinforcing and withdrawal effects of nicotine (N) and tobacco smoking associated with cognitive functions. In this study, blood-brain barrier (BBB) transport and capillary sequestration of AVP were examined in N-treated guinea pigs. N tartarate was administered s.c. by osmotic mini-pumps (Alzet 2002; 4.5 mg/kg of N free base/day) for 14 days; 14-day plasma levels of N and cotinine (N metabolite) were 11.4 and 101.8 ng/ml, respectively. [^{3}H]-AVP (3 nM) and [^{14}C]-sucrose (vascular space marker) were simultaneously infused into brains of N-treated animals (for 10 min) by vascular perfusion technique [Biochim. Biophys. Acta (1990) 1025: 191-98]. Capillary depletion method [J.Neurochem. (1990) 54: 1882-88] was used to distinguish between AVP BBB transport vs. binding. The rate (K_{in}) of [^{3}H]-AVP entry into brains of N-treated animals was significantly increased in comparison to N-naive animals (control), i.e. by 117% as determined in capillary depleted brain tissue. A ten times greater rate of AVP capillary in situ sequestration was found as a result of N treatment. Compartmental brain uptake of sucrose was not significantly altered by N-treatment. [^{3}H]-AVP BBB transport and binding in N-treated animals were strongly inhibited (57% to 81%) by unlabeled AVP and the V_1-antagonist (TMeAVP) at 4.5 μM. It is concluded that N increases transport of AVP into the brain parenchyma and its sequestration at the BBB most likely by modifying a specific BBB AVP carrier and/or a V_1-like receptor at the luminal side of the BBB. (Supported by Tobacco Related Disease Research Program, grant 2RT0071).

627.6

MORPHOLOGIC ALTERATIONS IN NEUROHYPOPHYSEAL ENDOTHELIAL CELLS OF NICOTINE-TREATED GUINEA PIGS. E. Barrón[1], M.N. Lipovac[2], B.V. Zlokovic[2], L.V. Johnson[3]* and L.S. Perlmutter[1]. USC School Medicine; Neurol[1], Neurol Surg[2] and Anat[3], Los Angeles, CA 90033.

Chronic exposure to nicotine has been linked to morphologic changes in aortic and umbilical cord endothelial cells. To study its effects in brain, we treated guinea pigs with nicotine for 14 days (Alzet minipumps; final plasma level: nicotine = 11.4 ng/ml; cotinine (nicotine metabolite) = 101.8 ng/ml) and examined endothelial cells either within (cerebral cortex) or outside (neurohypophysis) the blood-brain barrier (BBB). Animals were perfused with aldehyde fixative and processed for electron microscopy. Qualitative comparisons of cortical endothelial cells from nicotine-treated (n=3) and control (n=3) animals revealed no obvious morphologic differences; capillaries from nicotine-treated neurohypophyses did, however, show a striking increase in microvillous-like projections into the capillary lumen. Quantitative ultrastructural morphometric analyses of neurohypophyseal capillaries determined luminal perimeter, endothelial cell area, endocytotic vesicle number and the density of microvillous-like luminal projections.

We found a significant increase in microvillous-like luminal projections (#/luminal perimeter) in nicotine-treated versus control animals ($t = 12.5$, $p < 0.001$). There were no consistent changes in the other parameters. The lack of morphologic alteration of BBB endothelial cells may reflect either subthreshold exposure to nicotine, or a different response of these cells to the toxin. Nicotine exposure alters both circulating vasopressin levels (Larose et al, J Pharm Exp Ther, 1988, 244:1093) and blood-brain vasopressin transport (Lipovac et al, Soc Neurosci Abstr, 1992). Thus, neurohypophyseal changes may reflect either nicotine-induced alterations specifically related to vasopressinergic changes, or a generalized response of non-BBB endothelial cells to nicotine. (Tobacco Related Disease Research, CA, #2RT0071)

627.7

IMMUNOLOCALIZATION OF IMMUNOGLOBULIN G (IgG) IN THE NORMAL BRAIN OF ADULT RAT. H. Tanno*, R. P. Nockels, L. H. Pitts and L. J. Noble. Dept. of Neurosurgery, Univ. of California and San Francisco General Hospital, San Francisco, CA 94110.

Recent studies suggest that plasma proteins may enter the brain by vascular route or via the CSF. In this study we immunolocalized IgG in neurons of the normal rat brain and examined the relationship of these labelled cells to the blood-brain and blood-CSF barriers.

IgG was immunolocalized in brain sections using a biotinylated rabbit anti-rat IgG. Immunolabel was detected with the avidin-biotin-horseradish peroxidase complex and diaminobenzidine tetrachloride as the chromogen. Immunocytochemical controls consisted of the same procedures in the absence of antibody and in antibody preabsorbed with rat IgG.

No staining was detected in the immunocytochemical controls. In contrast, IgG was immunolocalized in regions of the brain considered outside the blood-brain barrier and in brain stem nuclei whose long axons project into the periphery. A limited number of blood vessels, scattered throughout the brain, exhibited immunoreactivity. The label was associated with the vascular wall and in the adjacent extracellular space. Neurons adjacent to these blood vessels were frequently immunolabelled. IgG was also immunolocalized in neurons in the external granular layer of the parasagittal cortex, pyramidal neurons in the hippocampus, and in Purkinje cells in the cerebellum. These immunolabelled neurons were randomly distributed and were not typically bilateral in their distribution. The most unifying feature of these labelled neurons was that they were either intimately associated with an immunolabelled blood vessel or that their processes were in close proximity to the subarachnoid space or ventricles.

These findings suggest that 1) the blood-brain barrier expresses limited permeability to IgG and 2) neurons may accumulate IgG from these permeable vascular sites and/or from the CSF.

627.8

CHANGES IN THE BRAIN PERMEABILITY OF IgG IN CHRONIC RELAPSING EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (CR-EAE) IN THE GUINEA PIG. M.B. Segal* and D. Skundric. Dept. Physiology, UMDS, St. Thomas' Hosp., London UK, SE1 7EH.

CR-EAE was induced in 20 day old strain 13 guinea pigs by 0.1ml SC inoculation into both hind limbs, with homologous whole spinal cord homogenate in Freund's complete adjuvant (1:1) and 9mg killed mycobacterium tuberculi. Of the 35 animals treated 77% developed the chronic relapsing form of EAE, 18% a hyperacute and 5% no signs. The vascular brain perfusion method of Zlokovic (1986) was used in the various stages of CR-EAE to study the entry of homologous IgG and albumin into the brain. From 7 days post inoculation and during periods of maximum relapse, discrete areas of positive anti-IgG staining were seen in the perivascular spaces of small blood vessels. In these areas anti-albumin staining was negative. There were also scattered regions of demyelination. In controls, weak anti-IgG staining was seen on the endothelium, but not in the perivascular spaces and also, in both groups, in the choroid plexuses ependyma and subpia. The passive restriction of the blood-brain barrier is preserved in CR-EAE while the transfer of IgG is increased in some areas. Zlokovic BV et al (1986) J.Neurochem 46 1444-1451.

627.9

INTRA-ISCHEMIC MILD HYPOTHERMIA PROTECTS AGAINST BLOOD-BRAIN BARRIER DAMAGE. Z.G. Huang*, E. Preston, D. Xue, K.E. Smith and A.M. Buchan. Neuroscience Unit, Ottawa Civic Hospital and National Research Council, Ottawa, Canada, K1Y 4E9.

This study was conducted to explore the effects of intra-ischemic mild hypothermia on blood-brain barrier (BBB) opening in a rodent model of MCA occlusion. Male SHR rats were subjected to permanent right common carotid artery occlusion and 2 hrs of reversible right MCA occlusion. Normothermic and halothane control groups were kept at 37.5°C throughout ischemia and reperfusion (RP). The hypothermia group was kept at 32°C under anesthesia during the 2 hrs of ischemia, but raised to 37.5°C throughout RP. Following RP at 2 min, 22 hrs or 46 hrs, a ^{3}H-sucrose solution (20 μCi/100gm) was administered IV, circulated for 30 min, and the animals immediately sacrified. Cortical transfer constants (Ki) for BBB permeation of sucrose were calculated from the ratio of parenchymal ($dpm \cdot g^{-1}$) and time-integrated plasma ($dpm \cdot s \cdot mL^{-1}$) sucrose concentrations. A one-way ANOVA was used.

Ki, Mean ± SE (n): *p<0.05,**p<0.01,***p<0.001.

	RP-2 min	RP-22hrs	RP-46hrs
Normothermia (6)	3.8 ± 0.5	6.7 ± 1.0	25.5 ± 3.4
Halothane (4)	2.6 ± 0.2*	7.0 ± 1.7	
Hypothermia (5)	1.7 ± 0.2**	2.5 ± 0.6*	2.7 ± 0.7***

Intra-ischemic mild hypothermia protects against BBB breakdown, affording an explanation for hypothermic cytoprotection. Mild hypothermia should be employed to prevent vasogenic edema during thrombolytic therapy for stroke patients.

627.10

ULTRASTRUCTURE OF THE BLOOD-BRAIN BARRIER IN ALZHEIMER'S DISEASE BRAIN BIOPSIES. L. Claudio*, C.F. Brosnan and D.W. Dickson. Division of Environmental Medicine, Mount Sinai Medical Center, New York, NY 10029 and Department of Pathology, Albert Einstein College of Medicine, New York, NY 10461.

The blood-brain barrier (BBB) is characterized by interendothelial tight junctions, few pinocytotic vesicles and high mitochondrial content in CNS capillary endothelium. We have analyzed ultrastructural and morphometric features of capillaries from five cases of Alzheimer's disease brain biopsies. The data were expressed as the percentage of endothelial cell cytoplasmic area occupied by the respective organelles. The values for vesicular content ranged from 0.49% (± 0.4) to 1.17% (± 0.1) and were inversely correlated with mitochondrial content, which ranged from 7.04% (± 1.31) to 2.88% (± 0.39). These values of mitochondrial content were much lower than those found in normal CNS (from 11% to 13%) and resemble those reported for the systemic circulation. We have shown previously that in inflammatory models of BBB disruption, low levels of endothelial mitochondria are associated with increased transcytotic activity and increased BBB permeability. In Alzheimer's disease, endothelial cells also showed necrotic changes, which were circumscribed to specific cells and did not involve all cells of the microvessels. Other significant features of the BBB in these tissues were alterations of the basement membrane, including large collagen deposits, intense binding of ruthenium red to the luminal and adventitial laminae and detachment of the adventitial laminae. In addition, perivascular astrocytes appeared reactive with extensive accumulation of filament bundles and intracellular lipid granules. This work adds to the increasing amount of evidence suggesting that BBB changes are a component of the pathogenesis of Alzheimer's disease.

627.11

PROTEIN KINASE C (PKC) IN ISOLATED MICROVESSELS (MVS) IN AGING AND ALZHEIMER'S DISEASE (AD). P Moore[1], P Grammas[1], T Botchlet[1], AE Roher[2], MJ Ball[3], R Leech*[1]. Univ of Okla HSC, OKC, OK, 73190[1], Wayne St Univ, Detroit, MI 48201[2], Oregon HSC, Portland, OR 97201[3]

PKC, an important enzyme in signal transduction, is a primary factor in determining cellular responsiveness to receptor activation. Several studies have shown changes in enzyme activity, distribution or isoforms in the brain in aging and AD. The objective of this study is to examine PKC in the cerebral microcirculation from AD patients and controls and young and aged (>18 mths) rodents. MVs were isolated from the frontal, temporal, and parietal cortex of AD patients and age-matched controls (post-mortem time 6-12 hrs) and cytosolic and particulate fractions prepared. Evaluation of PKC activity after partial purification on Q-Sepharose indicates that PKC activity in AD MVs (8.64±2.9 pmol/mg/min) was significantly ($p < 0.05$) less (54.7%) of that seen in control MVs (16.1 ± 6.1). In contrast, analysis of MVs from the cerebral cortex of young and aged rodents, by phorbol ester binding, indicates no significant difference in PKC. Our results demonstrate that PKC activity can be determined in human post-mortem samples and that AD microvessels demonstrate significantly less PKC activity compared to age-matched controls. In addition, PKC appears comparable in MVs from young vs. aged rodents. These data suggest that PKC function and responsiveness may be abnormal in AD. (Supported by AHAF, OCAST, NIH NS 30457, and NIH P30AG08017).

627.12

PROTEIN KINASE C (PKC) ISOFORMS IN HUMAN AND RAT BRAIN MICROVESSELS (MVs). D Cooper[1], P Grammas[2], J Watson[1], P Moore[2], O Hanson-Painton[2], R Brumback*[2], VA Hosp, Tampa, FL, 33612[1], U of OK HSC, OKC, OK, 73190[2]

PKC, a cytosolic enzyme important for receptor-mediated cell activation, translocates to the membrane upon activation. The distribution of PKC isoforms appears to be tissue and cell specific. The objective of this study was to determine PKC isoforms in the cerebral MVs. MVs were isolated from the cerebral cortex of young and aged (>18 mths) rats as well as from human autopsy specimens (parietal, temporal, frontal cortex) from Alzheimer's (AD) patients and controls. Cytosolic and particulate fractions were prepared, solubilized in SDS buffer and Western blots run using polyclonal antibodies to PKC isoforms α, β and ɣ. The results indicate that in both rat and human MVs, β is most abundant and is present in both membrane and cytosolic fractions. The α isoform is also present although to a much lower level than the β and it is localized primarily in the cytosolic fraction in both rat and human MVs. Finally, the ɣ isoform was undetectable in rat and human MVs in either fraction. These data demonstrate that both α and β isoforms are present in the cerebral microcirculation and that β is the predominant species. Similarities in the distribution of isoforms in aged and young MVs as well as AD and control MVs suggest that altered PKC responsiveness in aging or AD may reflect changes in enzyme levels or activity rather than a change in isoform type. (Supported by AHAF, OCAST, NIH NS 30457 and VA Res).

627.13

BALB/c AND FVB/N MOUSE CNS SUBCORTICAL ENDOTHELIAL CELLS [ECs] CONTAIN INCREASED ALKALINE PHOSPHATASE WHICH IS INDUCIBLE BY MATRIGEL⁻ AND DEXAMETHASONE. J. Vann and B.R. Brooks*, Neurology Service, Wm. S. Middleton VA Hosp. and Neurology Dept., Univ. of Wisconsin, Madison, WI 53705-2286

Alkaline phosphatase, present on CNS microarterioles and capillaries but not venules, is increased during inflammation. Differential alkaline phosphatase activity of CNS ECs in situ was first described by Bannister and Romanual, 1962, who commented on increased alkaline phosphatase in subcortical regions. CNS ECs were prepared from 5-9 day old mice by tituration and collagenase-DNase digestion followed by Dextran-Percoll step gradient sedimentation from mouse cortex, subcortex and cerebellum. ECs are otherwise similar with respect to factor VIII, actin and vimentin antigen as well as lectin binding by immunoblotting and FACS. Compared with murine embryonic SC, fibroblasts [0.3 ± 0.3 (SD) IU/10^5 cells] in vitro, alkaline phosphatase is significantly [$p \leq 0.05$] increased in cortical and cerebellar ECs [2.0 ± 0.8] as well as subcortical ECs [17.0 ± 4.3]. Alkaline phosphatase in subcortical ECs is significantly [$p \leq 0.01$] induced by Matrigel⁻ [27.0 ± 6.5] as well as dexamethasone at 10 [28.5 ± 1.1] or 100 [24.5 ± 3.3] nM. Regional differences in EC alkaline phosphatase activity in vitro suggest that different ECs are isolated from different regions.

627.14

PGD_2 STIMULATES SECRETION OF $PGF_{2\alpha}$, $9_\alpha,11_\beta$-PGF_2 AND THROMBOXANE B_2 IN CAPILLARY ENDOTHELIUM OF HUMAN BRAIN. M. Spatz, D. Stanimirovic, S. Uematsu[1], H. Wagner,* L. J. Roberts II,[2] R. M. McCarron, and J. Bembry. SB, NINDS, NIH, Beth., MD 20892; [1]Johns Hopkins Univ Sch. Med., Baltimore, MD; [2]Vanderbilt Univ., Nashville, TN

Prostaglandin D_2 (PGD_2) is a cyclooxygenase product of arachidonic acid metabolism. Recently, we demonstrated that vasoconstrictive peptides stimulate the production of $PGD_2 > PGF_{2\alpha}$ and thromboxane B_2 (TBX_{B2}) in cultured endothelial cells (EC) derived from capillaries of human brain. Since PGD_2 can be metabolized to $9_\alpha,11_\beta$-PGF_2 in the brain, we examined the possibility that this metabolite is formed among other prostanoids by EC exposed to exogenous PGD_2. Serum-free medium obtained from untreated and treated capillary EC (3-8 experiments in triplicate) served for the determination of prostanoids by immunoassays. The presence of $9_\alpha,11_\beta$-PGF_2 was analyzed by mass spectrometry. PGD_2 (10 nM) induced a 2-5-fold increase of $PGF_{2\alpha}$ and TBX_{B2} (controls = 2.94 ± 0.4 and 1.59 ± 0.2 ng/mg protein, respectively). The PGD_2 stimulation of $PGF_{2\alpha}$ was dose-dependent and was greater than that of TBX_{B2}. Dexamethasone (phospholipase A_2 inhibitor) partially blocked formation of TBX_{B2} but not $PGF_{2\alpha}$. The presence of $9_\alpha,11_\beta$-PGF_2 was confirmed by mass spectrometry. These results indicate that PGD_2 can be converted to its metabolite $9_\alpha,11_\beta$-PGF_2 (a known vasoconstrictor) and stimulate the formation of $PGF_{2\alpha}$ and TBX_{B2} in EC. These findings may have important implications concerned with PGD_2 function in cerebral capillaries under normal and disease conditions.

627.15

ENDOCYTOSIS OF HORSERADISH PEROXIDASE (HRP) IN CEREBRAL ENDOTHELIAL CELLS. L.J. Noble*, D. Bunch, M. Nishimura, S. Panter, and J.J. Hall. Dept. of Neurosurg., Univ. of Calif., San Francisco Gen. Hosp., San Francisco, CA 94110.

Recent studies suggest that anionic charges associated with the blood-brain barrier may present a repulsive interface to similarly charged plasma proteins. We have begun to address this relationship by exposing primary cultures of rat cerebral endothelial cells to charged forms of HRP.

Endocytosis of HRP II by endothelial cells was compared to a fibroblast cell line. In addition, endothelial cultures were exposed to HRP isoenzymes (VIII, IX, 200 u/ml) and anionic (aHRP) and cationic (cHRP) derivatives of HRP (1mg/ml) that were initially characterized by isoelectric focussing. Cells were exposed to these proteins for 1 h at 37 C in culture medium containing serum. The cells were lysed, and the amount of HRP was estimated by a kinetic, colorimetric assay.

The amount of endocytosed HRP was significantly higher in fibroblasts than endothelial cells (0.132±0.015 ug HRP/mg protein and 0.060 ± 0.029 ug HRP/mg protein, respectively). The major pI ranges for HRP types were as follows: II and IX (7.2-8.2), VIII and aHRP (<4.65) and cHRP (>8.2). There were no significant differences in the amount of endocytosis between HRP types.

In summary, endocytosis of HRP is relatively low, a finding consistent with *in vivo* studies. The charged nature of HRP, presented in this paradigm, does not appear to influence endocytosis of this protein.

627.16

PATCH CLAMP RECORDINGS FROM CULTURED EPENDYMAL CELLS PREPARED FROM FETAL RAT. D.E. Harold* and D.A. Mathers. Dept. of Physiology, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

A technique was developed for preparation of cultures with high ependymal cell content using brains isolated from 18-day fetal Wistar rats. Viable ependymocytes were identified by the presence of beating cilia. Single ependymal cells with beating cilia were observed as early as three days in vitro. The ependymocytes appeared to undergo proliferation. After several weeks in vitro the ependymal cells were observed to occur in small clumps or in larger arcs or circles. There appeared to be an absence of cells within the centre of these arcing or circular structures when viewed under a phase contrast microscope. Cultured ependymal cells were regularly maintained for 8 weeks in vitro.

Patch clamp recordings were made from ciliated ependymal cells after two to eight weeks in vitro. Bath saline contained (in mM): 110 Na^+, 3K^+, 1 Ca^{2+}, 1 Mg^{2+}, 92 Cl^-, 25 HCO^{3-}, and 10 HEPES, pH 7.3. Pipette saline contained (in mM): 110 K^+, 1 Na^+, 1 Mg^{2+}, 115 Cl^-, 1 Ca^{2+}, 10 EGTA, and 10 HEPES. Preliminary whole-cell recordings indicate whole cell resistance (33-38 MΩ) is similar to that reported for Necturus (1). Membrane potential ranged from -54 to -72 mV. Recordings of single channel activity were made in the cell-attached mode. Analysis of single channel activity indicated the presence of an inwardly rectifying channel with weak voltage sensitivity (90 pS conductance).

1)Loo D.F., P.D. Brown, and E.M. Wright, 1988. J. Membrane Biol. 105:221-231

LONG-TERM POTENTIATION V

628.1

ASSOCIATIVE CONDITIONING DOES NOT FACILITATE THE INDUCTION OF HETEROSYNAPTIC LONG-TERM DEPRESSION IN HIPPOCAMPAL FIELD CA1. D.S. Kerr* and W.C. Abraham. Department of Psychology and Neuroscience Research Centre, University of Otago, Dunedin, New Zealand.

In vitro studies were conducted in order to assess differences in non-associative and associative heterosynaptic long-term depression (LTD) in region CA1. Recent reports have indicated that the presence of negatively correlated co-activity in CA1 inputs during tetanization of separate, converging CA1 inputs, produces a voltage-dependent LTD of untetanized synapses that is differentiable from non-associative LTD (Stanton and Sejnowski, Nature:339,1989; Stanton et al., Neurosci. Lett.:127,1991). Two-pathway stimulation protocols were employed in which one stratum radiatum input received tetanizing stimulation consisting of trains of brief, high frequency bursts delivered 200 msec apart, sufficient to produce robust homosynaptic LTP while another (separate) stratum radiatum input received either single pulses interleaved between tetanizing bursts (associative condition) or no activity at all (non-associative condition). These basic procedures were conducted under a variety of conditions, including pre-conditioning ("priming") of the test pathway with 5 Hz stimulation, reduction of synaptic inhibition by perfusion with picrotoxin, and blockade of NMDA-receptor mediated responses with APV. LTP reversal studies were also carried out in order to assess associative conditioning procedures on non-naive pathways. Under no conditions were we able to induce associative LTD different from or greater than that non-associative LTD which has been generally observed in this region. However, the data do indicate that either mild, tonic disinhibion or prior synaptic activity at theta frequencies can affect subsequent synaptic weight changes. (Supported by New Zealand Health Research Council, Postdoctoral Fellowship grant to D.S.K.).

628.2

A KINETIC MODEL OF SHORT- AND LONG-TERM POTENTIATION. G.F. Ayala* and M. Migliore. I.A.I.F. - Nat.l Res. Council, via Archirafi 36, Palermo, Italy.

We present a kinetic model that can account for several experimental findings on Short- and Long-Term Potentiation (STP and LTP) and their pharmacological modulation. The model, which is consistent with Hebb's postulate, uses the hypothesis that part of the origin of LTP may be a consequence of an increased release of neurotransmitter due to a retrograde signal. The operation of the model is expressed by a set of irreversible reactions, each of which should be thought of as equivalent to a set of more complex reactions. We show that a retrograde signal alone is not sufficient to maintain LTP unless long-term change of the rate constant of some of the reactions involved are caused by high frequency stimulation. Pharmacological manipulation of LTP is interpreted as simple modifications of the rate constants of one or more of the reactions that are thought to be involved in a given mechanism. The model, because of its simplicity, can be useful to test more specific mechanisms by expanding one or more reactions as suggested by new experimental evidence.

628.3

KINETIC MODEL OF THE NMDA RECEPTOR-CHANNEL G. A. Chauvet*, N. Urban, and T. W. Berger *Dept. Biomedical Engineering and Program in Neural, Informational, and Behavioral Science, University of Southern California, Los Angeles, CA 90089, and Institute for Theoretical Biology, University of Angers, France*

A mathematical model of the kinetics of the NMDA receptor-channel complex is proposed which includes from three to five binding sites (one or two each for glutamate and glycine, and one site for Mg^{2+}) and three channel conformations (open, closed, and desensitized). Parameters of the model were constrained by available experimental findings, and were optimized to yield the best fit to experimental data. Individual and selected pairs of parameters then were varied in order to determine the sensitivity of the model to values of parameters chosen. The system was found to be most sensitive to changes in the channel conformation transition rates when glutamate and/or glycine was bound. Even for these parameters, however, the qualitative behavior of the system remained similar through a 10,000-fold variation in values. Model behavior for conditions equivalent to four published outside-out patch clamp experiments were simulated: 1. brief 5 ms application of 10 μM glutamate to an outside-out patch (Lester, et al., 1990); 2. a constant application of 100 μM NMDA to an outside-out patch (Sather et al., 1990); to simulate the rate of onset on desensitization; 3. a constant application of 100 μM NMDA to an outside-out patch followed by removal of NMDA for 750 ms followed by re-application for 700 ms (Sather et al., 1990); to simulate the rates of onset and recovery from desensitization; 4. a 100 ms pulse of glutamate is followed by AP5 or Mg2+ (Lester et al., 1990); to simulate the slow glutamate dissociation rate and the fast onset of the Mg^{2+} blockade of the channel. Results were consistent with the existence of two binding sites for glutamate, and with a minor change in parameters, could account for both glycine-sensitive and glycine-insensitive desensitization. Supported by NIMH (MH45156 and MH00343), ONR, AFOSR, and the AFOSR MRC.

628.4

FREQUENCY AND PATTERN DEPENDENT INDUCTION OF MONOSYNAPTIC AND TRANSSYNAPTIC LTP BY ENTORHINAL AFFERENTS *in vivo*. Mark F. Yeckel* and Theodore W. Berger, *Dept. of Biomedical Engineering, and Program in Neural , Informational, and Behavioral Science, University of Southern California. Los Angeles, CA 90089-2520.*

Layer II entorhinal cortical neurons are the origin of a monosynaptic projection to both the hippocampal dentate gyrus and the CA3 subfields. Pyramidal neurons of CA3 also receive disynaptic excitation from layer II neurons via mossy fiber axons of dentate granule cells. We investigated the possibility, *in vivo*, that different frequencies and patterns of stimulation delivered to entorhinal cortical axons or mossy fibers can differentially induce LTP in the three pathways that comprise this feedforward circuit.

Stimulating electrodes were placed into the perforant path and/or the mossy fibers of halothane anesthetized rabbits. Analysis of input/output functions for monosynaptic population responses recorded in the dentate gyrus and/or in the CA3 pyramidal cell region revealed that: i) 50-400 Hz impulses delivered to entorhinal afferents induced monosynaptic LTP of both the dentate and CA3; ii) 50-400 Hz stimulation of the mossy fibers also induced LTP of monosynaptic input to the CA3 subfield; iii) 15 Hz stimulation did not induce LTP of perforant path input to either the dentate or CA3; iv) however, 15 Hz stimulation of mossy fibers was capable of inducing LTP of CA3 pyramidal cells; v) furthermore, while 15 Hz stimulation of the perforant path did not induce LTP of the monosynaptic projection from the entorhinal cortex to CA3, it did *transsynaptically* induce LTP of the mossy fiber input to CA3; vi) finally, a pattern of tetanization incorporating both high and low frequencies (200 Hz bursts at a 10 Hz frequency) delivered to the perforant path, induced monosynaptic LTP of perforant path input to the dentate and CA3, and transsynaptic LTP of mossy fiber input to CA3. These results demonstrate the possibility that patterns of afferent excitation, generated by entorhinal cortical neurons, can selectively determine the spatial distribution of potentiated synapses within the hippocampus, and thus have profoundly different consequences on the global functional properties of the hippocampal system. Supported by ONR, AFOSR, MH45156, MH00343, and MH10222 (MFY predoc. fellowship).

628.5

SIMULTANEOUS EXPRESSION OF LTP BY AMPA AND NMDA RECEPTOR-MEDIATED EPSP IS OF SIMILAR MAGNITUDE X. Xie*[1], T.W. Berger[1] and G. Barrionuevo[2]. [1]Dept. of Biomedical Engineering and Program in Neuroscience, Univ. of Southern California, Los Angeles, CA 90089, and [2]Depts. of Behavioral Neuroscience & Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260.

We previously demonstrated that robust LTP could be induced and expressed by the CNQX-isolated NMDA receptor-mediated EPSPs at the perforant path/granule cell synapse in rabbit hippocampal slices (Xie et al., J. Neurophysiol. 67:1009-1013, 1992). LTP continued to be expressed by the AMPA receptor-mediated EPSPs after removal of CNQX. In this study we tested whether a proportional magnitude of LTP can be expressed simultaneously in the EPSP components mediated by both receptor subtypes. In 0.1 mM Mg^{2+}, control field EPSPs (fEPSPs) were obtained that either included both NMDA receptor- and AMPA receptor-mediated components, or only the AMPA receptor-mediated component isolated by the addition of D-APV (50 μM) in the perfusion medium. Tetanic stimuli (50 Hz) then were delivered either in the presence of D-APV, or 20 min after D-APV was removed. When D-APV was present, neither PTP or LTP was expressed. In the absence of D-APV, PTP developed rapidly and was subsequently followed by LTP. At the end of the experiments, D-APV was applied again. In all 10 slices tested, LTP was expressed by both components of fEPSPs. In 4 out of these slices the fEPSP was not contaminated by positive-going population spike. Results from subtractions of the fEPSP from these four slices showed that the total LTP magnitude of the fEPSP area was 121 $\pm$ 50% (SEM) (51 $\pm$ 6% and 49 $\pm$ 7% for the AMPA and NMDA receptor-mediated components respectively). The ratio AMPA/NMDA LTP was 1.04. These results suggest that a common mechanism of LTP expression is shared by these two receptor subtypes. Supported by AFOSR, NS01196, NS24288, MH45156, MH00343 and MRC grant (AFOSR-91-044).

628.6

DENDROTOXINS INDUCE A FORM OF LONG LASTING SYNAPTIC POTENTIATION IN HIPPOCAMPAL PYRAMIDAL NEURONES. A.P. Southan & D.G. Owen*, Wyeth Research (UK), Huntercombe Lane South, Taplow, Berks. SL6 OPH, UK.

Brief application of K^+ channel blockers to CA1 neurones of the *in vitro* rat hippocampal slice has been reported to induce a long lasting (TEA, MCDP) or decremental (4-AP, caesium) potentiation of synaptic responses (Aniksztjein & Ben-Ari,1991; Nature 349, 67-69). In this study we have extended these observations to include dendrotoxin (Dtx) homologues extracted from the venom of green and black mambas, which are potent and selective blockers of voltage-activated K^+ currents.

Extracellular field potential responses were recorded from the CA1 region of hippocampal slices using conventional methods and solutions. Potentials were evoked by constant stimulation of the Schaffer collaterals at 0.1Hz/ 0.02ms duration, at 30°C. Drugs were applied in the perfusing medium for a period of ten minutes, slices would then be returned to the control solution. 4-AP (10μM), Toxin I (100nM) and TEA (25mM) induced repetitive firing and increased the amplitude of the first population spike by *ca* 165%, *ca* 24% and *ca* 23% respectively, immediately following a ten minute exposure (n=3 in each case). The effect of 4-AP was rapid in onset but slowly reversed during washout. The enhancement due to exposure to TEA (peak *ca* 225% at 15 min wash) was slightly slower in onset and was poorly reversible (*ca* 150% enhancement at 60 min wash). Slices treated with Toxin I exhibited a slowly developing persistent potentiation which peaked 15 min after removal of the toxin (100% enhancement at 60 min wash). Although MCDP (1μM), γ-Dtx (60nM) and δ-Dtx (10nM) did not induce multiple population spikes (n=3 for each drug), MCDP promoted a rapid and persistent enhancement of the population spike amplitude (*ca* 200% at 60 min wash) and δ-Dtx produced a slowly developing enhancement taking up to 60 min to reach a maximum (*ca* 100% enhancement). γ-Dtx had similar actions to δ-Dtx but was much less potent (*ca* 40% enhancement at 60 min wash). The qualitative differences in activity of the toxins may reflect relative selectivities for pre- and post-synaptic elements.

628.7

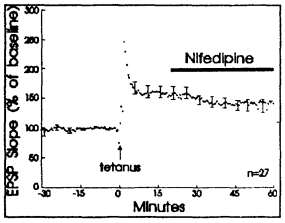
ETHANOL-INDUCED SUPPRESSION OF LONG-TERM POTENTIATION IN THE DENTATE GYRUS IS REVERSED BY LESIONS TO THE SEPTOHIPPOCAMPAL NUCLEUS. Steffensen, S.C.*, Yeckel, M.F., Miller, D.M. and Henriksen, S.J., The Scripps Research Institute, La Jolla, CA 92037.

Acute intoxicating levels of ethanol decrease long-term potentiation (LTP) and increase recurrent inhibition in the dentate gyrus of anesthetized and unanesthetized rats. To determine if the ethanol-induced effects result from direct actions on the dentate gyrus or on subcortical inputs to the dentate we studied the effects of systemic and local administration of ethanol on evoked population spike (PS) amplitudes and latencies, population EPSP slopes and amplitudes and single-unit activity in halothane-anesthetized rats following electrolytic lesions to the septohippocampal nucleus (SHN). Intraperitoneal ethanol (1.2 g/kg) increased putative GABAergic interneuron discharges (IDs, ↑144%), increased paired-pulse (PP) inhibition and markedly decreased induction and maintenance of LTP of PS amplitudes. Microelectroosmotic application of ethanol also increased IDs (↑138%) but had no effect on PP responses or LTP. Stimulation of the SHN evoked a small field potential in the dentate and produced interval-dependent depression and facilitation of perforant path to dentate PS amplitudes and PP responses. Electrolytic lesions of the SHN (±3.0 mA, 5.0 s duration) were considered complete when subsequent SHN stimulation failed to evoke any field potential or affect dentate PS amplitudes or PP responses. Intraperitoneal ethanol, administered 30 min following lesioning of the SHN, increased IDs (↑105%) but had no significant effect on PP responses or LTP. These findings demonstrate that septohippocampal input is necessary for ethanol to increase recurrent inhibition and block LTP in the dentate and suggest a role for local and remote GABAergic modulation of hippocampal plasticity.

628.8

EFFECTS OF NIFEDIPINE ON LONG TERM POTENTIATION IN AREA CA1. K.D. Parfitt* and D.V. Madison, *Dept. of Molecular & Cellular Physiology, Stanford Univ. Sch. of Med., Stanford CA 94305-5426.*

We have previously reported that phorbol esters (PE) cause an increase in the frequency of spontaneous miniature EPSCs in hippocampal CA1 pyramidal cells, without altering their amplitude, suggesting that PKC activation leads to an increase in transmitter release. This PE stimulation of glutamate release was attenuated approximately 50% by application of nifedipine. Based on our earlier findings that phorbol ester application causes L-type calcium channels to open at negative potentials, we hypothesized that a PE-stimulated increase in resting L-channel activity may underlie part of the PE stimulation of glutamate release. If PE-induced and tetanic stimulation-induced potentiation share a common expression mechanism, then application of nifedipine should also reduce tetanus-induced LTP expression. We have examined the effects of nifedipine on the expression of LTP. Our preliminary results suggest that nifedipine (10 μM), 20 min after LTP induction, may reduce LTP by up to 20%, without a similar decline in basal transmission. In contrast, nifedipine 60 minutes after induction of LTP had no apparent effect on the magnitude of potentiation. We are currently pursuing experiments to show if presynaptic L-type calcium channels play a role in the expression of LTP.



628.9

INTRACELLULAR INJECTIONS OF Ca^{2+} CHELATORS FACILITATE LTP OF HIPPOCAMPAL IPSPs. W. Morishita* and B. R. Sastry. Neurosci. Res. Lab., Dept. of Pharmacology and Therapeutics, Univ. of B. C., Vancouver, Canada, V6T 1Z3.

In the present study, the effects of the Ca^{2+}-chelators, EGTA and BAPTA, on the development of long-term potentiation (LTP) of hippocampal inhibitory postsynaptic potentials (IPSPs) were investigated. Postsynaptic potentials evoked by stratum radiatum stimulation were recorded from CA1 neurons in guinea-pig hippocampal slices. Tetanic stimulation of the input (two trains of 100 Hz lasting 1s, 20s interval) produced LTP of the excitatory postsynaptic potential (EPSP) as well as the $GABA_A$ receptor-mediated fast IPSP but not of the $GABA_B$ receptor-mediated slow IPSP (n=8). In contrast, in neurons where postsynaptic Ca^{2+} was chelated with either EGTA (n=6) or BAPTA (n=4), LTPs were observed for both the fast and the slow IPSPs but not the EPSP. During LTP the input resistance of the CA1 neurons and reversal potentials of the fast and the slow IPSPs were not significantly altered in control and Ca^{2+}-chelated cells. In experiments where the fast IPSP was blocked by picrotoxinin (20 μM), tetanic stimulation of the stratum radiatum induced LTP of the EPSP but a depression of the slow IPSP (n=6). In neurons injected with EGTA (n=6) or BAPTA (n=8), the slow IPSP, but not the EPSP displayed LTP; a subsequent application of phaclofen (500 μM) depressed the slow IPSP and increased the height and duration of the EPSP. These results indicate that chelation of postsynaptic Ca^{2+} facilitates LTP of hippocampal IPSPs and that such potentiations may, in turn, affect the degree of LTP of the EPSP. (Supported by grants from the Canadian M.R.C. and the B. C. Health Care Research Foundation).

628.10

THE EXPRESSION OF LONG-TERM POTENTIATION IN HIPPOCAMPAL AREA CA1 DOES NOT CHANGE PRESYNAPTIC CALCIUM. P. Saggau* and L.G. Wu, Div. of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

We investigated whether changes in transients or residual levels of presynaptic calcium could be responsible for the maintenance of long-term potentiation (LTP) in area CA1 in hippocampal slices of guinea pigs. A novel technique for filling synaptic structures with indicators (Saggau et al., Biophys J 61, 1992) was used. A membrane permeant calcium indicator (Fura-2 AM, 1 mM) was injected into the *stratum radiatum* (SR) resulting in local loading and subsequent diffusion via the Schaffer collateral/ commissural pathway (SCC) to presynaptic terminals. Fluorescence from presynaptic structures, emerging from the region of apical dendrites of area CA1, the postsynaptic target of the loaded terminals, was detected. Presynaptic calcium transients before and after the expression of LTP were investigated. Expression was confirmed about 20 min. after tetanic stimulation of SCC (100 Hz for 1 sec): single-pulse stimulation of SCC (0.1 Hz) then resulted in a 40-70% increase of the slope of the field EPSP, while the presynaptic calcium transients showed no change. We also tested residual levels of presynaptic calcium in relation to LTP. Double-pulse stimulation was used to control the residual presynaptic calcium and to assure the number of recruited SCC fibers to be constant throughout the facilitation. A calibration curve was generated to relate the facilitation of the second field EPSP to the residual presynaptic calcium from the first stimulus. The expression of LTP did not result in any significant residual presynaptic calcium, while the calibration curve predicted a clear residual level. We performed two controls for the sensitivity of our techniques: first, following double-pulse stimulation changes in presynaptic calcium affecting transmitter release were readily detected; second, we added cadmium to the bath and observed a dose-dependent block of synaptic transmission and presynaptic calcium transients. These results directly demonstrate for the first time that the expression of LTP in the area CA1 of the guinea pig hippocampus is independent of any detectable persistent changes in presynaptic calcium.
Supported by grants of the Cain Foundation and the Whitaker Foundation to P. Saggau.

628.11

HIPPOCAMPAL LONG-TERM POTENTIATION (LTP) SELECTIVELY MODIFIES THE BINDING PROPERTIES OF GLUTAMATE RECEPTORS.

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Several lines of evidence indicate that LTP is associated with a change in some properties of postsynaptic glutamate receptors. In the present study we have used quantitative autoradiography of radiolabeled ligands selective for the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) and NMDA (*N*-methyl-D-aspartate) subclasses of glutamate receptors to examine the binding properties of glutamate receptors in frozen brain sections obtained from rats in which perforant path LTP was induced.

Eleven adult male Long-Evans rats were anesthetized and implanted with a stimulating electrode in the perforant path and a recording electrode in the dentate hilus. LTP was induced by delivering either two 25 msec 400 Hz bursts separated by 200 msec (n = 6) or ten 25 msec 400 Hz bursts separated by 10 sec (n = 5) at a current intensity sufficient to elicit a 1-2 mV population spike. One hour following stimulation the rats were sacrificed and their brains rapidly dissected and frozen.

Perforant path LTP induction resulted in a selective increase in [^{3}H]-AMPA binding in the molecular layer of the dentate gyrus ipsilateral to the perforant path stimulation as compared to the contralateral side. The increase in AMPA binding in the dentate gyrus was highly correlated ($r = .93$, $p < .0002$) with the LTP-induced change in EPSP slope recorded in this structure one hour after LTP induction. No changes in the binding of either [^{3}H]-CNQX (6-nitro-7-cyano-quinoxaline-2,3-dione), an antagonist of the AMPA receptor, or [^{3}H]-TCP (*N*-{1-[thienyl]cyclohexyl}piperidine), a ligand for the NMDA receptor/channel, were observed. Together, these results indicate that a modification in postsynaptic AMPA receptors plays a critical role in the expression of synaptic enhancement following LTP induction in the hippocampus. Supported by the NIH (AG01542) and the McKnight Foundation to RFT and NSF (96284) to MB.

628.12

***IN VIVO* ANXIOLYTIC PRETREATMENT DISRUPTS LONG-TERM POTENTIATION ACQUISITION.**

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The effects of various doses of anxiolytics on long-term potentiation (LTP) at the perforant path-dentate granule cell synapse were determined. Male, Sprague-Dawley rats under urethane anesthesia received 1.0, 1.75, 2.5, 4.0, 6.0, and 8.0 mg/kg diazepam (DZ) by intraperitoneal injection 15 minutes prior to tetanic stimulation of the perforant path. The effects of the atypical anxiolytic, buspirone, were also investigated: 10.0, 20.0 and 40.0 mg/kg. The interaction of both drugs with ethanol were also investigated: 0.5, 1.0, 1.5, and 2.0 g/kg of 25% ethanol by gavage. Both DZ and ethanol decreased the induction of LTP in a dose dependent manner. Baseline pEPSP amplitudes were not affected under both experimental and control conditions. Enhanced interactive effects were observed with combined doses.

628.13

GABA-A INHIBITION OPPOSES MONOSYNAPTIC PERFORANT-PATH EXCITATION OF CA1 PYRAMIDS.

C.M. Colbert* and W.B Levy, Dept. of Neurosurgery, Univ of Virginia, Charlottesville VA, 22908.

Compared to the Schaffer collaterals (SCH), stimulation of the monosynaptic perforant path (PP) input to s. lacunosum-moleculare of CA1 (s. l-m.) is weak and rarely capable of firing CA1 pyramids. PP stimulation does, however, evoke a glutamate-receptor mediated pEPSP in s. l-m. that is distinguishable from the SCH response by several criteria (Colbert & Levy, J. Neurophys., in press). Here we report removal of a GABA-A inhibition reveals PP evoked EPSP's that excite CA1 pyramids to fire. Transverse hippocampal slices from 7 rats were maintained in an interface chamber. Region CA3 and the dentate gyrus were dissected away. Intracellular recording revealed that: PP stimulation in standard perfusion medium evoked an IPSP or no response; distal SCH stimulation evoked an EPSP followed by an IPSP; and simultaneous stimulation of both pathways no more effectively fired cells than SCH stimulation alone. Switching to a medium containing 20μm bicuculline decreased the early IPSP evoked by stimulating either pathway and increased the EPSP evoked by the PP. In 5 of 7 cells, single test pulses to the PP evoked cell firing. Compared to SCH stimulation, the PP latencies were long and variable, but the threshold for firing was similar for both the SCH and the PP. Neither 2-OH-saclofen, DAGO, 4AP, nor TEA alone allowed the PP to fire pyramids. However, each of these agents enhanced the effect of bicuculline on PP evoked cell firing. These results suggest that GABA-A mediated inhibition determines the ability of the excitatory input to s. l-m. to fire the CA1 pyramids. Thus, the inability of the PP to excite the CA1 pyramids may be explained by strong inhibition rather than by weak excitation. Supported by NIH 15488, NIMH 48161 and NIMH 10019.

628.14

ASSOCIATIVE POTENTIATION OF SCHAFFER COLLATERALS BY PAIRED CONDITIONING WITH THE PERFORANT PATH IN HIPPOCAMPAL CA1.

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As shown in the accompanying abstract (Colbert & Levy), removal of GABA-A inhibition increases the potency of perforant path (PP) stimulated excitation of CA1 pyramids. Using the same dissection, we report that, in the presence of 20μm bicuculline and 2μm DAGO, the PP input can associatively potentiate a weak Schaffer collateral (SCH) response. There are 5 electrodes in these experiments: a recording electrode in CA1 s. lacunosum moleculare (s. l-m.), a recording electrode in distal s. rad., a stimulating electrode in s. l-m., and two nonoverlapping stimulating electrodes in s. rad. (a control electrode that was never conditioned and a test electrode that was conditioned). Conditioning consisted of 3 trains (1/5s) of 8 bursts (1/200ms). Each burst consisted of 2 SCH pulses at 100Hz either alone or combined with 5 PP pulses at 100Hz. For paired conditioning the SCH burst led by 10 ms. The SCH test stimulation intensity was set to produce a small response (<0.3 mV). The paradigm sequentially consisted of a baseline test period, SCH conditioning alone, a second test period, paired SCH+PP conditioning, and a final 30' test period. SCH conditioning alone resulted in no significant changes in any of the pathways. Paired PP and SCH conditioning resulted in statistically significant potentiation at 30' of both the SCH test pathway (29.4 ± 7.1%) and the PP (22.5 ± 6.5%; μ ± sem; N = 5). The unconditioned SCH control did not increase. Although these experiments were run in the presence of two drugs, bicuculline alone is sufficient to permit demonstration of the associative interaction. Supported by NIH 15488, NIMH 48161 and 10019.

628.15

ASSOCIATIVE POTENTIATION OF PERFORANT PATH RESPONSES BY CONDITIONING WITH THE SCHAFFER COLLATERALS IN HIPPOCAMPAL CA1.

D.X. Zhang*, C.M. Colbert and W.B Levy, Dept. of Neurosurgery, Univ. of Virginia, Charlottesville VA 22908.

As shown in another abstract (Levy & Colbert), weak Schaffer collateral responses can be associatively potentiated by paired conditioning with the perforant path (PP) in disinhibited slices. Here we report that, in the presence of 10μM bicuculline, paired conditioning of the PP and Schaffer inputs can associatively potentiate PP responses. Transverse hippocampal slices from 6 rats were maintained in an interface chamber. Region CA3 and the dentate gyrus were dissected away. Recording electrodes were placed in s. lacunosum moleculare of CA1 (s. l-m) and in distal s. rad. Stimulating electrodes were placed in s. l-m and in s. rad. Conditioning consisted of 8 trains (1 train/5 sec.). Each train consisted of 1 PP pulse alone or combined with 5 Schaffer pulses at 400Hz. For paired conditioning the PP pulse was given at the same time as the first pulse of the Schaffer pulses. The paradigm consisted of a baseline test period, PP conditioning alone, a second test period, paired PP and Schaffer conditioning and a final test period. Each test period was 15 min. No significant changes were induced in either pathway by conditioning of the PP pathway alone. Paired conditioning of the Schaffer and the PP pathways resulted in statistically significant potentiation of the PP test pathway (+33.31 ± 10.72%, mean ± S.E.M., $p<0.05$) and the Schaffer pathway (+24.53 ± 7.34%, $p<0.05$). There was no associative potentiation of the PP responses by the Schaffer pathway without the disinhibition induced by bicuculline. This experiment shows that the PP can be associatively potentiated. Supported by NIH 15488, NIMH 48161 and 10019.

628.16

THE FREQUENCY-DEPENDENT ACTIVATION OF OPIOID RECEPTORS IS A NECESSARY FACTOR IN THE INDUCTION OF MOSSY FIBER LTP.

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Mossy fiber LTP (mf LTP) requires both mu opioid receptor activation (Soc. Neurosci. Abst. 16:980, 1990) and high-frequency mossy fiber stimulation (J. Neurophysiol. 64:948, 1990) for its induction. Because opioid peptides are released by the mossy fibers primarily during high-frequency activity (JPET 23:112, 1990), high-frequency activity may be required for induction of mf LTP as a result of the frequency-dependent release of opioid peptides. This possibility was investigated in vivo in anesthetized adult male Sprague-Dawley rats. Induction of mf LTP was found to be insensitive to systemic injections of the NMDA antagonist (+/-) CPP (10 mg/Kg), although this dose was effective in blocking LTP of CA3 responses evoked by stimulation of the contralateral CA3 area (commissural-CA3 responses). Using bursts of 10-200 pulses (at 100 Hz), mf LTP induction was found to have a strict dependence on burst size, with bursts of 25-30 pulses being necessary for LTP induction. Although application of the mu opioid receptor agonist DAGO (1 μmol) in area CA3, or delivery of bursts of 15 pulses, alone were ineffective in inducing mossy fiber LTP, delivery of 15 pulses following application of DAGO reliably induced mossy fiber LTP. Thus, one factor underlying the requirement for high-frequency mossy fiber stimulation for the induction of mossy fiber LTP is opioid receptor activation, and, presumably, the frequency-dependent release of opioid peptides.

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628.17

NALOXONE BLOCKS THE INDUCTION OF LONG-TERM POTENTIATION OF LATERAL, BUT NOT MEDIAL, PERFORANT PATH RESPONSES IN AREA CA3 OF THE HIPPOCAMPUS

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The activation of opioid receptors is suggested to play an important role in LTP induction at lateral perforant path-dentate gyrus and mossy fiber-CA3 synapses. Recently, the monosynaptic perforant path projection to area CA3, whose lateral aspect is suggested to contain and release opioid peptides, has been characterized and shown to display LTP. (PNAS 34:345, 1990). In the present study, the sensitivity of LTP induction in perforant path-CA3 responses to naloxone was assessed in vivo in anesthetized adult male Sprague-Dawley rats. Lateral and medial perforant path responses were evoked by stimulation of the angular bundle as determined by field EPSP rise times measured in the dentate gyrus. Perforant path-CA3 responses were recorded in the pyramidal layer of area CA3. Application of naloxone (10 nmol) into the CA3 region 10 min prior to tetanization blocked LTP of lateral, but not medial, perforant path-CA3 responses. Additionally, LTP of lateral perforant path-CA3 responses developed slowly and showed little decay over a 1 hr period, parallelling the time course of LTP observed at the mossy fiber-CA3 synapse (Adv. Biosci. 75:213, 1989). These results suggest that medial and lateral perforant path projections to area CA3 utilize distinct mechanisms of LTP induction and expression and therefore evidence distinct forms of LTP.

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628.18

THE GENERATION OF AN LTP cDNA LIBRARY FROM THE MOSSY FIBER-CA3 SYNAPSE.

D.T. Rivera, B.E. Derrick and J.L. Martinez, Jr.*, Department of Psychology, University of California, Berkeley, CA 94720.

Long-term synaptic changes (*e.g.* long-term potentiation, LTP) must be supported by changes in gene expression. Previous studies indicated that induction of FOS-related antigens (FRAs) linked to opioid receptor activation is associated with the expression of mossy-fiber (MF) LTP (Draganow *et al.* 1988 *Soc. Neurosc. Abs.* 14:225.8; Martinez *et al.*, 1990. *Soc. Neurosc. Abs.* 16:423.2). The genes that code for FRAs and other immediate-early genes likely regulate the expression of *later genes* which maintain LTP. In an effort to identify these *late genes* (> 3 h), we generated a cDNA library from a rat hippocampus in which mossy fiber LTP was induced *in vivo* in the MF-CA3 area, and the formation of NMDA-dependent LTP was prevented with the administration of CPP (10 mg/kg). The LTP cDNA library will be characterized in various ways including subtraction of cDNAs from the non-tetanized contralateral hippocampus of the same rat.

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628.19

NONLINEAR SUMMATION OF NMDA RESPONSES TO BURST STIMULATION IN PIRIFORM CORTEX SLICES REQUIRES $GABA_A$ BLOCKADE. A. Kapur*, E.D. Kanter and L.B. Haberly, Neuroscience Training Program and Department of Anatomy, Univ. of Wisconsin, Madison, WI 53706

As previously reported (Kanter and Haberly, Soc Neurosci Abstr, 17:385, 1991) associative LTP in piriform cortex can be induced only after blockade of $GABA_A$ (but not $GABA_B$) receptors. We are now investigating the role of the interaction of GABA-mediated and NMDA-mediated responses in the regulation of synaptic plasticity. Intracellular recordings were made from layer II pyramidal cells in piriform cortex slices. In the presence of the non-NMDA glutamate receptor antagonist DNQX (20 μM), stimulation of either afferent or association fibers evoked responses that consisted of a fast, $GABA_A$-mediated IPSP (depolarizing at resting membrane potential), an NMDA-mediated EPSP and a slow, $GABA_B$-mediated IPSP, as revealed by specific receptor blockers. Responses to bursts of 2-6 pulses were obtained in DNQX with or without the $GABA_A$ antagonist bicuculline methiodide (BIC, 10 μM). After $GABA_A$ blockade the NMDA-mediated responses to bursts were greatly enhanced, exhibiting a nonlinear summation. This phenomenon was observed from stimulation of both afferent fibers (layer Ia) and association fibers (layer Ib or layer III) and was optimally observed at stimulus frequencies of 100 to 200 Hz (30°C). Bursts with inhibition intact produced at best a linear summation of the depolarizing response ($GABA_A$-mediated IPSP + NMDA-mediated EPSP). In some cells the size of the burst response after $GABA_A$ blockade was several fold larger than with $GABA_A$ inhibition intact. Blockade of the slow IPSP with the $GABA_B$ antagonist CGP-35348 (Ciba-Geigy) did not by itself enhance NMDA responses to bursts and did not alter the optimal stimulus frequency for the enhancement produced by $GABA_A$ blockade. Supported by grant NS19865 to LBH.

628.20

APICAL DENDRITIC LTP IN CA1 IN BEHAVING RATS: HIGH-FREQUENCY DEPENDENT AND APV-RESISTANT. L. Stan Leung* and B. Shen. Dept. Clin. Neurol. Sci. and Physiology, Univ. Western Ontario, London, N6A 5A5 Canada.

In a previous study on behaving rats, we reported that patterned primed burst (PB) stimulation of the contralateral CA1 or CA3 resulted in robust long-term potentiation (LTP) at the basal dendritic synapse of CA1, but only weak or no LTP at the apical dendritic synapse (Leung et al., Neurosci. 48:63,1992). This abstract reports factors that may enhance the apical dendritic LTP in behaving rats. Septal co-stimulation, at an intensity which caused desynchronization of the hippocampal EEG, or 50 mg/kg i.p. atropine sulfate did not significantly enhance the success of PB-induced LTP. High frequency (HF) trains of 100-200 Hz, at 3x threshold intensity, were successful in eliciting LTP in 8 of 10 rats, a significantly higher success rate than PBs of a similar intensity in the same rats (0%). HF stimulations, however, almost always elicited afterdischarges and posticta depression. The mean apical dendritic LTP peaked at about 115% (of baseline) at >2 hr post-tetanus. The non-decremental time course and low magnitude of the apical dendritic LTP is clearly different from the decremental, high-amplitude basal dendritic LTP in CA1. Furthermore, the N-methyl-D-aspartate antagonist APV did not significantly change the HF-induced apical dendritic LTP in 5 rats, while it strongly attenuated the basal dendritic LTP induced by HF or PB (Leung and Shen, Neurosci. Abstr.17:511). The time course, magnitude and HF-dependency of the apical dendritic LTP are consistent with the properties of an APV-resistant LTP in vitro (Grover and Teyler, Nature 347:477). However, in vitro, the propensity and properties of LTP seem to be the same at the basal and apical dendrites in CA1. Ipsilateral stimulation and recording in CA1, placed as in an in vitro slice, was done in 16 behaving rats. The results corroborated those using contralateral hippocampal activation, in that LTP was robust at the basal but not the apical dendrites. (Supported by NSERC).

628.21

K^+-INDUCED FACILITATION OF HIPPOCAMPAL LONG-TERM POTENTIATION (LTP) MAY BE MEDIATED THROUGH AN ACTION ON NMDA RECEPTORS. B.A. Ballyk* and J.W. Goh, Department of Pharmacology & Toxicology, Queen's University, Kingston, Canada K7L 3N6.

In the hippocampus, repetitive activation of CA_1 afferents, which induces LTP, results in elevated extracellular K^+ concentrations. We have shown that elevating extracellular K^+ during a weak tetanus either by bath application or by iontophoresis at the CA_1 synaptic zone facilitates the induction of LTP. We hypothesize that K^+ exerts this facilitation through an action on the NMDA receptor. Dendritic population EPSPs were recorded in the CA_1 region of guinea pig hippocampal slices by stimulation of stratum radiatum. Weak EPSPs (<300μV) failed to exhibit LTP following a 100Hz, 1s tetanus (104± 3% (SEM) of control, 30min post tetanus, n=6). K^+, iontophoretically applied at the site of recording for 10s, did not in itself produce long-lasting effects on EPSPs (98± 2% of control, 30min post K^+, n=6). In the presence of 25μM D-2-amino-5-phosphonopentanoic acid, delivery of the weak tetanus with simultaneous iontophoretic (200nA) application of K^+ in the dendrites did not result in significant potentiation of EPSPs (105± 3% of control, 30min post K^+ & tetanus, n=6). However, following washout of the NMDA receptor antagonist, the combination of weak tetanus and K^+ resulted in significant potentiation of EPSPs (135± 6% of control, 30min post K^+ & tetanus, n=6). In other experiments, intracellular records were obtained from CA_1 pyramidal neurons, and responses to NMDA, applied iontophoretically at the dendrites, recorded. Increasing bath K^+ from 3.1mM to 15mM reversibly increased the duration of the NMDA response by 85± 8% (n=3). We suggest that elevated extracellular K^+ at the synaptic zone resulting from an afferent tetanus enhances NMDA receptor activation required for successful induction of LTP.

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628.22

LONG-LASTING PLASTICITY OF EXCITATORY SYNAPTIC INPUT IN SEPTAL NEURONS OF THE RAT. T. DeFazio, R. Dunia, F. Hefti, and J.P. Walsh*. Andrus Gerontology Center & Department of Biological Sciences, USC, Los Angeles, CA 90089-0191.

The plasticity of synaptic input to the septum was investigated in an *in vitro* slice preparation. Septal neurons were recorded intracellularly and labelled with biocytin. An extracellular stimulating electrode was placed in the medial septum of coronal slices and a recording electrode was placed about 1 mm lateral to the stimulating electrode. Extracellular stimulation evoked a rapid EPSP followed by an IPSP that lasted 50-200 msecs. Addition of the $GABA_A$ receptor antagonist bicuculline (BIC) (30 μM) revealed an EPSP that lasted 40-80 msec and reversed in polarity near 0 mV. The EPSP was blocked by kynurenic acid (2 mM) indicating that excitatory amino acids mediate this synaptic potential.

We next investigated plasticity of the EPSP using two separate paradigms, both in the presence of BIC. First, the slices were briefly (5-10 min) exposed to 10-100 nM kainate. This caused a potentiation of the EPSP that lasted for 20-30 min. The second paradigm consisted of high frequency tetanic stimulation of afferents to determine whether excitatory synaptic input to the septum could express long-term forms of use-dependent synaptic plasticity. A population of cells that received the tetanus in the presence of BIC expressed a long-term enhancement of the EPSP that lasted for 30 min. This study was funded by a grant from the American Federation for Aging Research (AFAR).

628.23

FREQUENCY-DEPENDENT ENHANCEMENT OF MONOSYNAPTIC INHIBITORY POSTSYNAPTIC EVENTS IN THE RAT NUCLEUS TRACTUS SOLITARIUS (NTS).

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Whole cell patch recordings in the NTS were made in transverse slices of rat brainstem (age 2-8 weeks). Postsynaptic potentials and currents were evoked by low frequency (0.2Hz), electrical stimulation in the area of the tractus solitarius. Since most NTS neurones received a mixed excitatory and inhibitory input, inhibitory postsynaptic potentials (IPSPs) and currents (IPSCs) were studied in isolation by applying blockers of excitatory amino acid receptors. CNQX or DNQX completely blocked excitatory synaptic responses in most neurones but in some cases APV or AP-5 were also applied to block a residual excitatory component. The inhibitory synaptic events were completely blocked by bicuculline. No evidence of a $GABA_B$- mediated IPSP was obtained although baclofen had both pre- and postsynaptic effects.

IPSPs and IPSCs were studied at potentials of -50 to -60mV during and after stimulation at higher frequencies (1-20Hz). In 8 out of 12 cases the IPSc or IPSP was increased in amplitude after stimulation at frequencies of 5Hz or higher. Depression of the inhibitory event after the higher frequency stimulation was not observed. The potentiation was long-lasting (up to 1 hour) and could be evoked more than once during a recording. The reversal potential of the inhibitory IPSP/IPSC was not altered after potentiation.

The induction and maintenance of the potentiation was resistant to blockade of NMDA receptors with AP-5 and in addition was not affected by the $GABA_B$ antagonist 2-OH-saclofen.

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628.24

LTP EXPRESSION MECHANISMS: EVIDENCE FOR A TIME WINDOW DURING WHICH LTP CAN BE REVERSED.

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One hypothesis concerning LTP expression mechanisms is that biochemical events could take place during a transitory phase to generate more stable modifications responsible for the increase in synaptic efficacy. If this is the case, then one would expect to be able to reverse LTP during this specific time window. Using a specially designed recording chamber built with peltier elements, we have tested the effects of fast and brief cooling shocks on the maintenance of LTP in area CA1 of hippocampus. Slices were prepared and maintained at a standard temperature of 33°C and brief shocks to 30°C, 27°C and 24°C for 2 or 5 min were applied. These cooling episodes resulted in marked modifications of the size and time course of synaptic responses. Changing the temperature for 5 min from 33°C to 24°C reduced by about 80% the size of evoked synaptic responses and prolonged by a factor of 2-4 their rise time. These two parameters fully recovered over a period of 10-20 min. Applying these cooling shocks 10-20 min after LTP induction completely reversed the potentiation effect. A second application of high frequency stimulation re-instated LTP. Several induction / abolition sequences could thus be generated. In contrast, application of the cooling shocks later than 20 min after LTP induction did not affect the degree of potentiation. These results indicate that LTP can be reversed during a specific time window following induction and that a metabolic process might be involved during an initial phase of 10-20 min to generate more stable modifications. Work supported by FNRS 31-30980.91.

628.25

ACTIVITY-DEPENDENT PLASTICITY OF INHIBITION IN THE DENTATE COMMISSURAL PATHWAY: RELATIONSHIP TO EPSP/SPIKE DISSOCIATION

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We tested the hypothesis that a decrease in feed-forward inhibition contributes to LTP-associated EPSP/spike (E-S) dissociation in the hippocampus. Because the dentate commissural pathway (CP) and the perforant path (PP) activate a common pool of interneurons, a change of synaptic efficacy due to activation of one input could induce changes in the other.

In urethane-anaesthetized rats we measured the inhibition of PP population spikes by the CP at interstimulus intervals of 6 and 12 msec. This measure and an E-S function were obtained before and after a) PP tetany (400 Hz, 18 msec) and b) CP tetany (200 Hz, 30 msec).

Low intensity PP conditioning (spike threshold) led to decreases of CP inhibition and large E-S shifts to the left; higher intensity PP tetany produced increases in CP inhibition and smaller leftward E-S shifts. CP conditioning led to increases in CP inhibition and variable E-S shifts. Post-hoc analysis of the CP-tetany cases revealed that leftward E-S shifts accompanied depression of the PP pEPSP (0 to -9%), and right E-S shifts accompanied potentiation of the PP pEPSP (2 to 8%).

The inhibitory circuit of the dentate gyrus expresses activity-dependent plasticity under conditions which alter the E-S relationship. This suggests that changes in inhibition contribute to EPSP/spike dissociation. The pattern of changes is best explained by independent plasticity of excitatory and inhibitory synapses. Supported by K08NS01438 to RT and BNS 8818766 to OS. WBL was supported by NIH 15488 and NIMH 48161.

628.26

A DEVELOPMENTAL ANALYSIS OF LONG-TERM POTENTIATION IN THE FREELY-MOVING RAT.

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The ability of rats to establish and support LTP of the perforant path/dentate granule cell synapse was examined at 15, 30 and 90 days of age. Hippocampal dentate granule cell field potentials were recorded, before, and at timepoints out to 24 hrs after tetanization of the perforant pathway. Waveforms were analyzed for changes in population EPSP slope (a measure of synaptic drive) and population spike amplitude (a measure of population discharge). 90-day old animals exhibited an 18% enhancement of the population EPSP slope 30 mins after tetanization, rising to approximately 40% above baseline 5 hrs. after tetanization and remaining at this level at 24 hrs. In contrast, EPSP slope measures obtained from 30-day olds declined nearly 25% from baseline 30 mins after tetanization, failing to regain baseline over the 24 hr test period. Population spike amplitude measures in the 30-day old group enhanced beginning 1 hr post-potentiation. This measure rose to > 100% after 24 hrs. The rate of population spike enhancement obtained from 30-day old animals closely paralleled that of the 90-day old group, however, 90-day old animals consistently attained levels of enhancement 20% higher than those in the 30-day old group. Preliminary results in 15-day olds also indicate potentiation of the population spike component in association with decreases in EPSP slope measures. The results indicate a dissociation between the EPSP slope and population spike components in younger animals, which may reflect functional immaturity of transmitter systems modulating dentate granule cell excitability.

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628.27

HOMOSYNAPTIC NMDA RECEPTOR-DEPENDENT LONG TERM DEPRESSION IN RAT HIPPOCAMPAL SLICES.

R.M. Mulkey* and R.C. Malenka. Dept's of Psychiatry & Physiology, Univ. of California, San Francisco, CA 94143.

Low frequency stimulation (LFS) has been reported to induce long-term depression (LTD) in area CA1 of hippocampus (Bear et al., Soc Neurosci Abst, 17, 1329). Experiments were performed to examine the mechanisms underlying LTD. In hippocampal slices prepared from young rats (12-21 days), afferent stimulation in stratum radiatum at 1 Hz for 5 to 15 min produced a synapse-specific depression of synaptic transmission (31 ± 1.7%; n = 53) that lasted for at least 30 minutes. NMDA receptor activation appears necessary for LTD as 25 μM APV reversibly blocked this phenomenon (n = 7), whereas the voltage-dependent calcium channel antagonist nifedipine (20 μM) did not (n = 5). To investigate whether an increase in postsynaptic calcium is required for LTD induction, the calcium chelator BAPTA (10 mM) was included in whole cell patch pipettes while simultaneously recording field EPSPs. LTD was blocked in 8 of 11 cells even though it was reliably elicited in the field EPSPs. In addition, strong hyperpolarization (to -105 mV) during intracellular recording reversibly blocked LTD (n = 3). As one control for excitotoxicity at individual dendritic spines, the effects of manipulating extracellular calcium ($[Ca^{2+}]_o$) were examined. Applying a tetanus (20 Hz, 30 sec) after decreasing extracellular calcium to 0.5 mM caused LTD in slices in which the same tetanus in 2.5 mM $[Ca^{2+}]_o$ previously elicited LTP (n = 2) or no change in synaptic transmission (n = 3). Paired-pulse facilitation was unaffected during LTD. These findings suggest that this form of LTD requires NMDA receptor activation and an increase in postsynaptic calcium distinct from that required for LTP.

628.28

EPSPS IN HIPPOCAMPAL INTERNEURONES EXHIBIT LONG-TERM ENHANCEMENT.

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Tetanic stimulation of Schaffer collaterals has been reported to potentiate some EPSPs evoked in CA1 basket cell (BC) interneurones (1). Because synapses from Schaffer collaterals on BCs are not formed on spines (2), whereas they are largely found on spines in pyramidal cells, the ability to potentiate EPSPs in CA1 BCs, and the specificity of that potentiation, was further examined. Intracellular recordings were made from BCs in conventional hippocampal slices. Control and test EPSPs were evoked by extracellular stimulation of stratum radiatum at 1 Hz. Conditioning procedures consisted of 100 pairs of 1 Hz stimulation with post-synaptic depolarization. If the EPSP did not potentiate, a tetanus of 20 stimuli at 100 Hz was paired with post-synaptic depolarization four times (.1 Hz). Depolarizing currents were always sufficient to discharge the neuron repetitively. Under these conditions, ten EPSPs (53%) exhibited potentiation of 50 to 100% with either pairing or tetanization. Seven of these EPSPs were recorded for 25 to 60 minutes after conditioning with no sign of a return to control values. The cells of the other potentiated EPSPs were lost within 10 minutes after conditioning but the recordings showed no return to control values in this period. In the nine remaining BC recordings, seven demonstrated an initial potentiation of as much as 100%, but returned to control values within 10 minutes. Two EPSPs exhibited depression. Preliminary results have shown that when two inputs were tested, the untetanized one did not potentiate. These results indicate potentiation of these EPSPs resembles LTP in CA1 pyramidal cells. The results also indicate that it is not necessary for the synapses to be formed on spines for this potentiation to occur.

1. Taube, J.S. and Schwartzkroin, P.A. (1987) Br. Res. 419, 32-38.
2. Schwartzkroin, P.A. and Kunkel, D.D. (1985) J. Comp. Neurol. 232, 205-218.

628.29

REVERSIBLE INACTIVATION OF THE LOCUS COERULEUS, BUT NOT THE MEDIAL SEPTUM, PREVENTS HIPPOCAMPAL LONG-TERM POTENTIATION. E.J. Barea*, S.E. Krahl, and D.C. Smith. Department of Psychology and School of Medicine, Southern Illinois University, Carbondale, Illinois 62901.

Several studies have suggested that norepinephrine (NE) may play an important modulatory role in the induction of long-term potentiation (LTP) in the dentate gyrus (DG) of the hippocampus (cf. Neuman & Harley, *Brain Res.*, *273*:162, 1983). The present study investigated whether the microinfusion of 2% lidocaine hydrochloride, a reversible local anesthetic, into the locus coeruleus (LC) prevents the induction of DG-LTP in the urethane-anesthetized, adult male rat.

Long Evans rats were implanted with a unilateral guide cannula immediately above the LC or medial septum (MS), and with an electrode in the ipsilateral DG from which EPSPs, evoked by 80-300 μA biphasic square-wave pulses from a perforant path (PP) bipolar stimulating electrode, were recorded. Following a 1-hr stabilization period, both groups were microinfused with 0.5 μl of saline or lidocaine over a 2-min period. Eight minutes later, the animals received high-frequency stimulation (8 trains of 8 pulses at 400 Hz) of the PP.

Saline-infused controls displayed significant EPSP potentiation following high-frequency stimulation in both the LC, $F(1,9)=5.14$, $p<.05$, and MS groups, $F(1,7)=10.51$, $p<.05$. In contrast, lidocaine infused into the LC prevented EPSP potentiation when compared to pre-potentiation levels, $F(1,9)=1.21$, $p>.05$, or saline-infused potentiated controls, $F(1,26)=3.98$, $p<.05$. Lidocaine infused into the MS did not alter EPSP potentiation as compared to saline-infused controls, $F(1,24)=0.00$, $p>.05$. These findings suggest that the release of NE from the locus coeruleus is necessary for the induction of DG-LTP.

628.30

A MULTI-ELECTRODE ARRAY CAPABLE OF DELIVERING TETANIC STIMULATION TO AN ENTIRE HIPPOCAMPAL CA1 MINI-SLICE. C.I. Moore, B.A. Duff, E.M. Dudek, T.V. Dunwiddie, G.M. Rose and M. Browning*, Dept. of Pharmacology, U. Colorado Health Sci. Ctr, Denver, CO.

Recently our laboratory has found that several pharmacological treatments which produce LTP-like synaptic enhancement also produce increases in synapsin phosphorylation. Greengard's group has shown that synapsin I may play a role in regulation of transmitter release. Thus it is possible that synapsin may play a role in the increased transmitter release seen in LTP. A critical test of this hypothesis is to determine whether synapsin phosphorylation is correlated with LTP induced by classical means, i.e. tetanic stimulation. Unfortunately, tetany delivered via a single electrode induces LTP in only small fraction of the synapses in a slice, resulting in signal detection problems in biochemical studies. To address this problem we have begun using a mini-slice preparation in which the CA1 region is microdissected from a typical hippocampal slice. In addition, we have also begun using an electrode array (Longreach Scientific Resources, Orr's Island, ME) 1consisting of four monopolar stimulating electrodes placed side by side and spaced 100 microns apart. When the stimulating electrodes are separated in the array by 150 microns or less, such electrodes can be paired to produce paired pulse facilitation when a 50 msec inter-stimulus interval is used. Moreover, tetanic stimulation delivered to one of the electrodes produced LTP in the adjacent unstimulated electrode in 13 of 19 experiments. These effects can be seen in stratum radiatum when the electrode is positioned either parallel or perpendicular to the CA1 cell layer. We have examined the biochemical effects of synchronous tetany (400 Hz for 1 sec) delivered to all four electrodes at two positions in the stratum radiatum of a CA1 mini-slice. Such stimulation produced an increase in synapsin I phosphorylation at its CAM kinase II sites in 7 of 10 experiments.

628.31

NMDA-ACTIVATED CONDUCTANCES PROVIDE SHORT-TERM MEMORY FOR DENDRITIC SPINE LOGIC COMPUTATIONS. Roderick V. Jensen+ and Gordon M. Shepherd* (Section of Neurobiology,Yale University School of Medicine, New Haven, CT 06510) (+Department of Physics, Wesleyan University, Middletown, CT 06457)

Active conductances in or near dendritic spines may permit elaborate computational processing of multiple synaptic inputs long before these signals reach the soma. Numerical models of dendritic trees indicate that the interactions of postsynaptic potentials in active spines can generate simple logic operations such as AND, OR and NAND gates. However, because the spine head EPSP's closely follow the underlying, short-duration (1-5 ms), synaptic conductances, previous studies concluded that precise timing of synaptic inputs would be critical for these logic operations to occur. We show that this temporal limitation on dendritic computation can be relaxed by the inclusion of slow (100-300 ms), voltage-dependent, NMDA-receptor mediated conductances in the spine heads. Our numerical simulations show that this simple mechanism provides a short term memory (~100 ms) for logic AND gates with time-delayed inputs on one or more spines.
Supported by ONR and NIDCD.

628.32

HOMOSYNAPTIC LONG-TERM DEPRESSION IN CA1 *IN VITRO*: DEVELOPMENT, MECHANISM, AND INTERACTION WITH LTP. S.M. Dudek* and M.F. BEAR. Center for Neural Science, Brown University, Providence, RI 02912.

Last year, we showed that 900 pulses delivered to the Schaffer collaterals at 1-3 Hz consistently yielded a depression of the CA1 population EPSP that persisted without signs of recovery for > 1 hour following cessation of the conditioning stimulation. This long-term depression (LTD) was specific to the conditioned input, ruling out generalized changes in postsynaptic responsiveness or excitability. LTD was dependent on the stimulation frequency; 900 pulses at 10 Hz caused no lasting change, and at 50 Hz a synaptic potentiation was usually observed. This, coupled with the observation that the depressed synapses continued to support long-term potentiation in response to a high frequency tetanus, suggested that LTD is accounted for by a modification of synaptic effectiveness rather than damage to or fatigue of the stimulated inputs.

We have extended these results in three ways. *First*, we have found that AP5 blocks induction of LTD. Because the release of neurotransmitter at the Schaffer collateral - CA1 synapse is not directly affected by AP5, this observation suggests that depletion of neurotransmitter is not a likely explanation for LTD. Moreover, our data suggest that synaptic depression can be triggered by prolonged NMDA receptor activation that is below the threshold for inducing synaptic potentiation. *Second*, we have found that LTD, like LTP, is of greater magnitude during early postnatal development. *Third*, we have evidence that LTP may be "unsaturated" by LTD, suggesting that the site of modification for LTP and LTD is the same. We propose that this mechanism is important – perhaps as important as LTP - for the modifications of hippocampal response properties that underlie some forms of learning and memory.

(Supported by ONR Young Investigator Award to M.F.B.)

628.33

COMMON FORMS OF PLASTICITY IN HIPPOCAMPUS AND VISUAL CORTEX *IN VITRO*. A. Kirkwood, C. D. Aizenman and M.F. Bear*. Department of Neuroscience, Brown University, Providence, RI 02912.

The best studied model for synaptic plasticity *in vitro* is the CA1 region of adult rat hippocampus, where both homosynaptic long-term potentiation (LTP) and homosynaptic long term depression (LTD) have been shown to occur. Using a novel stimulation-recording arrangement in visual cortex, we have found that remarkably similar forms of plasticity can be elicited with precisely the same types of stimulation that are effective in hippocampus.

Previous work in visual cortex has shown that the synaptic responses of layer III cells to white matter (WM) stimulation can undergo LTP, but only under conditions in which synaptic inhibition is partly blocked by bicuculline. We have found that LTP can be induced reliably without bicuculline if layer IV rather that WM is stimulated. "Theta-burst" stimulation (TBS) induces non-decremental LTP in the amplitude of layer IV->III field reponses. Measured at 20 min after TBS, the average increase was 34 ± 4% (n = 42); and in 35 out the 42 cases (>80%) the increase exceeded 15%. In contrast, the same stimulation (without bicuculline) potentiated the WM->III responses by only 6 ± 4 % (n=17). However, in agreement with previous studies, if synaptic inhibition was blocked by including 10 mM bicuculline in the recording electrode then WM->III responses did potentiate by 30 ± 6% (n = 15).

Recent work in this laboratory has shown that homosynaptic LTD can be induced by low frequency stimulation (LFS) in CA1. Similarly, depression of the layer IV->III responses can be induced by LFS (900 pulses at 1 Hz). The average magnitude of the LTD at 30 min. after LFS was -29 ± 7% (n=5). As in CA1, the LTD was dependent on the frequency of conditioning stimulation; 900 pulses at 4 Hz produced no change (-3 ±10%, n=5).

The combined results suggest that hippocampus should no longer be considered a privileged site for synaptic plasticity in the adult brain. Even the mature primary visual cortex can exhibit plasticity of comparable magnitude and robustness.
Supported in part by the NEI, ONR, and the HFSP.

629.1

EFFECTS OF NEUROTENSIN ON MAGNOCELLULAR CHOLINERGIC NEURONS FROM THE NUCLEUS BASALIS. R.Farkas, J.J.Grigg, S.Nakajima and Y.Nakajima* Dept. of Anat. and Cell Biol. and Dept. of Pharmacol., Univ. of Illinois, College of Med. at Chicago, Chicago, IL, 60612.

The magnocellular cholinergic neurons of the rat basal forebrain are known to be rich in neurotensin (NT) receptors. The whole-cell clamp method was used to investigate the effect of NT on dissociated cultured neurons from the nucleus basalis (NB) of newborn rats. Membrane potential was held at -74mV and recurring depolarizing (20mV, 100ms) and hyperpolarizing (50mV, 100ms) voltage pulses were imposed. Application of NT (1μM) produced a long-lasting decline of conductance (154s for 90% recovery, n=9) together with a slow inward current, reflecting cellular excitation. This NT sensitive current revealed inwardly directed rectification with a reversal potential approximately equal to or more negative than E_k. Occasionally the reversal potential was substantially more negative than E_k, suggesting the concomitant activation of a non-selective ion channel. In cells preloaded with the hydrolysis resistant GTP analog GTP-γS (100μM), application of NT produced an almost irreversible reduction in membrane conductance and inward shift of the base-line current. In NB cultures pretreated with pertussis toxin (500ng/ml, 15-22 hrs) the NT effect was not decreased from controls. These findings suggest that the conductance decrease produced by NT is mediated through a pertussis toxin-insensitive G protein. Supported by PHS grants AG06093 and 1F30MH10167.

629.2

DOPAMINE SENSITIVE AND INSENSITIVE K^+ CHANNELS IN RAT STRIATAL NEURONS. Gabriela J. Greif*, Yong-Jian Lin and Jonathan E. Freedman. Dept. Pharmaceutical Sciences, Northeastern Univ., Boston, MA 02115.

We have performed cell-attached patch-clamp recordings on freshly dissociated rat corpus striatum (caudate and putamen) neurons in order to characterize the different types of K^+ channels present, and to determine whether they are modulated by dopamine receptors. As previously described, an 85 pS channel was observed when dopamine or the $D_{2/3/4}$ agonist quinpirole was present in the patch pipette, but was not observed in the absence of drug, or when agonist was applied via a macropipette to the cell membrane outside the patch. Two other classes of channels were also observed near resting membrane potential with 140 mM KCl in the patch pipette. First, there were inwardly-rectifying K^+ channels with conductances between 8-30 pS. Secondly, there were voltage-sensitive K^+ channels of 100-200 pS, which appeared to be large conductance Ca^{2+}-activated K^+ channels. Neither of these two types appeared to be modulated by dopaminergic drugs. These results may help clarify which subtypes of K^+ conductances are involved in dopamine receptor function in the caudate and putamen. (Supported by the Pharmaceutical Manufacturers Association, Tourette Syndrome Association, and NIH FIRST award MH-48545.)

629.3

SULFONYLUREA BLOCKADE OF DOPAMINE D_2 RECEPTOR ACTIVATED K^+ CHANNELS. Yong-Jian Lin*, Gabriela J. Greif and Jonathan E. Freedman. Dept. Pharmaceutical Sciences, Northeastern Univ., Boston, MA 02115.

In order to further characterize the 85 pS K^+ channel which we have previously reported to be activated by D_2 or related dopamine receptors in rat corpus striatum, we have used cell-attached patch-clamp recordings to study the effects of sulfonylurea drugs on single channel currents. Channels were recorded with 10 μM quinpirole (a $D_{2/3/4}$ agonist) in the patch pipette, in the presence and absence of glibenclamide (glyburide). There was no apparent effect of 1 μM glibenclamide on channel current. At 10 μM, there was a partial blockade. In some patches, blockade was progressive over ~10 minutes of exposure. The most prominent feature of blockade was a change to shorter open times with longer closed times, as opposed to the long burst openings seen in the absence of blockade. These results suggest some similarity between this channel and the sulfonylurea-sensitive ATP-modulated K^+ channels described by others, although we needed relatively high concentrations of glibenclamide for blockade. (Supported by the Tourette Syndrome Association, Pharmaceutical Manufacturers Association, and NIH FIRST award MH-48545.)

629.4

EGF INDUCES EXPRESSION OF FUNCTIONAL DOPAMINE RECEPTORS IN GH3/B6 CELLS. J. R. Schwarz , S. Reichmann and C. K. Bauer (SPON: European Neuroscience Association), Inst. Physiol., Univ. Hamburg, 2000 Hamburg 20, Germany

The GH3 cell line serves as a model system to study the cellular control of TRH-induced prolactin secretion. One difference between this cell line and normal lactotrophs is the lack of dopamine (DA) receptors in GH3 cells. Recently, the expression of DA receptors in GH3 cells induced by treatment with epidermal growth factor (EGF) was shown by binding studies (Missale et al., Endocrinol 128, 1991).

In normal rat lactotrophs, DA induces a transient hyperpolarization and stop of action potentials (Israel et al., J. Physiol. 390, 1987). In GH3/B6 control cells (whole cell patch clamp), no changes in the electrical activity could be induced by the application of 5 μM DA. After prolonged (>4 d) treatment with 100 nM EGF and 100 μM progesterone, the GH3/B6 cells consistently responded to DA with a decrease in frequency of action potentials, and an increase in action potential duration leading to more hyperpolarized afterpotentials. This effect lasted several minutes.

D2 receptors present in rat lactotrophs are shown to be coupled to pertussis toxin (PTX)-sensitive G-proteins (Lledo et al., Brain Res. 558, 1991). In EGF-treated GH3/B6 cells, the DA response was not inhibited by preincubation of the cells with PTX (500 ng/ml, 5h).

Our results show that EGF treatment resulted in the functional expression of DA receptors in GH3/B6 cells, but differences in electrophysiological response and PTX-sensitivity suggest that these DA receptors are not identical to those of normal rat lactotroph cells.

629.5

MODULATION OF AN OUTWARD MEMBRANE CURRENT BY OXYGEN IN RAT PHEOCHROMOCYTOMA (PC12) CELLS . W. H. Zhu,* M.J. Stutts, M.F. Czyzyk-Krzeska, E.E. Lawson and D.E.Millhorn. University of North Carolina, Chapel Hill, NC .

PC12 cells are dopamine secreting cells that are morphologically and chemically similar to the Type I oxygen-sensitive cells of mammalian carotid body. Type I cells depolarize and release dopamine when exposed to low O_2 (hypoxia). It was reported recently that depolarization of type I cells occurs as a result of a decrease in conductance of a O_2 sensitive potassium channel (Science 241,1988). However, the actual mechanism by which type I cells detect low O_2 and tranduce this signal into altered cellular function remains unknown. The present study was undertaken to determine if PC12 cells respond to hypoxia in a manner similar to type I cells. If so, this cell line might prove valuable for studying fundamental mechanisms associated with O_2 detection and signal transduction. Whole-cell recordings were performed on rat PC12 cells exposed to control gas (hyperoxia; 95% O_2; room air, 21% O_2) or hypoxia (<10% O_2). Membane potential was voltage clamped at -80mv and change in current was measured in response to positive voltage steps (20mv, 300ms) from -80mv to +80mv. We measured a voltage sensitive outward current that was maximum (800-900 pA) at the largest voltage step. The magnitude of this outward current was decreased substantially when cells were exposed to hypoxia and recovered to prehypoxia levels when reexposed to control gas. Current-voltage plots showed that this outward current is voltage-sensitive and has reversal potential of about -80mv. These findings suggest that the oxygen-sensitive current in PC12 cells is a voltage-dependent potassium current. In addition, we measured dopamine concentration in media of cells exposed to control gas and low O_2 and found that hypoxia evoked a 2-3 fold increase in dopamine release from PC12.

629.6

MEMBRANE POTENTIAL CHANGES INDUCED BY ANOXIA IN RAT DORSAL VAGAL MOTONEURONES ARE INFLUENCED BY INTRACELLULAR pH. A. I. Cowan, R. L. Martin & S. J. Redman*. Division of Neuroscience, JCSMR, and Division of Botany and Zoology, Australian National University, Canberra, A.C.T. 2601, Australia.

This study was undertaken to investigate the influence of intracellular pH (pH_i) on the membrane potential changes induced by anoxia. Intracellular recording from dorsal vagal motoneurones (DVMs) in brainstem slices prepared from rats aged 35-45 days demonstrated that 44% of DVMs hyperpolarized and 45% depolarized during anoxia in bicarbonate/CO_2 buffered artificial cerebrospinal fluid (ACSF). However, in *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES) buffered ACSF (which is expected to cause an increase in intracellular pH (1)), anoxia resulted in a depolarization of 9.5±1.2 mV (sem) in all 31 neurones tested. There was an increase in input resistance of 24.3±0.1% in 42% of the neurones and a decrease in input resistance of 15.2±0.1% in the remainder. The effects persisted when spike dependent synaptic transmission was blocked with tetrodotoxin. Addition of tetraethylammonium chloride, 4-aminopyridine, tetrodotoxin, ouabain or manganese, singly or in combination, showed that the membrane potential changes involve an increase in a calcium current which is counteracted to some extent by a small increase in the delayed rectifier current. A residual depolarization associated with an increase in input resistance is not due to an effect on the A current or on chloride channels. Inhibition of the Na-K ATPase also does not appear to be involved. These results contrast with the effects of anoxia on membrane potential in bicarbonate/CO_2 buffered ACSF (2) in which pH_i is presumed to be lower. It is concluded that changes in pH_i of neurones during anoxia may be responsible for the early changes in their electrical properties.

1. Gaillard, S. & Dupont, J.-L. (1990) J. Physiol. 425, 71-83.
2. Cowan, A. I. & Martin, R. L. (1992) J. Physiol. (In Press).

629.7

ANOXIC CHANGES IN MEMBRANE NOISE OF HIPPOCAMPAL NEURONS. M. Glavinović, P. Miu and K. Krnjević*. Anaesthesia Research Dept., McGill University, Montréal, Qué, H3G 1Y6 Canada.

Brief periods of anoxia elicit a characteristic hyperpolarization of pyramidal cells (Hansen et al. 1982, *Acta physiol. scand.* 115: 301). To obtain further information on the nature of the underlying conductance changes, we recorded in CA1 neurons, by single electrode voltage-clamp, the currents and (at higher gain) the changes in electrical noise generated by anoxia (2-3 min of 95% N_2 + CO_2), in the presence of TTX. The experiments were done in slices (from Sprague-Dawley rats) kept at 33°.

In the majority of cells, the anoxic outward currents seen at holding potentials (V_H) between -70 and -30 mV were associated with an **increase** in variance (σ^2) of the base-line noise. This indicates the opening of ionic channels, in keeping with the observed simultaneous increase in macroscopic conductance. Also in keeping with the macroscopic currents, the unitary currents reversed from outward to inward at -78 ± 6.6 mV (n=3; mean ± SD). The corresponding single channel conductance varied over a wide range: 20.2 ± 16.4 pS.

In two cells, the anoxic outward current was accompanied by a **reduction** in electrical noise. This inverse relation between σ^2 and current confirms previous evidence that anoxia can elicit both opening and closing of certain ionic channels.

Supported by the Canadian Medical Research Council.

629.8

POTENTIAL-DEPENDENCY OF ASTROCYTIC ANION CHANNELS ACTIVATED BY OSMOTIC GRADIENTS

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During astrocytic swelling caused by extracellular hypoosmotic or high K^+ solutions non-selective cation channels open, astrocytes become depolarized, Ca^{2+} ions enter the cell and this in turn is followed by cell regulatory volume decrease (RVD) when both K^+ and Cl^- ions and amino-acids flow out of the cell. A high-conductance (200-250 pS) anion channel has been shown to be more readily activated in astrocytes when hypoosmotic rather than isoosmotic recording solutions are used. To study the potential-dependency of the increased anion conductance we have recorded the activity of single anion channels from primary astrocytes in cultures using the patch-clamp technique. With symmetrical and non-symmetrical Cl^- concentrations in the pipette and bath the channels in excised inside-out patches showed the highest probability to stay open at or near zero membrane potential, thus indicating that membrane depolarization induces the channel to open. The activated Cl^- channel is able to attain at least five discrete open sublevels. When the maximal open state is reached the channel has a tendency to stay open for seconds and then closes abruptly in one step. Opening to any of the sublevels leaves the channel in an unstable state which is characterized by fast flickering openings and closures. The channel is blocked by the anion transport inhibitor L-644,711, which also blocks RVD. Such a channel may function to let Cl^- out during RVD. To verify the existence of these channels in other than cultured mammalian astrocytes we are also using cortical tissue print cells from 21-day-old rats identified by GFAP staining to be astrocytes. Supported by NS 23750 (H.K.K.), and the H.Schaffer Foundation (A.J.P.).

629.9

GLIAL MODULATION OF TRANSIENT POTASSIUM CURRENT EXPRESSION IN CULTURED MOUSE HIPPOCAMPAL NEURONS. R.-L. Wu* and M. E. Barish. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Rodent hippocampal neurons express multiple transient potassium currents that influence action potential duration and accommodation to sustained depolarization. We have studied pyramidally-shaped neurons in dissociated cell cultures prepared from mouse hippocampus on embryonic days 15-16 using whole-cell gigaohm-seal voltage clamp techniques. These neurons exhibit two transient potassium currents, termed A- and D-currents, that can be separated based on steady state inactivation characteristics and sensitivity to 4-aminopyridine (Wu and Barish, *J. Neurosci.*, in press). We have observed that after 5-7 days in culture A-current density (current normalized to membrane area) is larger than D-current density in neurons growing on glial cells (primarily GFAP-immunoreactive astrocytes), while D-current density is larger in neurons growing only on the poly-lysine/laminin-coated glass substrate. This variation is approximately reciprocal; the sum of A- and D-current densities is similar in both types of neurons. The glia-derived signal probably acts by cell surface interaction or restricted diffusion of a soluble factor, as neighboring cells show differences in potassium current expression based on glial contact. Living glia may be required, as currents in neurons growing on dried glial membranes or methanol-fixed glia resemble those in neurons that do not touch glia. We suggest glia-induced plasticity of A- and D-current expression as a novel mechanism of long-term modulation of hippocampal neuron excitability.

629.10

NOVEL ETHANOL ENHANCEMENT OF THE DELAYED RECTIFIER POTASSIUM CURRENT IN RAT HIPPOCAMPAL CA1 NEURONS.

J.L. Weiner*, L. Zhang and P.L. Carlen. Playfair Neuroscience Unit, Toronto Hospital, Department of Pharmacology, University of Toronto & Addiction Research Foundation, Toronto, Ontario, Canada.

Recent biochemical studies have demonstrated that ethanol, at relatively high concentrations (>50 mM), interacts with several second messenger systems. The role of these effects in the mediation of ethanol intoxication remain unknown.

We have recently characterized the sequence of events coupling muscarinic receptor activation to the potentiation of the delayed rectifier potassium current (I_K) in rat hippocampal CA1 neurons. I_K was recorded from mature rat CA1 neurons in brain slices using the whole cell patch clamp recording method. Under voltage clamp recording conditions in which other ionic currents were suppressed, bath application of 10 μM carbachol reversibly potentiated I_K (mean=80%) with an onset of action of 1-3 minutes. A reversible decrease in holding current with no associated change in resting conductance was also observed during carbachol application. The intracellular mediators of this enhancement were shown to include a G protein, IP_3, DAG, and PKC.

This kinase-modulated current was used as an assay to characterize possible actions of ethanol on second messenger systems. Bath application of 20 mM ethanol also enhanced I_K with a slower onset of action and recovery rate than carbachol and no associated change in holding current. In addition, incubation of slices with 20 mM ethanol for 10-25 minutes did not consistently inhibit subsequent carbachol enhancement of I_K. Ethanol pretreatment did however reduce the carbachol-induced reduction in holding current.

These preliminary results demonstrate that ethanol, at a clinically relevant concentration, can potentiate I_K in central mammalian neurons. The cellular mechanisms underlying this enhancement are currently under investigation.

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629.11

Multiple components of I_{AHP} following trains of action potentials in bullfrog sympathetic ganglion neurons. P. Pennefather* and M.V. Sanchez-Vives[1]. MRC Nerve Cell on Synapse Group, University of Toronto, Toronto, Canada, M5S 2S2, [1]Dept. of Physiology, University of Alicante, 030380, Alicante, Spain.

B neurons were recorded from using 20-60 MΩ standard electrodes. When the recording mode is switched from single electrode current clamp to single electrode voltage clamp (hydrid clamp) immediately following a train of evoked action potentials, a calcium and apamin dependent K current is observed that decays slowly back to baseline. This current, I_{AHP}, underlies the slow afterhyperpolarization observed in these neurons. We have analyzed the time course of I_{AHP} at -60 mV using the Clampfit program (Axon Instruments) and have detected multiple components whose amplitude and time course are a function of the amount of calcium entering per action potential and the number of action potentials in the train used to evoke I_{AHP}. We conclude that I_{AHP} is made up of two main components. A major component with an amplitude of ≈ 1nA and a τ ranging for 60-1500 ms and a minor component with an amplitude < 70 nA and a τ of ≈ 5000 ms. Under certain conditions an apparent fast component due to the activation of I_M is observed. Using a versatile interactive simulation program (see K. Miller and P. Pennefather this meeting), we will show how these properties can be explained by the modulation of the calcium signal responsible for I_{AHP} by two pools of calcium buffers, a high capacity (≈ 300 μM) slow buffer and a lower capacity (≈ 50 μM) fast buffer. Uptake into the slow buffer determines the limiting τ of the major component of I_{AHP} following long trains as well as the amplitude of the late slow component. Release of calcium from that pool determines the τ of the late slow component. As explained previously, (Pennefather, Sala and Hernandez-Cruz, 1990, Soc. Neurosci. Abst. XVI, 187) the changes of amplitude and τ of the major component with action potential number is determined primarily by the properties of the fast buffer. Thus a detailed analysis of the relation between I_{AHP} time course and calcium load induced by action potentials can provide insights into how calcium entering neurons during action potentials is buffered.

629.12

EFFECTS OF PHOSPHOLIPASE A_2-INHIBITORS ON COUPLING OF α_2-ADRENOCEPTORS TO INWARDLY RECTIFYING POTASSIUM CONDUCTANCE IN SUBMUCOSAL NEURONES. R.J.Evans & A. Surprenant*. Vollum Institute, O.H.S.U., Portland, OR 97201

Noradrenaline and somatostatin hyperpolarise enteric submucosal neurones by activating a set of inwardly rectifying potassium channels. Receptor-channel coupling appears to involve only a pertussus toxin-sensitive G-protein because agonists activate the K channels in outside-out membrane patches (Shen et al. J.Physiol. **445**, 1992). This study investigated whether the production of arachidonic acid and its metablolites may be involved in mediating the response using the PLA_2 inhibitors quinacrine and 4-bromophenacyl bromide (4-BPB), and the cycloxygenase and lipoxygenase inhibitor eicosatetraynoic acid (ETYA). Quinacrine (10 μM) reduced the noradrenergic IPSP and hyperpolarisations to the α_2 adrenoceptor agonist UK 14304 (100 nM) and somatostatin (10 nM) by 85, 70 and 65% respectively. A similar reduction in the IPSP and the UK 14304 response was found with 4-BPB (10 μM). Quinacrine had no effect on the slow EPSP or the depolarisation in response to substance P, which result from closure of resting and calcium-activated potassium channels, nor on the nicotinic fast EPSP. The agonist induced current to UK 14304 and somatostatin shows substantial inward rectification at potentials negative to E_K; this rectification was reduced by 90% in the presence of quinacrine. ETYA (20 μM) had no effect on the response to UK14304. Our results to date indicate that prostagliandin and eicosanoid metabolites of arachidonic acid are not involved in mediating signal transduction from α_2-receptor to potassium channel activation.

629.13

PLUGGING THE LEAK: ADRENERGIC MODULATION OF LEAKAGE POTASSIUM CHANNELS IN RAT THALAMUS.
Peter B. Reiner* and Xue-Ping Wang, Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, BC V6T 1Z3 Canada.

The predominant determinant of the resting potential of excitable cells is a voltage-independent "leakage" potassium current, I_{KL}. Neurotransmitters are capable of inhibiting I_{KL} in mammalian neurons, resulting in a depolarization accompanied by an increase in whole-cell input resistance. Using cell-attached and cell-free patch clamp recordings, we have characterized I_{KL} channels in thalamic neurons studied in slices of rat brain. The channel exhibits a slope conductance of ~24 pS and is highly selective for potassium ions. I_{KL} channels are active at the resting potential and exhibit little voltage-dependence. $Alpha_1$ adrenergic agonists, known to inhibit macroscopic I_{KL}, reduce the probability of opening of single I_{KL} channels. Modulation of I_{KL} appears to be mediated by a soluble intracellular messenger, as bath application of agonist alters channel kinetics in cell-attached patches. In thalamic neurons, transmitter-induced modulation of I_{KL} channels results in a depolarization which is critically involved in desynchronization of the cortical EEG.

(Supported by MRC)

629.14

SENSITIVITY OF CALCIUM AND POTASSIUM CURRENTS IN DROSOPHILA EMBRYONIC NEURONS TO CYCLIC AMP.
W.B. Alshuaib* and L. Byerly, Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089-2520.

Modulation of membrane currents by intracellular cAMP has been connected to learning in *Aplysia*. Abnormal cAMP metabolism in *Drosophila* learning mutants suggests that a similar mechanism may also underlie learning in *Drosophila*. We are studying the effect of cAMP on Ba and K currents in cultured *Drosophila* embryonic neurons.

Since these neurons are only 5 μm in diameter, Ca current and modulation mechanisms involving intracelluar molecules are likely to washout rapidly when the conventional whole-cell patch clamp technique is used. Therefore, we employ the perforated-patch whole-cell technique, using amphotericin-B to permeabilize the patch. Permeabilization was unreliable in small-opening patch electrodes required for the 5 μm neurons; however, it works much better in large-opening patch electrodes that are used on the 10-15 μm "giant" neurons obtained by arresting cell cleavage with cytochalasin-B.

Our present results suggest that cAMP enhances Ba current but has no effect on K current. With the perforated-patch technique *Drosophila* Ba current shows no washout. Supported by NSF Grant BNS-8903312.

629.15

β-Adrenergic Receptor Stimulation and Cell Density Regulate the Level of Connexin43 mRNA in C_6 Glioma Cells
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The gap junction is a route of rapid intercellular communication among astrocytes and between astrocytes and neurons. The gap junction protein, connexin43, is expressed endogenously in C_6 glioma cells. We investigated the regulation of connexin43 mRNA levels in C_6 cells in response to a variety of treatments to better understand the factors controlling gap junction expression in astrocytes and neurons. Connexin43 mRNA levels per cell increased with time in culture, reaching levels 62-fold greater than those expressed on the first day of subculture. 1 μM isoproterenol stimulated a 3 to 4 fold increase in connexin43 mRNA levels within 4 hours in cells of moderate density. β-Adrenergic receptor mRNA was simultaneously down-regulated by this treatment. Somewhat less stimulation by isoproterenol of connexin43 mRNA accumulation was obtained from cells grown at higher cell densities. The increase in connexin43 mRNA induced by isoproterenol could be attenuated 50% or more by the presence of 10 μM colchicine during isoproterenol stimulation, while colchicine alone had little effect. Additional studies using β-adrenergic antagonists and cyclic AMP analogs are underway to determine in more detail the nature of the induction of connexin43 mRNA levels.

629.16

INTERACTIONS BETWEEN SP AND NMDA-ACTIVATED CURRENTS IN SPINAL TRIGEMINAL NEURONS. Y. Gu*[1] and L-Y. M. Huang[1,2]. Marine Biomedical Institute[1] and Department of Physiology and Biophysics[2], The University of Texas Medical Branch, Galveston, Texas 77555-0843.

Substance P (SP) is present in small primary afferent fibers and participates in the transmission of sensory information, including pain, in the caudal medulla and the spinal dorsal horn. To understand the role of SP in nociception, we studied the interaction between SP and the excitatory amino acid, N-methyl-D-asparate (NMDA) in dissociated trigeminal neurons. The cells were isolated from the spinal trigeminal nuclei in the caudal medulla of rats, and the whole cell currents were measured with patch clamp pipets. The fast perfusion method was used to deliver NMDA to the cell. SP increased NMDA responses in 56% of the cells tested, and had no effect on the rest. For those cells in which NMDA responses were enhanced, the effect of SP was rapidly reversible in 60% of the cases. In the other 40% of the cells, the enhancement by SP persisted for 10-30 min after SP was removed from the external solution. We also found that the enhancing effect of SP was sensitive to external glycine. The increase in NMDA responses by SP was abolished when glycine was added to the external solution, and the enhancement reappeared when the glycine antagonist 7-chlorokynurenic acid was introduced in the solution. The mechanism underlying the SP actions will be discussed. (Supported by NIH NS11255 and NS012050).

629.17

DYNORPHIN REDUCES NMDA-ACTIVATED CURRENTS. L. Chen[1] and L-Y. M. Huang*[1,2]. Marine Biomedical Institute[1] and Department of Physiology and Biophysics[2], The University of Texas Medical Branch, Galveston, Texas 77555-0843.

Opioids, such as morphine, has been used widely in the control of pain. Mu-opioid receptors, the preferential binding sites for morphine, clearly play a role in analgesia. We have previously shown the μ-opioid receptor agonist D-Ala^2-MdPhe^4-Gly-ol^5-enkephalin (DAGO) causes a sustained increase in N-methyl-D-asparate (NMDA)-activated currents by activating protein kinase C inside trigeminal neurons [Neuron, 7:319-326, 1991]. Recent studies of κ-opioid receptors indicate that those receptors may also participate in pain modulation. To understand how a κ-opioid receptor agonist affects the excitability of dorsal horn neurons, we examined the action of dynorphin on excitatory amino acid-activated currents. The experiments were performed on trigeminal neurons acutely isolated from 10-15 day rats. The whole cell currents were measured using the patch clamp recording technique. The amino acids were applied to the cell with a fast perfusion method. Dynorphin had no effect on kainate-activated currents, but it reduced NMDA-activated currents in almost all the cells tested. It decreased NMDA responses by 10% at 10 nM, by 20% at 100 nM and by 40% at 1 μM. The inhibition was rapidly reversed when the opioid was removed from the external solution. Dynorphin changed neither the voltage-dependence nor the kinetics of the NMDA-activated currents. Thus, the effect of κ-opioid receptor agonist dynorphin on NMDA-activated currents is quite different from that of μ-opioid receptor agonist. (Supported by NIH NS23061, NS01050).

629.18

ELECTRICAL PROPERTIES OF GLUTAMATE-RESISTANT AND GLUTAMATE-SENSITIVE CEREBELLAR GRANULE CELLS.
C.Zona*,M.T.Ciotti,D.Mercanti,A.Angelini, P.Calissano - *Institute of Physiology, University of Roma "Tor Vergata", Institute of Neurobiology C.N.R., Roma, Italy

Cerebellar granule cells in vitro, in the presence of a protein complex isolated from rabbit serum (NOAC) develop a phenotype which is in several properties identical to that ensuing in 10% FCS but is markedly different in terms of glutamate sensitivity. NOAC-cultured neurons exhibit a full resistance to the otherwise lethal action of excitatory aminoacids (EAA-phenotype). This EAA- phenotype can be induced to become EAA sensitive (EAA+) when neurons are incubated with another protein complex isolated from rabbit serum. Membrane ionic currents have been recorded in EAA- and in EAA+ neurons using the whole cell patch-clamp techinique. When K currents were blocked, depolarization commands evoked Na and Ca currents in EAA- and EAA+ neurons. The amplitude of the Na currents was significantly bigger in EAA+ neurons than in EAA- neurons while there was not significantly difference in amplitude in Ca currents in the two groups of neurons. Our preliminary data show that the electrical properties of EAA- and EAA+ neurons are different.

629.19

ZINC POTENTIATES ATP-ACTIVATED INWARD CURRENT IN RAT NODOSE GANGLION NEURONS. Chaoying Li*, Robert W. Peoples, Zhiwang Li and Forrest F. Weight. Laboratory of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

The effects of micromolar zinc on membrane inward current activated by extracellular adenosine 5'-triphosphate (ATP) were studied in freshly isolated adult rat nodose ganglion neurons using the whole-cell patch-clamp technique. In 48 of 60 neurons tested, zinc, 5 or 10 μM, increased the peak amplitude of current activated by 10 μM ATP by 30% (n=9) and 120% (n=16), respectively, and reduced the rate of decay of the current simultaneously. In 12 of 60 neurons, zinc decreased the rate of decay of ATP-activated current but had little effect on the peak amplitude of the current. Enhancement of peak amplitude of ATP-activated current by zinc (1-50 μM) was concentration-dependent with an EC_{50}=11 μM. In this concentration range zinc did not have any other detectable effect, and did not change the reversal potential of ATP-activated inward current. The potentiation of membrane conductance was not voltage-dependent between -80 and +60 mV (P>0.25, n=5). Zinc shifted the concentration-response curve for ATP to the left and significantly decreased the EC_{50} of ATP from 30 to 8 μM. These observations suggest that the modulatory site for zinc action may be located on or near the exterior surface of the ATP receptor-ion channel complex. They also suggest that zinc may enhance the ATP-activated current by increasing the affinity of the receptor for ATP.

629.20

EFFECTS OF CALCIUM AND MAGNESIUM IONS ON SPONTANEOUS OSCILLATION OF MEMBRANE CURRENT IN MAMMALIAN PARASYMPATHETIC NEURONS. T. Nishimura* & T. Akasu. Department of Physiology, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830, Japan.

A spontaneous rhythmic outward current (Iso) was recorded from neurons in rabbit vesical parasympathetic ganglia (VPG), using single electrode voltage clamp techniques in vitro. After penetration of cells with an electrode containing 3 M KCl, cells were clamped at a given resting potential (-45 to -65 mV). The Iso occurred at fairly constant intervals ranging between 30 s and 5 min in the Krebs solution. Ionic mechanisms of the Iso are activation of calcium-dependent potassium conductance and inactivation of calcium-dependent chloride conductance (Nishimura et al., J. Physiol., 437: 673-690, 1991). The frequency of the Iso was increased by removal of magnesium ions (1.2 mM) from the Krebs solution, while it was decreased by raising magnesium ions to 6 mM. In either case removal of calcium ions (2.5 mM) from the perfusate eliminated the Iso. Application of a calcium-rich solution (4 mM) or caffeine (1-3 mM) increased the frequency of the Iso. Effects of the calcium-rich solution and caffeine on the Iso were antagonized by raising external magnesium ions (6 mM). Nifedipine (10 μM), verapamil (10 μM) and ω-conotoxin (1 μM) did not alter the Iso. Application of BAPTA-AM (300 μM, 30 min), a calcium-chelator acting in cytosol, eliminated the Iso. Subsequent calcium-load into cells restored the Iso. Inhibitors for calcium-induced calcium release (CICR), ryanodine (0.1-1 μM)and procaine (30-300 μM)reversibly blocked the Iso. An inhibitor for calcium-pump, cyclopiazonic acid (3-10 μM)also reversibly blocked the Iso. These data imply that the magnesium-sensitive calcium influx occurring at resting potential regulates the CICR from ryanodine-sensitive calcium-store sites and the calcium-extrusion system which generate the Iso in rabbit VPG neurons.

629.21

EFFECTS OF INTRACELLULAR SODIUM AND CHLORIDE ACTIVITIES ON THE MEMBRANE PROPERTIES OF CA1 NEURONS Kevin J. Staley* Neurology Dept, University of Colorado Health Sciences Center, Denver, CO 80262

Whole cell recordings from different laboratories using various concentrations of electrode Na^+ and Cl^- have produced quite different values for the resting membrane potential (RMP) and input resistance (R_N) of hippocampal neurons, suggesting that the intracellular activities of Na^+ and Cl^- may modulate these "membrane properties".

The RMP and R_N of CA1 neurons were measured in adult hippocampal slice preparations using whole-cell recordings. All electrode solutions were pH 7.25 and included (in mM) K^+HEPES 10, $MgCl_2$ 2, and variable Na^+ and Cl^- (tabulated below); K^+gluconate was added so that the sum of K^+, Na^+, Cl^-, and gluconate was 284 meq. RMPs were corrected for junction potentials. 34ºC, pH 7.4 artificial cerebrospinal fluid (ACSF) included NaCl, 126; KCl, 2.5; $NaHCO_3$, 26; $CaCl_2$, 2; $MgCl_2$, 2; NaH_2PO_4 1.25; and glucose, 10. 800 μM ethacrynic acid (ECA) was added to the ACSF in some experiments.

ECA	-	-	-	-	-	+
Cl^-	4	12	40	80	139	139
Na^+	0	8	0-8	8	8	8
RMP (mV)	-68	-72	-67	-68	-60	-63
R_N (MΩ)	70	45	87	84	85	46
N	10	5	10	7	6	4

In the high chloride cells, ECA both decreased the R_N and diminished a large, slow depolarizing shift of the RMP. Effects of intracellular calcium buffering (10 mM K_2EGTA) were small. These results suggest that electroneutral NaCl cotransport may reduce internal Na^+ in cells recorded with high Cl^- electrode solutions, thereby decreasing a Na^+ -dependent K^+ conductance which is active at RMP and which significantly affects the R_N.

629.22

COMPUTER SIMULATION OF A CA3 HIPPOCAMPAL NEURON. M. Migliore*, David Jaffe and Daniel Johnston. Div. of Neuroscience, Baylor College of Medicine, Houston, TX.

Computer simulations of the firing properties of a hippocampal CA3 pyramidal neuron were performed using the program NEURON/NMODL, which was developed by Mike Hines at Duke University. The model neuron (Jaffe et el., Soc. Neurosci. abstr. **17**, 1991) was constructed from a camera lucida drawing of a golgi stained CA3 neuron and consisted of 149 compartments, representing the soma and apical and basal dendrites. To study the interplay among ionic currents during spike frequency adaptation and bursts, several ionic currents have been included in the model. Based on available voltage- and current-clamp experimental data, we modeled the Ca-independent potassium currents IK_A, IK_{DR} and IK_M, the Ca-dependent potassium currents, IK_C and IK_{AHP}, a fast Na current, *INa*, and three Ca currents, ICa_N, ICa_L and ICa_T. Calcium buffering, pumping and radial and longitudinal diffusion have also been incorporated into the model. Using a combination of electrophysiological and fluorescence imaging results, distributions of several of these channels were nonuniformly placed in different regions of the dendrites. The results from the simulations can qualitatively reproduce a number of the repetitive firing characteristics of these pyramidal neurons under different conditions of injected current and pharmacological manipulations. (Supported by NIHM grants MH44754 and 48431 and the Keck Foundation.)

629.23

THE EFFECT OF AXOTOMY ON THE ELECTRICAL PROPERTIES OF NEUROSECRETORY CELLS. R. Godínez, R.F. Valdiosera and P. Huizar*. Dept. of Physiology, CINVESTAV-IPN. Apartado Postal 14-740, México, D.F. 07000. MEXICO.

The effect of axotomy on the electrical properties of X-organ neurons of the crayfish was investigated with microelectrode techniques. These neurons are monopolar and their axons start branching at about 200-500 μm from the cell body to form a neuropil where they recieve their synaptic inputs. The axons were cut at about 150 μm from the cell body so most or all of their connections were removed. The isolated X-organ was placed in a bath and recordings were made 15 to 30 min afterwards. After axotomy, the synaptic activity disappeared and the resting potential increased from a control value in intact neurons of -52.6 ± 6 mV (n=10) to -80.6 ± 5 mV (n=7). This hyperpolarization was accompanied by a reduction of input resistance to about half the control value. The action potential, either disappeared or a slow depolarizing response remained. Under these conditions, the perfusion with Dibutyryl cyclic AMP (2 mM) from a micropipette nearby produced a depolarization near to control levels accompanied by a threefold increase in input resistance. The action potential either reappeared or increased in size and became faster. These results indicate that axotomy has important effects on the state of K^+ and Ca^{++} channels.

629.24

A MUTATION AFFECTING THE SODUM PUMP OF *DROSOPHILA*. M. Schubiger[1], D.M. Fambrough[2] and J. Palka[1]*. [1]Dept. Zoology, Univ. Washington, Seattle, WA 98195 USA; [2]Dept. Biology, Johns Hopkins Univ., Baltimore, MD 21218 USA.

In a behavioral screen of 2,000 enhancer trap lines we isolated one (designated 2206) in which the P-element had inserted into region 93B of the salivary gland chromosomes, the site of the previously cloned gene for the α-subunit of the *Drosophila* Na^+,K^+-ATPase. The following evidence indicates that 2206 is a hypomorphic mutation resulting from the insertion of the P-element into the regulatory region of the gene: (1) 2206 homozygotes are nearly an order of magnitude more sensitive to ouabain, a highly specific pump poison, than are control flies. (2) They are bang-sensitive, undergoing brief paralysis in response to mechanical agitation. This behavior is mimicked by injection of ouabain into wild type flies. (3) Excision of the transposon leads to a reversion of the bang-sensitive phenotype and restores wild type resistance to ouabain. (4) A monoclonal antibody to the α-subunit stains much less intensely in certain tissues of 2206 than it does in control flies. Quantitative protein immunoblots show that in the mutants only about 30% of the wild type quantity of pump protein is made. (5) In Northern blots the cDNA to the coding region recognizes the previously reported mRNAs but shows quantitatively reduced hybridization. (6) While no novel restriction lengths within the pump gene coding region are seen in Southern blots of 2206 DNA, a cosmid has been isolated that spans the coding region and the site of insertion of the P-element. This will enable us to localize the insert. Thus, the evidence argues that 2206 carries an insertion that disrupts the normal functioning of the regulatory region of the α-subunit gene. The isolation of a hypomorphic mutation with a behavioral phenotype invites further studies on the regulation of pump gene expression.

630.1

OVER EXPRESSION, AFFINITY PURIFICATION AND CRYSTALLIZATION OF RECOMBINANT RAT CHOLINE ACETYLTRANSFERASE.

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Department of Biochemistry, UT. Southwestern, Dallas, Texas 75235-9038.

Choline acetyltransferase (ChAT, E.C. 2.3.1.6) catalyzes the biosynthesis of the neurotransmitter acetylcholine using choline and acetylCoA and serves as the most specific marker yet known for cholinergic neurons. The detailed characterization of this enzyme at the molecular level has been hampered by its low abundance from natural sources. Previous attempts to express recombinant *Drosophila* or rat ChAT in E. coli resulted in high levels of inactive insoluble enzyme and only modest levels of soluble active enzyme. We report here the optimization of expression of rat ChAT in its active form in E. coli, yielding approximately 50 mg of enzyme per liter of culture (estimated to be equivalent to the amount of enzyme contained in 20,000 rat brains). A facile purification scheme was developed for the recombinant enzyme using a poly-histidine affinity tag which permits homogeneous enzyme to be obtained in a single day. Finally, the crystallization of the recombinant enzyme has been achieved which should pave the way for the determination of the enzyme structure.

630.2

FEEDBACK REGULATION OF CHOLINE ACETYLTRANSFERASE EXPRESSION IN *DROSOPHILA*.

V. Andrisani, T. Kitamoto and P. M. Salvaterra*. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA91010.

We have examined the possibility that transcriptional regulation plays an important part in feedback regulation of the *Drosophila* choline acetyltransferase (ChAT) gene. We constructed transgenic flies carrying a *lacZ* reporter gene whose expression was directed by the 5' flanking DNA of the ChAT gene. The fusion gene was introduced into *Drosophila* with either a wild type or temperature sensitive *Cha* genetic background. Compared to wild type flies, temperature sensitive *Cha* mutants show lower ChAT activity and higher ChAT mRNA levels. At a restrictive temperature, ChAT activity and ChAT mRNA levels decrease in mutants but increase in wild-type flies. If the 5' flanking DNA directing reporter gene expression contains elements responsible for this feedback regulation, the levels of mRNA coding for reporter gene should change in parallel with those of ChAT mRNA. It will also be possible to map the elements responsible for feedback regulation by using different amount of 5' flanking DNA. We are now analyzing the reporter gene expression directed by 7.4 kb of 5' flanking DNA. This DNA has been shown to contain most, if not all, of the cis elements required for correct temporal and spatial expression of ChAT. Our preliminary results indicate that higher β-galactosidase activity is present in temperature sensitive *Cha* mutants relative to wild type at a permissive temperature. The β-galactosidase activity also decrease in flies at a restrictive temperature. These results may suggest that 7.4 kb of 5' flanking DNA is involved in feedback regulation of ChAT.

630.3

DISTRIBUTION OF CHOLINERGIC NEURONS IN WHOLE MOUNT PREPARATIONS OF *DROSOPHILA*.

T. Kitamoto*, K. Ikeda and P.M. Salvaterra. Div. Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

We have analyzed the distribution of putative cholinergic neurons in whole mount preparations of *Drosophila melanogaster*. Cholinergic neurons were visualized by X-gal staining of P-element transformed flies constructed with a fusion gene containing bacterial *LacZ*. *LacZ* expression was controlled by various amounts of the 5' flanking DNA of the *Drosophila* choline acetyltransferase gene. We have previously demonstrated that cryostat sections of transgenic flies containing 7.4 kb of 5' flanking DNA express *LacZ* in a detailed pattern similar to the known distribution of ChAT protein. Whole mount staining of these same flies should thus represent the overall distribution of cholinergic neurons in the fly. X-gal staining could be observed in most, but not all, areas of the CNS and PNS. Removal of the distal part of the 5' flanking DNA resulted in a dramatic reduction of X-gal staining in the PNS. For example, the 7.4 kb DNA directed strong *lacZ* expression in leg sensory neurons but the 1.2 kb DNA did not. Our results suggest that ChAT expression is regulated differentially in the CNS and PNS.

630.4

EXPRESSION AND LOCALIZATION OF CHOLINE ACETYLTRANSFERASE AND THE *unc-17* GENE PRODUCT IN THE NEMATODE, *C. elegans*.

J. S. Duerr*, H.-P. Han, and J. B. Rand, Program in Molecular and Cell Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

In *C. elegans*, *cha-1*, the gene that encodes choline acetyltransferase (ChAT), is part of a complex genetic locus with *unc-17*. The function of the *unc-17* gene product is unknown, but defects in this gene, like defects in *cha-1*, cause uncoordinated locomotion and resistance to cholinesterase inhibitors. To study the function and distribution of these two gene products, we are expressing them as fusion proteins and generating specific antibodies for immunolocalization studies.

The full length coding sequences from the *cha-1* and *unc-17* cDNAs were cloned into pMAL plasmid expression vectors to produce fusion proteins in *E. coli*. The fusion proteins were induced, purified, and cleaved to yield ChAT or unc-17 protein; these cleaved proteins were used as immunogens. Peptides designed from the known cDNA sequences were also used as immunogens. Polyclonal sera were produced in rabbits and chickens; a subset of these sera specifically recognized the ChAT or unc-17 fusion protein on Western blots. However, the sera exhibited high nonspecific staining of *C. elegans*. Therefore, they have been affinity purified with the fusion proteins before use in immunocytochemistry. The affinity-purified sera are being used to identify the ChAT and unc-17 containing neurons in the ventral nerve cord and ganglia, as well as to identify staining in any non-neuronal cells. This identification will be simplified by the fact that the identity and origin of all of the cells in *C. elegans* have been described. Supported by grants from NSF and NIGMS.

630.5

MOLECULAR ANALYSIS OF TWO NESTED GENES INVOLVED IN ACETYLCHOLINE METABOLISM AND RELEASE: THE *cha-1/unc-17* GENE COMPLEX IN THE NEMATODE *C. elegans*.

A. Alfonso*, K. Grundahl, J.M. Asbury, J.R. McManus and J.B. Rand, Program in Molecular and Cell Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104

cha-1 and *unc-17* are parts of a complex locus and transcription unit. *cha-1* encodes choline acetyltransferase, and *unc-17* encodes a unique 58 kDa protein of unknown function, believed to be involved in acetylcholine metabolism or release. *unc-17* is nested within a long *cha-1* intron, and the two transcripts share some 5' sequences. The entire genomic region (~11.5 kb) has been sequenced.

We screened 3 cDNA libraries, and isolated 3 independent *cha-1* cDNAs and 4 independent *unc-17* cDNAs. Although *cha-1* and *unc-17* contain no coding sequence in common, all of the cDNAs share a 66 bp 5'-untranslated exon. The 2 cDNAs extending furthest in the 5' direction (1 *cha-1* and 1 *unc-17*) also contain sequences apparently derived from the trans-spliced leader SL1. Primer extension analysis reveals heterogeneity within each transcript class. A *cha-1* specific primer yields a minor product which is the predicted length for a trans-spliced RNA, and a major product ~60 bases longer. The *unc-17* specific primer gives rise to several extension products: the most abundant one is the predicted size for trans-spliced RNA, and a minor product corresponds to the length of the major *cha-1* extension product. Supported by a grant from NIGMS.

630.6

NEW CHOLINERGIC GENES IN THE NEMATODE *C. elegans*.

J. B. Rand*, A. Alfonso, and M. Nguyen. Program in Molecular and Cell Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

We have identified and mapped 4 new genetic loci in *C. elegans*, which are characterized by resistance to inhibitors of cholinesterase (the *ric* phenotype). We are interested in determining the roles of these genes in acetylcholine metabolism and cholinergic function. These genes are unlinked to each other, and give rise to recessive drug resistance, uncoordinated locomotion, and slow growth. For mutants in each of these genes (and also in the 14 previously identified genes), we performed growth curves and acetylcholine measurements, and we quantified the resistance to two different AChE inhibitors: the carbamate aldicarb and the organophosphate trichlorfon.

Mutations in each of these 18 genes confer comparable resistance to aldicarb and trichlorfon, which suggests that all of these genes encode gene products involved in cholinergic metabolism and/or function. Mutations in *ric-1* through *ric-4*, as well as 8 other genes, lead to 2- to 4-fold increases in acetylcholine levels, suggesting that these genes act presynaptically. Molecular analysis of *ric-2* is now in progress.

Supported by a grant from the Oklahoma Center for the Advancement of Science and Technology.

630.7

FIRST AND SECOND GENERATION CHOLINESTERASE INHIBITORS: THEIR EFFECTS ON CORTICAL ACETYLCHOLINE. E. Giacobini*, E. Messamore and X-D. Zhu. Dept. Pharmacology, Southern Illinois Univ. Sch. Med., Springfield, IL 62794-9230.

Several cholinesterase inhibitors (ChEI) are presently being clinically evaluated for enhancement of cholinergic function in Alzheimer disease (AD) patients. We postulated that the ability of ChEI to ameliorate the cholinergic deficit in AD is related to their ability to maintain long-lasting, non-toxic steady state levels of ACh in cortex (Becker and Giacobini, 1988). We have modified the HPLC-ECD method for acetylcholine (ACh) to detect femtomole levels of ACh in microdialysates from rat frontal cortex without using a ChEI in the probe to elevate ACh levels. Using this methodology we have compared the effects of two first generation ChEI, physostigmine (PHY) and tetrahydroaminoacridine (THA) and two second generation ChEI, heptyl-physostigmine (HEP) and MDL 73,745, in increasing and maintaining cortical extracellular ACh levels in the rat. Dissimilar magnitudes of ChEI and effects on ACh levels were seen. Although all four ChEI are capable to significantly raise cortical ACh levels, the effects of HEP and MDL 73,745 on ACh are more long-lasting and are associated to less cholinergic side effects than PHY and THA at doses producing comparable AChE inhibition. It appears that ChE activity inhibition is not the sole determinant of extracellular ACh levels and cholinergic side effects. Physostigmine or HEP elicited the same maximal effect on ACh levels in the dialysate, however, ChE inhibition differed markedly between the two. This may reflect differences in the pharmacological profiles of these ChEI. Therefore, measurement of ChE inhibition as currently used is not sufficient to predict effects on extracellular cerebral ACh levels. Additional factors may influence this relationship and identification of these factors may improve prediction of therapeutic efficacy of ChEI treatment in AD. (Supported in part by NIA Core Grant #P30 AG08014). REFERENCE: Becker, R. and E. Giacobini, Mechanisms of cholinesterase inhibition in senile dementia of the Alzheimer type: clinical, pharmacological and therapeutic aspects. Drug Dev. Res. 12:163-195, 1988.

630.8

STANDARDS FOR ACETYLCHOLINESTERASE HISTOCHEMISTRY PERMIT QUANTIFICATION OF ENZYME IN TISSUE SECTIONS USING COMPUTER AIDED DENSITOMETRY. B. W. Fenton*, M. B. Moss, D. L. Rosene. Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA, 02118.

Acetyl cholinesterase (AChE) histochemistry is an important method for determining the distribution of cholinergic nerve terminals, but is impractical for quantification of AChE in regions unavailable for dissection and biochemical assay, such as the subfields of the hippocampus. We have developed a series of AChE standards that can be verified biochemically and used to calibrate the optical density of AChE reaction product in tissue sections. Membrane bound AChE (EC 3.1.1.7 Sigma) was extracted using Triton X100 and tolulene, suspended in a gelatin solution which was then frozen in isopentane, cut on a cryostat at 15 µm and processed histochemically by the method of Tago along with 15 µm fresh frozen brain sections. The concentration of AChE in the standards was determined by ^{14}C-acetylcholine degradation (measured as µmol ACh degraded /min /gram at 37°C and pH 8). The variability of optical density in different slides of the standards was less than 7%, indicating uniform distribution of the enzyme. The relationship of optical density to the biochemically determined AChE concentrations in the standards was best fit by a linear model ($p<0.01$). Optical density of standards is also linearly related ($p<0.05$) to duration of histochemical incubation. Measurements of hippocampal sections calibrated to standards provided results that are within 10% of previously reported radiochemical measurements. Using standards to calibrate optical density, AChE concentration was determined in 25 hippocampal subfields of normal adult rats. Highest concentrations of AChE were found in the pyramidal layers of Ammon's horn (13 to 11 units of AChE) and the infragranular region of the dentate gyrus (11 units), while stratum radiatum contained the lowest concentrations (3.7 to 5.2 units). The ability to detect subtle quantitative changes can help refine knowledge of the hippocampus, which may have several smaller routes of cholinergic innervation other than the fornix. "Supported by NIH training grant NS07152, research grants AG00001, NS16841, AG04321, and Alzheimer's Association Grant RG-89-116."

630.9

DISTRIBUTION OF BUTYRYLCHOLINESTERASE IN THE RAT BRAIN. S. Darvesh, A.J. Smereczynsky and D.A. Hopkins*. Department of Anatomy and Neurobiology, Dalhousie University, Halifax, N.S. Canada B3H 4H7.

Butyrylcholinesterase (BuChE, EC 3.1.1.8) has a widespread distribution not only in brain blood vessels and glia but also in neurons and neuropil of selected regions of the central nervous system (Friede, 1967). The function of this enzyme in neurotransmission is unknown but BuChE has been implicated in neural development, neuropathology and drug metabolism. The distribution of BuChE in the rat was mapped following standard histochemical methods and compared with the distribution of cholinergic neurons. BuChE is co-localized with some but not all cholinergic neurons and is found in distinct populations of non-cholinergic neurons. Similar relationships were seen for neuropil staining. In the medulla oblongata, BuChE staining was prominent in some but not all motoneurons, the subnucleus interpolaris of spinal V, medial reticular formation and parts of the vestibular complex. In the pons, BuChE was concentrated in the caudal cholinergic cell column and reticular formation. In the mesencephalon, the enzyme was present in parasympathetic motoneurons and the neuropil of the interpeduncular nucleus and superior colliculus. Several thalamic nuclei were heavily stained as were parts of the basal forebrain. Sporadic neurons of the neocortex were intensely stained as was the neuropil of the cingulate cortex. A detailed map of BuChE distribution in the rat brain will provide a guide for investigations into possible neural functions of this enzyme. Supported by MRC (MT-7369) and the Scottish Rite Charitable Foundation of Canada.

630.10

REACTIVATION OF TABUN-INHIBITED AChE BY BISQUATERNARY OXIMES, RELATED TO PYRIDOSTIGMINE PRETREATMENT. G. Amitai, I. Rabinovitz, G. Cohen, G. Zomber, R. Adani and L. Raveh, IIBR, P.O.Box 19, 70450 Ness Ziona, Israel.

Certain oximes such as toxogonin and HI-6 together with anticholinergics serve as antidotes against poisoning by organophosphates. Pretreatment with pyridostigmine (PYR) increases their protection ratio (PR). We have studied the reactivation of tabun-inhibited FBS-AChE elicited by toxogonin, HLö-7, AB-13, HI-6 and AB-8. The bimolecular rate constants for reactivation obtained for toxogonin, HLö-7 and AB- 13 were: 157, 18.7 and 12.5 $M^{-1} min^{-1}$, respectively, whereas AB-8 and HI-6 showed low reactivation potency. The reactivation data correlated well with the PR values obtained in conjunction with atropine and benactyzine in mice and guinea pigs following tabun poisoning. When PYR was added as a pretreatment the PR values markedly increased (e.g. in guinea pigs, HI-6 from 3.8 to 50 and AB-8 from 3.3 to 45.8) but could not be correlated to the reactivation data. Reactivation of PYR-inhibited FBS-AChE displayed similar kinetics for HI-6, HLö-7 and toxogonin ($t_{1/2}$=15-30 min) whereas the rate obtained for AB-8 and AB-13 was equal to spontaneous reactivation ($t_{1/2}$=60 min). Thus, it seems that the antidotal efficacy against tabun with PYR pretreatment can not be related only to the reactivation rate of carbamoyl-AChE. It is conceivable that different conformational changes occur upon formation of ternary binding complexes comprised of PYR-AChE-oxime.

630.11

THE EFFECT OF NORPYRIDOSTIGMINE (3-PYRIDINOL DIMETHYLCARBAMATE), ON ACh AND AChE IN THE RAT BRAIN. M.E. Bach*, A.B. Naini, L.J. Côté, S. Ginsburg, J.L. Bach[2] & M.Sano. Dept. of Neur., College of P& S, Columbia Univ., NY, NY 10032 & [2]Ciba-Geigy Co., Summit, NJ

Norpyridostigmine, is a new centrally active, reversible acetylcholinesterase (AChE) inhibitor. Norpyridostigmine is a derivative of pyridostigmine, the latter does not cross the blood-brain barrier. We assessed the effectiveness of Norpyridostigmine, on the level of acetylcholine (ACh) and the activity of AChE in rat brain. ACh and AChE were measured following an injection (i.p.) of Norpyridostigmine tosylate (30mg/kg). Control rats received a saline injection. ACh levels were measured across five time intervals (15-180 min) following injection. Rats were killed with a focused microwave apparatus, to instantly denature AChE. A HPLC-EC technique, with an immobolized enzyme column (Bioanalytical Systems, Inc.), was used to measure the level of ACh. AChE activity was measured across four time intervals (15-120min), using a radioenzyme assay. ACh was increased by almost 50% at 20 min, and was still above control at 3 hr. AChE activity was decreased by almost 70% at 30 min, and was still inhibited by 37% at 120 min. Norpyridostigmine effectively inhibits the breakdown of ACh by AChE for several hours. Behavioral studies done in our laboratory, demonstrated that Norpyridostigmine attenuated the learning and memory deficit produced by a lesion in the nucleus basalis magnocellularis in the rat. In Alzheimer's disease (AD), a profound cholinergic deficit exists, which has been linked to the memory impairment associated with the disease. Our data suggests that Norpyridostigmine might be useful in the treatment of AD.

631.1

NICOTINIC RECEPTOR SUBUNIT EXPRESSION AND FUNCTIONAL BLOCK BY ANTISENSE IN CHICK CNS NEURONS X. Yang, A. Brussaard, J. Doyle, D. Colman* & L. Role. Anat. & Cell Biol. & Ctr for Neurobiol. & Behav., Columbia Univ., P&S, 630 W.168 St. NY,NY 10032

Previous studies on peripheral neurons reveal important developmental changes in nAChRs (Moss et al., *Neuron* 3:597,1989; see abstract Devay & Role, this volume) leading us to test for similar regulation of AChR channel expression in the chick CNS. AChR subunit gene expression in the habenular complex of ED11 chick embryos was initially assessed by the PCR technique. We observed cDNA dependent PCR products in reactions which contained synthetic oligonucleotide primers specific for α3, α4, α5, α8, β2 and β4 AChR subunit genes. By Southern blot and/or restriction digestion maps, we have confirmed the identity of these amplified fragments. Examination of the expression of other subunits is underway.

In biophysical studies we have investigated the role of expressed AChR α subunit genes in AChR channel function. Four classes of nicotine-gated channels are seen in chick habenular neurons, all of which are blocked by neuronal bungarotoxin. Treatment of the neurons with a specific antisense oligonucleotide to the α4 start sequence (Listerud et al *Science*:254:1518,1991) selectively deletes the smallest conductance channel. Both the developmental profile of AChR subunit expression and developmental changes in the functional role of individual subunits will be evaluated. (Funding: NS29071 & Council for Tobacco Research).

631.2

REGULATION OF nAChR SUBUNIT EXPRESSION IN CHICK LUMBAR SYMPATHETIC GANGLIA DURING EMBRYOGENESIS. P. Devay*, X. Qu, S. Peng and L. Role. Dept. of Anat. and Cell Biol. & Cntr. for Neurobiol. & Behav., Columbia Univ., P&S, NY, NY 10032

The biophysical properties and distribution of ACh-activated channels (AChRs) in chick lumbar sympathetic neurons change during embryonic development (Moss et al Neuron 3: 597, 1989). To examine the relative role of presynaptic input vs target contact in the regulation of AChR expression, we have studied the time course of establishment of both pre and postsynaptic contacts concurrent with changes in AChR subunit expression.

Following injection of DiI into the sympathetic chain we could detect anterogradely labelled sympathetic nerve endings in some target tissues (skin, renal capsule) as early as embryonic day (ED)10. At the time of these first contacts with target <5% of the sympathetic neurons have received presynaptic input (as indicated by their being surrounded by fluorescent boutons following injection of DiI into the region of the preganglionic nucleus). Examination of the array of AChR subunit genes expressed by the lumbar sympathetic neurons during embryogenesis reveals that α3, α4, α5, α7, β2 and β4 are detected at stages both before and after synaptogenesisRNase protection assay reveals increases in the level of expression of specific nAChR subunit mRNAs over the same period of development. The relative role of presynaptic input vs target contact in AChR subunit expression and AChR channel function will be examined *in vitro*. Funding: NIH (NS29071) & Council forTobacco Research.

631.3

ANTISENSE OLIGONUCLEOTIDE UPTAKE INTO NEURONS C. Yu, X. Yang, & L. Role*. Dept. of Anat. & Cell Biol. in the Ctr for Neurobiol. and Behav., Columbia Univ., P & S, 630 W. 168 St. New York, NY 10032

Previous work using antisense oligonucleotides to selectively delete individual AChR subunits have revealed distinct functional contributions of the α3, α4 and α7 subunits to the AChRs in peripheral and central neurons (Listerud et al, *Science* 254:1518,1991; Yang et al., abstract this volume). To further characterize the mechanism of antisense oligonucleotide block of AChR subunit gene expression, we are examining the mechanism of oligonucleotide uptake. Our initial studies examine the stability and time course of oligonucleotide uptake into primary neurons.

Antisense oligonucleotides (15 bases) were added directly to the bathing medium of dispersed sympathetic neurons and uptake was measured within 3-48 hrs. Intact 15mer can be detected in the neurons within 6 hrs and the uptake plateaus within 24 hrs. After 48 hrs, the time at which functional block of AChR channels was assayed, the oligonucleotide can still be detected in the neurons as intact 15mer. Unmodified 15mers are detectably degraded within 3 hours in normal culture media containing heat inactivated horse serum and 5% chicken embryo extract (CEE). Heat inactivation of the CEE decreased the degradation rate by 2-5 fold. Comparison of the time course and pharmacology of the neuronal uptake of oligonucleotides with that previously described in non-neuronal cells suggest the transport systems might be quite similar. Funding: NIH(NS29071) & Council for Tobacco Research.

631.4

THE α5 GENE PRODUCT ASSEMBLES WITH MULTIPLE ACETYCHOLINE RECEPTOR SUBUNITS TO FORM DISTINCTIVE NEURONAL RECEPTOR SUBTYPES *IN VIVO*. **W.G. Conroy, A.B. Vernallis, and D.K. Berg*, Dept. of Biology, UC San Diego; La Jolla, CA 92093.**

The acetylcholine receptor (AChR) α5 gene has been classified as a member of the AChR gene family based on sequence homology, but expression studies have yet to confirm a function for the encoded protein. We find that the α5 gene product is identical to a 49 kD protein that co-purifies with AChRs from brain and ciliary ganglia. It combines with several combinations of AChR subunits.

The 49 kD protein was identified as the α5 gene product by showing that the protein shares three epitopes associated with the α5 gene product translated *in vitro*. The epitopes were absent from all other neuronal AChR gene products tested (six). Immunoprecipitation experiments with subunit-specific monoclonal antibodies indicated that in ciliary ganglia much of the α5 gene product is co-assembled with α3 and β4 subunits together, but not with α4 subunits. In brain the α5 gene product was found associated with α4 subunits, with α3 subunits, and, to a small extent, with both together. No α5 was found associated with α7 or α8 subunits. Other AChR gene products have yet to be tested for co-assembly with α5. The results show that neuronal AChRs can have as many as three kinds of subunits with at least two being of the α-type. (NS 12601 & 25916)

631.5

COORDINATE EXPRESSION OF ACH RECEPTOR GENES DURING DEVELOPMENT IN CILIARY GANGLIA. **R.A. Corriveau* and D.K. Berg. Dept. of Biology, University of California, San Diego; La Jolla, CA 92093.**

Chick ciliary ganglion neurons have at least two classes of nicotinic acetylcholine receptors (AChRs). One class is largely synaptic in location, generates nicotinic responses mediating synaptic transmission, and contains the AChR gene products α3, β4, and α5. The other class is 5- to 10-fold more abundant, is located primarily in non-synaptic membrane, and increases intracellular calcium when activated. It contains the AChR α7 gene product. Other possible AChR gene products have yet to be identified. The known AChRs increase 12- and 6-fold, respectively, per neuron between embryonic days 8 (E8) and 17-19 (E17-19).

Quantitative RNAase protection experiments showed that E17-19 ganglia contain moderate mRNA levels from AChR genes α3, α5, α7, β2, and β4. α7 transcripts are most abundant (ca. 2000 copies/neuron) and β2 least abundant (ca. 150 copies/neuron). In each case transcript number per neuron increases 4- to 8-fold between E8 and E17-19. Northern blot analysis detected no differences in transcript sizes between E8 and E17-19. mRNAs from several other AChR genes were either undetectable or present in only trace amounts. The proportionality between transcript levels and receptor number suggests that during development receptor mRNA may, in part, be rate-limiting for AChR accumulation in the ganglion. (NS 12601 & 25916)

631.6

MUSCLE MEMBRANE BUT NOT FORSKOLIN RESTORES ACh SENSITIVITY TO CULTURED CILIARY GANGLION CELLS.

J.M. Spitsbergen* and J.B. Tuttle, Department of Neuroscience, University of Virginia School of Medicine, Charlottesville, VA 22908.

Ciliary ganglion neurons cultured in the absence of muscle cells rapidly lose sensitivity to ACh, while cultures grown with muscle or muscle cell membranes maintain sensitivity to ACh for extended periods of time. The present study asked whether sensitivity to ACh could be restored to cultured neurons, by addition of muscle cell membranes or stimulation of adenylate cyclase with forskolin, once sensitivity had been lost. Ciliary ganglion neurons from 11-13 day old chick embryos were grown on collagen coated substrate in the absence of muscle cells. Sensitivity to ACh was assessed by measuring current responses following application of 100 μM ACh to neurons under whole-cell voltage clamp. ACh induced currents decreased from peak values of 646±37 pA the day of plating to 116±11 pA after 4 days in culture. Exposure of cultures to forskolin (1 μM) and IBMX (1 mM), soon after plating, had a variable effect on peak ACh currents, increasing currents in some cells, while having no effect on current amplitude in others. However, by 3 to 4 days postplating forskolin and IBMX had no effect on currents induced by ACh. Exposure of neurons to muscle membranes for 24 hours increased ACh induced currents from 128±45 pA on day 3 in culture to 427±46 pA on day 4. In conclusion, growth of ciliary ganglion neurons in the absence of muscle leads to a decrease in ACh sensitivity within 3-4 days. At this time forskolin and IBMX have no effect on the amplitude of ACh induced currents, yet they still increase cyclic AMP levels. Conversely, exposure of ciliary ganglion neurons to muscle membranes, the normal target of these neurons, restores ACh responsiveness.

631.7

BOTH INNERVATION AND TARGET TISSUE INTERACTIONS REGULATE ACETYLCHOLINE RECEPTOR TRANSCRIPT LEVELS IN DEVELOPING NEURONS. M. Schwartz Levey*, C. Brumwell and M.H. Jacob. Worcester Fnd. for Exp. Biol., Shrewsbury, MA 01545.

Innervation regulates nicotinic acetylcholine receptor (nAChR) protein and mRNA levels in muscle. In developing motoneurons, innervation as well as intrinsic factors and retrograde signals from the target tissue may be important regulatory influences. To establish the role of cell-cell interactions in inducing nAChR expression in chick ciliary ganglion motoneurons *in situ*, we have surgically removed the preganglionic nucleus (at ED 3.5-4) or the target tissue (the eye, at ED 2) prior to synapse formation. Ganglia were sampled at ED 8. Previous studies have demonstrated that ciliary ganglion neurons develop normally in the absence of inputs or target tissue interactions up to ED 9.

Alpha3 and $\beta 4$ mRNA levels were quantitated using RT-PCR and mutated internal standards. In input-deprived ganglia, both $\alpha 3$ and $\beta 4$ mRNA levels are reduced 30% as compared to control ganglion values at ED 8. Target tissue-deprived ganglia have 20% lower $\alpha 3$ and $\beta 4$ mRNA levels relative to control ganglia. In comparison, ganglia deprived of both the source of inputs and the target tissue have even greater reductions in $\alpha 3$ and $\beta 4$ mRNA levels, exhibiting 80% declines relative to controls. Qualitatively similar changes were observed in internal nAChR levels by labeling frozen sections of operated and control ganglia with an anti-AChR mAb. The results demonstrate that both presynaptic input and retrograde signals from the target tissue regulate nAChR protein and mRNA levels in developing neurons. Supported by NIH NS 21725, the Pfeiffer Fnd. and MDA.

631.8

NICOTINIC NEURONAL ACETYLCHOLINE RECEPTOR α-3 SUBUNIT TRANSCRIPTION IN NORMAL THYMUS, MYASTHENIC HYPERTROPHIC THYMUS AND THYMOMA. M. Mihovilovic*, C. Hulette, J. Mittlestaedt and A.D. Roses. Dept. of Medicine/Neurology, Duke Univ. Med. Ctr., Durham, NC 27710.

A putative thymic nicotinic neuronal acetylcholine receptor (AcChR) may serve as modulator or transducer of signals delivered to the thymus through the autonomic nervous system. To investigate this hypothesis we are studying the relationship that may exist between the transcript expression of the AcChR α-3 subunit and thymic compartamentalization. Thymic tissues from normals and Myasthenia gravis (MG) patients have been studied.

Transcripts encoding both normal and variant forms of the α-3 subunit of an AcChR are expressed in thymic tissues. Normal and MG hypertrophic thymi express equivvalent levels of both normal and variant α-3 transcripts. On the other hand, thymomas express reduced levels of α-3 subunit transcripts. Histological examination of thymomas indicates that the reduction of the transcriptional level for this subunit is associated with expansion of the thymic epithelia. Trace amounts of α-3 transcripts have been found in a thymoma that upon histological examination has remnants of thymic tissue and large areas of fibrosis. Overall, these findings indicate that the disruption of the thymic cortical and medullary compartments is associated with a decrease in the level of AcChR α-3 subunit transcripts.

631.9

THE RAT $\alpha 7$ SUBUNIT ENCODES A NICOTINIC ION CHANNEL HIGHLY PERMEABLE TO CALCIUM. P. Séguéla, J. Wadiche, K. Miller, A.C.S. Costa#*, J. A. Dani# and J. W. Patrick. Division of Neuroscience, #Department of Molecular Physiology & Biophysics, Baylor College of Medicine, Houston TX 77030.

We isolated a full-length clone coding for the rat neuronal nicotinic receptor $\alpha 7$ subunit and tested its pharmacological and functional properties in the *Xenopus* oocyte expression system. Homo-oligomeric $\alpha 7$ nicotinic receptors displayed a characteristic profile of sensitivity to agonists (nicotine > cytisine > DMPP > acetylcholine) and antagonists (α-BTX> D-tubocurarine= strychnine). Permeability of $\alpha 7$ channels to Ca^{++} ions was suggested by a dramatic decrease of nicotine-induced current after specific blockade of the endogeneous Ca^{++}-activated chloride channels with niflumic acid and flufenamic acid. In the presence of high external Cl^-, application of agonist produces an inward current followed by an outward current sensitive to Cl^- channels blockers. We compared the reversal potential shift of the ligand-induced current in 1mM vs 10 mM external Ca^{++} under conditions where Cl^- currents are minimal. We measured a +25-30 mV shift, larger than previously reported for muscle and neuronal nicotinic receptors. We conclude that the $\alpha 7$ receptors are highly permeable to Ca^{++}, with an apparent ratio pCa/pNa comparable to the one of the NMDA subtype of glutamate receptor. The functional properties and the expression pattern of this subunit in the rat brain suggest that $\alpha 7$-containing acetylcholine-gated channels play a significant role in triggering post-synaptic Ca^{++}-mediated events in the limbic system in general and in the hippocampus in particular. Supported by NINDS, NIDA, NIH and DOD.

631.10

CHOLINERGIC STIMULATION AND CATION INFLUX INDUCE A DIFFERENTIAL PATTERN OF IMMEDIATE EARLY GENE EXPRESSION IN MUSCLE CELLS. S. Abu-Shakra*, R.N. Adams, A.J. Cole and D.B. Drachman. Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205

Muscle acetylcholine receptor (AChR) synthesis is closely regulated by the state of neuromuscular activity. Denervation causes an increase in AChR subunit gene expression, while direct electrical stimulation of muscle down regulates AChR subunits. We have previously shown that carbachol (a cholinergic agonist) decreases AChR α-subunit message levels in primary rat muscle cultures at 24 hours post-treatment. However, the early genomic regulatory events induced by cholinergic treatment are unknown. Immediate early genes (IEGs), that encode transcription factors and are induced rapidly after cell surface stimulation, are candidates for such a role. We studied the expression of 4 IEGs (zif268, c-jun, nur77 and junB) in the mouse skeletal muscle cell line - C2 - which normally fuses and expresses AChRs. Treatment of C2 mouse skeletal muscle cells with carbachol induces increased zif268, c-jun and nur77 mRNA levels, while junB message levels showed no consistent change. The mRNA levels were increased at 1 hour, peaked at 3 hours, and were back to basal levels by 6 hours. This effect was blocked by α-bungarotoxin pre-treatment. In addition, A23187 and Veratridine, which cause an influx of Ca^{+2} and Na^+ ions respectively, induced a similar pattern of IEG expression. This suggests a role for IEG expression in regulating skeletal muscle properties in response to neuromuscular activity.

631.11

Cyclophilin-mediated Prolyl Peptidyl Isomerization Modulates the Expression of the $\alpha 7$ Homo-oligomeric Neuronal nAChR in *Xenopus* Oocytes. Santosh A. Helekar* and Jim Patrick, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

One of the noteworthy features of the primary structure of oligomeric cell surface receptor subunits is the presence of a number of highly conserved proline residues. Since prolyl peptide bonds undergo cis-trans isomerization by an enzyme-mediated mechanism we tested the possibility that this mechanism might play a role in the synthesis and function of the nicotinic acetylcholine receptor (nAChR), using as a model the rat $\alpha 7$ homo-oligomeric receptor. Subunit cDNA subcloned into pcDNA plasmid vectors was intranuclearly injected into *Xenopus* oocytes and receptor expression was assayed on the 4th - 6th day following injection by recording acetylcholine- (ACh) and nicotine- (NIC) induced currents under two-electrode voltage-clamp. Incubation of the injected oocytes in Barth's saline containing cyclosporine (1 - 30 μM, CSP), a selective blocker of one class of prolyl isomerases, namely cyclophilins (CPH), showed a dose-dependent reduction in the expression of nAChRs. Short-term (1 - 2 hours) incubation in CSP did not block agonist-induced responses in control oocytes. CSP did not appear to nonspecifically block translation or transcription of plasmid DNA since injection of β-galactosidase (β-gal) cDNA-containing vectors resulted in normal levels of β-gal activity in the presence of this agent. Finally, the blockade of $\alpha 7$ receptor expression by 30 μM CSP was reversed by co-expression of CPH cDNA, indicating that CSP is likely to be acting specifically through its blockade of CPH activity. Furthermore, CPH co-expression in the absence of CSP produced greater than 90 % reduction in the expression of the receptor. These findings indicate that post-translational CPH-mediated isomerization of prolyl peptide bonds may be critically involved in the functional expression of the homo-oligomeric nAChR.

631.12

A ROLE FOR THE α-BUNGAROTOXIN/THYMOPOIETIN RECEPTOR IN NEURITE OUTGROWTH IN PC12 CELLS. J.Chan*, G.Goldstein, and M.Quik. Dept. Pharmacol., McGill U., Montreal, Can. and Immunobiol. Res. Inst., NJ, U.S.A.

The function of the neuronal nicotinic α-bungarotoxin (α-BGT) receptor is currently unclear however the α-toxin site has been implicated in growth related activities. Present studies show that nicotine (10^{-5} M and 10^{-4} M) decreased nerve growth factor induced neuritic outgrowth in PC12 cells in culture. This effect was observed as early as 1 day after exposure to the agonist and persisted for at least 7 days. Interestingly, α-BGT, at $3x10^{-8}$ M and 10^{-8} M, was able to prevent the decrease in process formation which occurred after nicotine exposure. These concentrations of the α-toxin correlated very well with those which resulted in a block of ^{125}I-α-BGT binding to PC12 cells. Thymopoietin (10^{-8} M), a thymic polypeptide, which interacts potently and specifically at the α-toxin site, also prevented the nicotine induced decline in process formation; again the concentration required to affect process outgrowth correlated well with that required to inhibit ^{125}I-α-BGT binding. Cell numbers were not altered after exposure to any of these agents, thus alterations in neurite outgrowth were not due to changes in cell proliferation. The effect of thymopoietin was also tested on nicotinic receptor mediated neurotransmitter release; no change was observed indicating that thymopoietin specifically affects the nicotinic α-BGT receptor population. The present results suggest a functional role for the neuronal nicotinic α-BGT receptor in regulating neurite outgrowth and for thymopoietin as an endogenous ligand with such a role at the α-BGT site. Supported by the MRC (Canada).

631.13

COMPARISON OF A VERTEBRATE AND AN INVERTEBRATE HOMO-OLIGOMERIC NICOTINIC ACETYLCHOLINE RECEPTOR. P Thomas, M Amar, M Goosey[1], S Wonnacott* and G G Lunt. Dept of Biochem, Univ of Bath, Bath, UK and [1]Shell Research Ltd, Sittingbourne, Kent.

A vertebrate α-bungarotoxin (αBgt)-sensitive nicotinic acetylcholine receptor (nAChR) subunit, α7, has been cloned and forms homo-oligomeric channels when expressed in Xenopus oocytes[1]. Expression of an analogous receptor subunit from a locust, ARL2, also results in functional homo-oligomeric channels sensitive to αBgt[2]. We have compared the properties of these two putative nAChR using cDNA expression in oocytes. In both cases, agonist-evoked current (V_H = -70 mV) for (-)nicotine, acetylcholine and cytisine produced sigmoid relationships between current amplitude and agonist concentration. α7 was much more sensitive to (-)nicotine than ARL2. Strong inward rectification of the current-voltage relationship was evident in both cases. Whereas α7 channels activate and desensitize rapidly over approx 2s, ARL2 activates slowly and shows no desensitization in the presence of agonist for 45s. Both types of channel are irreversibly blocked by α-cobratoxin and reversibly by methyllycaconitine. These expressed subunits allow direct comparison of the specific, activating pharmacophore of each receptor, using families of semi-rigid ligands including cytisine, anatoxin and piperazine-based compounds.

1. Couturier et al. (1990) Neuron. 5, 847-856.
2. Marshall et al. (1990) EMBO J., 9, 4391-4398.

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631.14

EXTERNAL Ca^{2+} POTENTLY ENHANCES NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR CURRENTS. Mariano Amador and John A. Dani*. Division of Neurosci., and Dept. of Molecu. Physiol. & Biop., Baylor College of Medicine, Houston, TX 77030.

Recently, we found that some subtypes of neuronal nicotinic acetylcholine receptors (NnAChRs) are highly permeable to Ca^{2+} and are modulated by external Ca^{2+} (Vernino et al., 1992 Neuron 8:127). As the external concentration of Ca^{2+} is increased, whole-cell currents through muscle nAChRs decrease but currents through neuronal nAChRs increase. As the external Ca^{2+} is decreased, whole-cell neuronal nAChR currents decrease, but small currents are still seen in Ca^{2+}-free solutions.

Patch-clamp studies of chromaffine cells indicate that Ca^{2+} acts externally on the NnAChRs rather than activating an intracellular enzyme cascade. Ca^{2+} enhancement of NnAChR currents is very rapid, is reversible and repeatable in cells internally perfused with 20 mM BAPTA, does not require exogenous enzymes or a source of phosphate, is not voltage dependent, and is seen in cell-free patches of membrane. In addition, the amplitude of the single-channel currents decreases (not increases) as external Ca^{2+} is increased. These results suggest that an influx of Ca^{2+} is not required to observe the enhancement of agonist-induced NnAChR responses.

Activity-dependent fluctuations in extracellular Ca^{2+} have been reported throughout the central nervous system, and the Ca^{2+}-dependent modulation of NnAChRs occurs over a range that includes physiologic levels of Ca^{2+}. Therefore, activity-dependent changes in the concentration of external Ca^{2+} could alter NnAChR responses and thereby influence the efficacy of neuronal nicotinic synapses.

631.15

REGIONS OF THE β SUBUNIT OF THE NEURONAL NICOTINIC RECEPTOR AFFECTING AGONIST SELECTIVITY. A. FIGL, B. N. COHEN, M. W. QUICK, X.-C. YANG*, AND H. A. LESTER, Division of Biology 156-29, Caltech, Pasadena, CA 91125.

To identify regions of the β subunit that influence the pharmacological selectivity of neuronal nicotinic receptors we constructed chimeras of the β2 and β4 subunits and expressed them with the α3 subunit in *Xenopus* oocytes. Peak macroscopic currents elicited by 30 μM acetylcholine (ACh), cytisine (CYT), nicotine (NIC), and 100 μM tetramethylammonium (TMA) were measured using a two-electrode voltage clamp. For the α3β2 wild-type receptor, the relative amplitudes of the CYT, NIC, and TMA responses compared to ACh were 0.02 ± 0.01 (3) [mean ± sd (n)], 0.3 ± 0.2 (2), and 0.6 ± 0.1 (5). For the α3β4 wild-type, the relative responses were 2.6 ± 0.2 (5), 1.3 ± 0.2 (3), and 1.8 ± 0.5 (3). The external regions of β2 and β4 (N-terminal to M1) account for almost all of the effect of the β subunits on selectivity. For TMA, chimeras with the first 109 or fewer amino acids from β4 and the remainder from β2 showed β2-like sensitivity. Chimeras with the first 111 or more amino acids from β4 showed β4-like sensitivity. For CYT, chimeras with the first 92 or fewer amino acids from β4 showed β2-like sensitivity, although there was an increase in the relative response for chimeras with ≥ the first 20 residues of β4. Chimeras with the first 109 or more amino acids from β4 behaved as the β4 wild-type. There was no distinct region that accounted for NIC sensitivity. The results show that the 109-111 region accounts for most of the difference in TMA sensitivity, the 92-109 region accounts for most of the difference in CYT sensitivity, and that no single region of the external portion of the β subunit accounts for the difference in NIC sensitivity. (Support: NS-11756, Ca. TRDRP, MDA)

631.16

SUBTYPES OF THE β SUBUNIT CONFER DIFFERENT ANTAGONIST SENSITIVITIES ON NEURONAL NICOTINIC RECEPTORS. B. N. COHEN, A. FIGL, M. W. QUICK, A. T. ISHIDA*, AND H. A. LESTER, Division of Biology 156-29, Caltech, Pasadena, CA 91125.

The β subunit partially determines the agonist sensitivity of neuronal nicotinic receptors. To determine if the β subunit also affects antagonist sensitivity, we examined block of the α3β2 and α3β4 receptors expressed in *Xenopus* oocytes by hexamethonium (HEX), mecamylamine (MEC), and trimethaphan (TRI). The oocytes were voltage clamped at potentials of -80 to -60 mV in a solution containing 98 mM NaCl, 1 mM $MgCl_2$, and 5 mM HEPES (pH 7.4). All antagonists inhibited α3β4 better than α3β2. HEX (30 μM) blocked 25 ± 25% (6) [mean ± sd (n)] of the α3β2 response to 30 μM acetylcholine (ACh) and 96 ± 3% (6) of the α3β4 response. The IC50 for HEX was 1.4 μM for α3β4 and 30 μM for α3β2 at 30 μM ACh. Raising [ACh] from 30 to 60 μM relieved the block of both receptors by 10 μM HEX suggesting that HEX competes with ACh binding. MEC (30 μM) blocked 94% of the α3β4 response to 60 μM ACh but had little effect on the α3β2 response. TRI (10 μM) blocked 87% of the α3β4 response to 30 μM ACh but hardly affected the α3β2 response. Block of α3β4 by 10 μM TRI was independent of [ACh] from 30-300 μM suggesting either that TRI binds with high affinity to α3β4 or that it blocks noncompetitively. TRI (10 μM) blocked 35-51% of the 30 μM ACh response from receptors formed from α3 and a chimeric subunit containing ≤ 114 N-terminal residues from β4 and the remaining C-terminal residues from β2; but, TRI blocked 90% of ACh response of a chimeric receptor with 120 N-terminal residues from β4. These results show that the β subunit of neuronal nicotinic receptors affects antagonist sensitivity and that positions 114-120 in β4 play a role in TRI antagonism. (Support: NS-11756, Ca. TRDRP, MDA)

631.17

GENE TRANSCRIPTS FOR THE nAChR SUBUNIT, B4, ARE DISTRIBUTED IN MULTIPLE AREAS OF THE RAT CNS. K. Dineley-Miller, S. B. Sands* and J. Patrick, Division of Neuroscience, Baylor College of Medicine, Houston TX 77030.

Previous *in situ* hybridization experiments found that beta4 (β4) neuronal nicotinic acetylcholine receptor (nAChR) transcripts were found only in the medial habenula (MHB). Co-expression in *Xenopus* oocytes of the β4 subunit and any one of three ligand-binding or alpha subunits results in the formation of functional nAChR's. Comparisons between the pharmacology of nAChR's expressed in oocytes and the pharmacology of nAChR's found in the rat CNS prompted a further investigation of the localization of transcripts encoding the β4 nAChR subunit. Using two β4-specific cRNA probes, *in situ* hybridization was performed in rat brain. β4 mRNA was detected at high levels in the presubiculum, parasubiculum, subiculum and dentate gyrus of the hippocampal formation, in layer IV of the isocortex, in the medial habenula, in the interpeduncular nucleus, and in the oculomotor and trigeminal motor nerve nuclei. Moderate hybridization signals were seen in the isocortex (layers I-III), in olfactory regions, in fields CA1 through CA4 of Ammon's horn and the entorhinal cortex of the hippocampal formation, in the supramammillary nucleus, in the pontine nucleus, in the cerebellum, and in the locus coeruleus. No hybridization above background was detected in the septum, basal ganglia, sensory portions of the brainstem, or spinal cord.

631.18

SUBUNIT SPECIFIC ANTISERA PREPARED AGAINST NEURONAL nAChR's. K. Dineley-Miller, S. E. Neff, D. Char, and J. Patrick*, Division of Neuroscience, Baylor College of Medicine, Houston TX 77030.

The nicotinic acetylcholine receptor (nAChR) gene family includes, to date, ten genes whose transcripts are found in a variety of central and peripheral nervous system structures. Molecular cloning has allowed the expression and study of these receptors *in vitro* but elucidation of receptor composition and localization in rat brain has been hampered by a lack of biochemical probes specific for the extracellular domain of the native form of each subunit.

Our strategy for preparing polyclonal antisera selective for seven of these highly related proteins consisted of 1) immunizing rabbits with bacterially expressed recombinant protein representing the N-terminal extracellular region of each nAChR subunit; 2) affinity purification of antibodies against a synthetic peptide prepared for each subunit in the region corresponding to amino acid residues 69-83 of the alpha (α1) subunit of the muscle nAChR (this portion of the α1 subunit is within the main immunogenic region recognized by antibodies of myasthenia gravis patients); 3) subtracting cross-reactive antibodies by adsorbing against recombinant protein of homologous subunits.

Subunit specificity of antisera have been evaluated by immunoblot of recombinant protein, immunohistochemistry, and affinity purification of receptor subunits from expressed in *Xenopus* oocytes rat brain.

631.19

LESSER RECTIFICATION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR) OF RAT HIPPOCAMPAL NEURONS DISTINGUISHES IT FROM OTHER NEURONAL SUBTYPES. E.X. Albuquerque* and M. Alkondon. Dept. Pharmacol. Exp. Ther., Univ. Maryland, Sch. Medicine, Baltimore, MD 21201.

Rectification is a property found in several neuronal subtypes of nAChR such that very small or no detectable outward macroscopic currents can be recorded. In the present study, we tried to characterize the extent of rectification found in the nAChR of fetal rat hippocampal neurons grown in culture (30-60 days), using the whole cell patch clamp technique. The external solution consisted of (mM): NaCl 165; KCl 5; $CaCl_2$ 2; HEPES 5; D-glucose 10 plus TTX (0.3 μM) & atropine (1μM), and the internal solution had (mM): CsCl 80; CsF 80; CsEGTA 10; HEPES 10. Several nicotinic agonists such as acetylcholine, (+)anatoxin-a, DMPP, cytisine and carbachol were used. The current-voltage plots revealed the following: i) the peak whole cell current grew with the transmembrane voltage on either direction, however, the outward currents were smaller than the inward currents at comparable potentials; ii) the ratio of the current amplitude at +50 mV to that obtained at -50 mV increased from ~0.2 at 15 min of the recording to ~0.75 during the later part of the recording (75 min or later); iii) the reversal potential derived from the linear segment between -20 and +10 mV gave values in the range of 4-7 mV; iv) addition of 2 mM Mg^{2+} to the intracellular solution abolished the outward currents to nondetectable levels. Thus, the presence of significant outward currents, and the sensitivity of the nicotinic currents to αBGT makes the nAChR of fetal rat hippocampal neurons a unique one among the different neuronal nAChR known. Support: U.S. Army Med. Res. & Devel. Comm. Cont. DAMD-17-88-C-8119 and USPHS NS25296.

EXCITATORY AMINO ACIDS: RECEPTORS VIII

632.1

CALCIUM DEPENDENT DESENSITIZATION OF HIPPOCAMPAL NMDA RECEPTORS. G. Lonart* and K.M. Johnson. *Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77555-1031.*

Prolonged stimulation of NMDA receptors can lead to desensitization. In this study, NMDA stimulated fractional release of [^{3}H]-norepinephrine ([^{3}H]-NE) from cross-chopped rat hippocampal slices was utilized as a functional measure of NMDA receptor activity. In the second 5 min fraction of continuous superfusion with 100 μM NMDA, [^{3}H]-NE release reached a maximum level (6-fold increase above baseline) and then rapidly declined. The response to 3 μM ionomycin, a calcium ionophore, did not decline during the same time course. Introduction of additional NMDA (300 μM, 30 sec pulse) at the 30th min of superfusion with 100 μM NMDA had no significant effect. However, addition of 300 μM kainate under the same conditions produced a significant increase in the [^{3}H]-NE release. These observations indicate that NMDA induces a homologous desensitization and that a non-specific change in the substrates underlying vesicular release does not account for the decay in the NMDA response. Under conditions of reduced extracellular Ca^{2+}, the response to 100 μM NMDA declined with a slower rate, and subsequent stimulation with 300 μM NMDA produced a significant increase in the [^{3}H]-NE release. This suggests that the NMDA receptors are desensitized by a mechanism which is dependent upon extracellular Ca^{2+} concentration. Ongoing experiments are being carried out to test possible involvement of protein kinases and phosphatases in this paradigm. Supported by DA-02073.

632.2

THE ROLE OF EXCITATORY AMINO ACID RECEPTORS AND CALCIUM POOLS IN THE REGULATION OF NITRIC OXIDE SYNTHASE (NOS) IN CORTICAL SLICES. S. Alagarsamy*, G. Lonart and K.M. Johnson. *Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77555-1031.*

Nitric oxide (NO) is a putative, calcium-dependent, diffusible second messenger, synthesized from arginine (ARG) by NOS in the brain. Cross-chopped cortical slices (450 X 450 μm) prepared from male Sprague-Dawley rats, were washed and incubated for 60 min in modified Krebs bicarbonate buffer containing 0.3mM CaCl. Gravity packed slices (50 μl) were added to 270 μl Krebs buffer containing ^{3}H-ARG. Additions of agonists/antagonists were made in a volume of 30 μl. Activity was measured by the conversion of ^{3}H-ARG to ^{3}H-citrulline. A five minute incubation with NMDA was found to stimulate NOS in a calcium-dependent and dose-dependent manner, with a maximal effect (160% of control) at 300μM with an approximate EC 50 of 50μM. The response to NMDA was stabilized by 10μM D-serine and was therefore added routinely in subsequent experiments. The response to 300μM NMDA was completely blocked by 10μM phencyclidine, 1.2mM Mg^{++}, 10μM CGS 19755 (NMDA receptor antagonists) and 10μM nitro-arginine (an irreversible inhibitor of NOS). Raising intracellular calcium levels by addition of 50mM K^+, 10μM ionomycin, 30μM A23187 stimulated NOS activity by 250-300% of control. However, 10mM caffeine was without effect, suggesting that different calcium pools may regulate NOS. 1mM 1S,3R-ACPD (a metabotropic glutamate receptor agonist) also stimulated NOS in a dose-dependent manner, with a maximal effect (150% of control) at 1mM. 100μM NMDA enhanced the 1S,3R-ACPD response, suggesting independent mechanisms for these two glutamate receptor agonists. Ongoing experiments are being carried out to verify this, as well as to establish the role of other amino acid and monoamine receptors in the regulation of this enzyme. Supported by DA-02073.

632.3

CHARACTERIZATION OF EXCITATORY AMINO ACID STIMULATED cGMP IN CEREBELLAR SLICES.
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Pharmacology, ICI Americas, Inc., Wilmington, DE 19897

In cerebellar slices from adult male Sprague-Dawley rats, kainate, AMPA, or NMDA increase cGMP levels. Noncompetitive NMDA receptor antagonists (MK-801), glycine antagonists (7-chlorokynurenic acid), or competitive NMDA antagonists (CPP) inhibit the NMDA stimulated cGMP. At concentrations that inhibit NMDA stimulated cGMP, NMDA receptor antagonists do not inhibit the increases in cGMP with kainate. The AMPA receptor antagonist NBQX inhibits kainate stimulated cGMP but not NMDA stimulated cGMP. The response to kainate is larger than that seen with AMPA, and AMPA inhibits the response to kainate, consistent with mediation through a single receptor. Treatment with the lectin concanavalin A can increase the magnitude of cGMP increases with AMPA, while not increasing the responses to kainate. Antagonists to voltage sensitive calcium channels inhibit the increase in cGMP with NMDA, but do not inhibit the binding of the noncompetitive NMDA antagonist [3H]TCP. The results are consistent with excitatory amino acid receptors regulating guanylate cyclase in a calcium dependent manner. Kainate and AMPA seem to interact with a single receptor in this preparation.

632.4

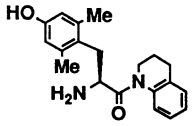
BOAA BLOCKS EXPRESSION BUT NOT INDUCTION OF LTP OF RAT MOTOR CORTEX. H. Son, N. Hori, M. Fejtl and D.O. Carpenter*. Wadsworth Laboratories and School of Public Health, Albany, NY 12201.

β-oxalylamino-L-alanine (BOAA) causes lathyrism, a human disease of upper motor neurons. BOAA is thought to be an excitatory amino acid agonist, and may act at the AMPA receptor. We have characterized long term potentiation (LTP) in rat motor cortex slices and evaluated the effects of bath perfusion of BOAA on the magnitude of the population evoked response on white matter stimulation, and on the induction and expression of LTP. Since NMDA receptors are required for LTP induction and AMPA receptors for LTP expression, our hypothesis is that if BOAA activates and desensitizes AMPA receptors on upper motor neurons, there should be a reduction in LTP expression without effect on induction. The peak of the population evoked response in motor cortex was reversibly reduced by 50% upon perfusion of BOAA at 10^{-5} M. Tetanic stimulation (TS 100 Hz, 2s) induced LTP of the population spike recorded in layer (L) II. This potentiation persisted for up to 5 hours and its induction was blocked by 10^{-5} M AP-5, when applied before TS. In L V however, the same TS induced LTP only when the normal Ringer solution was replaced with a solution containing low Mg^{2+} (0.5 mM), which should increase currents through NMDA channels. This may be due to the difference in the number of NMDA receptors within the cortical layers. The density of NMDA receptors is high in L II, whereas kainate receptors are more abundant in L V. When BOAA (10^{-5} M) was applied before, during and after TS, it did not block the induction of LTP, but did block the expression of LTP for as long as BOAA was present. Lower concentration of BOAA (10^{-6} M) had no effect on either induction or expression of LTP in L II. These data indicate that as at other sites, the induction of LTP in motor cortex requires NMDA receptor activation. BOAA blocks the expression but not the induction of LTP in a concentration-dependent manner, probably through desensitization of the AMPA receptor. Supported by NS23807.

632.5

TYROSYL AMIDES AS LIGANDS AT THE PHENCYCLIDINE RECEPTOR LOCATED ON THE NMDA-SENSITIVE GLUTAMATE SITE. B. S. Pitzele*, N. S. Chandrakumar, P. C. Contreras, S. E. Allen, M. Clare, M. A. Savage, D. L. Hammond, M. E. Nevins, S. A. Nash, P. M. Beardsley, D. Ragan, T. H. Lanthorn, Searle, 4901 Searle Pkwy, Skokie, IL 60077.

As part of an enkephalin analog program, a number of amides of (L)-2,6-dimethyltyrosine were synthesized and tested broadly. Since these compounds were superimposable on metazocine, testing included binding assays in sigma (^{3}H-PPP) and phencyclidine (^{3}H-TCP) assays. One compound, SC-48443, was found to bind potently, selectively, and competitively to the phencyclidine site. Structure-Activity data and physiological and behavioral results will be discussed.



SC-48443

632.6

INTERACTIONS OF 4-METHYLPYRAZOLE, AN ALCOHOL DEHYDROGENASE INHIBITOR, WITH SEVERAL LIGAND-GATED ION CHANNELS. M. Marchioro[1], Y. Aracava[1,2], E.S. Rocha[1,2], J. Yang*[2] & E.X. Albuquerque[1,2]. [1]Lab. Mol. Pharmacol., UFRJ, RJ, 21944 Brazil; [2]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

We have shown that 4-methylpyrazole (4-MP) interacts with the NMDA receptor of the cultured hippocampal neurons, activating single channel currents that resemble those evoked by NMDA (*Soc. Neurosci. Abs.*, 17: 1538, 1991). Applying the whole cell patch-clamp technique to cultured fetal hippocampal neurons from 18-to-20-day gestation rats, we further analyzed the interactions of 4-MP with the NMDA receptor and with the strychnine-sensitive glycine receptor. 4-MP (1 mM) blocks reversibly the strychnine-sensitive whole cell current activated by 50 μM glycine. Also, whole cell currents activated by 50 μM NMDA in the presence of 50 μM glycine and 100 μM strychnine were blocked by the same concentration of 4-MP. In addition, biochemical studies were carried out in rat brain synaptosomes. 4-MP alone caused a very small increase in the basal Ca^{2+} uptake into the synaptosomes, but, it clearly reduced the uptake induced by 100 μM NMDA. Moreover, at *Torpedo* nicotinic receptors (nAChR), in the absence of carbamylcholine, 4-MP enhanced the binding of perhydrohistrionicotoxin, an effect similar to that elicited by the nicotinic agonists. However, 4-MP did not displace α-bungarotoxin from its binding site, thus suggesting an allosteric activation of nAChR via a site distinct from that of ACh. These data indicated that 4-MP is recognized by different binding sites on several receptors, and that such effects could account for its therapeutic efficacy in the treatment of alcoholic intoxication.

Support: CNPq, FINEP, FINEP/UMAB Mol. Pharmacol. Training Program.

632.7

SENSITIVITY OF N-METHYL-D-ASPARTATE (NMDA) RECEPTORS TO 9-AMINOACRIDINES. M.E. Nelson[1], E.F.R. Pereira[1,2*], and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD, USA 21201; [2]Lab. Mol. Pharmacol. II, IBCCF, RJ, Brazil 21944.

The anticholinesterase, 1,2,3,4-tetrahydro-9-aminoacridine (THA; Tacrine), used with some success to treat Alzheimer's disease patients, has been shown to block NMDA-elicited whole cell and single channel currents with concentration and voltage dependence (*Neurosci. Soc. Abs.*, 15:1166, 1989). However, the concentrations of THA that blocked NMDA currents were greater than those shown to be clinically effective. We now characterize the effects of a series of alkylene bridged bis-9,9'-aminoacridines on NMDA receptors of cultured fetal rat hippocampal neurons. We have previously shown that these compounds are potent non-competitive antagonists of the muscle nicotinic receptor (*FASEB J.* 4:A470, 1990). Here, we tested 1,2-propyl- and 1,4-butyl-9,9'-aminoacridine (1,2-PAA and 1,4-BAA) as well as THA on NMDA-activated single channel currents in outside-out patches. The current-voltage relationship revealed that the single channels activated by NMDA (20 μM) had at least two conductance values. The 50-pS channels, being the most predominant, were chosen to test the effects of these drugs. At -80 mV, these currents had a mean open time (τ_{open}) of 1.88 $\pm$ 0.33 ms, and appeared as bursts whose time constants (τ_{burst}) were 1.42 $\pm$ 0.77 ms and 8.04 $\pm$ 1.88 ms. In the presence of either 50 μM THA, 10 μM 1,2-PAA, or 10 μM 1,4-BAA, τ_{open} was reduced to 0.54 $\pm$ 0.12, 0.78 $\pm$ 0.12, and 0.89 $\pm$ 0.16 ms, respectively. Additionally, only one type of burst was detected with time constants of 2.96 $\pm$ 1.45, 1.39 $\pm$ 0.31, and 1.14 $\pm$ 0.24 ms, respectively. These effects were voltage dependent, suggesting that these compounds act as open channel blockers at the NMDA receptor. Our findings indicate that the alkylene bridged bis-9,9'-aminoacridines are more potent blockers of NMDA receptors than the mono-9-aminoacridine, THA. (Support: US Army Med. Res. & Devel. Comm. Contr. DAMD17-88-C-8119; USPHS Grant NS25296)

632.8

EFFECTS OF DIPYRONE ON THE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR: A POSSIBLE MECHANISM OF ANALGESIA. A.M.N. Costa[1], Y. Aracava*[1,2], E.S. Rocha[1,2] & E.X. Albuquerque[1,2]. [1]Lab. Pharmacol. Mol. II, IBCCF, Rio de Janeiro, RJ, 21944 Brazil; [2]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

Dipyrone, a pyrazolone compound with analgesic, antipyretic and antiinflammatory properties, is reported to modulate nociceptive inputs to the central nervous system. Recently, single channel current studies on cultured rat hippocampal neurons, indicated that dipyrone activates the NMDA subtype of glutamate receptors (*Neurosci. Soc. Abs.*, 17:394, 1991). Dipyrone's actions were further evaluated at the single channel and whole cell currents recorded from cultured fetal hippocampal neurons from 18-to-20-day gestation rats. The conductances, τ_{closed}, and τ_{burst} of the single channel currents elicited by either NMDA or dipyrone were quite similar. In contrast to NMDA, increasing dipyrone concentration did not clearly enhance the channel opening frequency. Indeed, no discernable whole cell current was induced by dipyrone, up to 500 μM, either alone or in the presence of glycine (1-10 μM). However, dipyrone (50 μM-1 mM) blocked the whole cell currents induced by NMDA (10-50 μM) in the presence of glycine (1-50 μM) and strychnine (100 μM). In addition, dipyrone caused a small increase in the basal $^{45}Ca^{2+}$ uptake into the rat brain synaptosomes, whereas it reduced the Ca^{2+} flux induced by 100 μM NMDA. At the nicotinic receptors of *Torpedo* membranes, dipyrone did not displace α-bungarotoxin (αBGT), but it enhanced the perhydrohistrionicotoxin (H_{12}HTX) binding in the absence of nicotinic agonist. These results indicated that at the NMDA receptor, dipyrone is a weak agonist, acting as an antagonist when NMDA is present. Furthermore, the agonist-like increase of H_{12}HTX binding may arise from the interaction with a site distinct from that of αBGT. Our findings suggest implication of excitatory receptor systems in the central anti-nociceptive action of dipyrone.

Support: CNPq, FINEP, FINEP/UMAB Mol. Pharmacol. Training Program.

632.9

EXCITATORY AMINO ACIDS INCREASE INTRACELLULAR CALCIUM IN CULTURED PURKINJE NEURONS. D.L. Gruol*, K. L. Parsons and R. M. Krieger. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

The effect of selective excitatory amino acid receptor agonists on intracellular Ca^{2+} levels of cultured cerebellar Purkinje neurons was examined using digital imaging techniques and the Ca^{2+} sensitive dye fura-2. Several agonists were tested: (a) the ionotropic receptor agonists AMPA and domoate (Dom), (b) the metabotropic receptor agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD), and (c) quisqualate (Quis) which activates both the ionotropic and metabotropic receptors. The agonists were applied from drug pipettes by brief (200 msec) micropressure pulses. Ca^{2+} levels were measured at 3 sec intervals. In both the somatic and dendritic regions, Quis, AMPA and Dom evoked increases in intracellular Ca^{2+} consisting of an initial peak, occurring within ~ 4 to 11 sec from agonist application, and a prolonged recovery phase. The Dom response was smaller in amplitude and shorter duration than that of Quis or AMPA. Peak somatic levels were 133$\pm$9 nM (n=27), 136$\pm$9 nM (22) and 88$\pm$9 nM (16) for Quis, AMPA and Dom, respectively (rest = ~ 40 nM). High K+ saline applied in the same manner elicited Ca^{2+} responses 2-3 fold larger (411$\pm$5 nM, n=10). Peak dendritic levels were somewhat smaller for all agonists. With repetitive application, the second Ca^{2+} response was always smaller in amplitude than the first response. In contrast, ACPD evoked Ca^{2+} oscillations (5-50 nM) in ~ half of the neurons tested (n=20). The remaining showed small (5-10 nM), gradual Ca^{2+} increases or no response. These results suggest that changes in intracellular Ca^{2+} can play a prominent role in the ionotropic and metabotropic responses of the cultured Purkinje neurons. *Supported by AAA6665*

632.10

CALCIUM MEASUREMENTS AND ELECTROPHYSIOLOGY OF CHICK CEREBELLAR PURKINJE CELLS IN RESPONSE TO EXCITATORY AMINO ACIDS. Cliffer, K.D.*, Merritt, T., and Christensen, B.N. Department of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, TX 77555-0641.

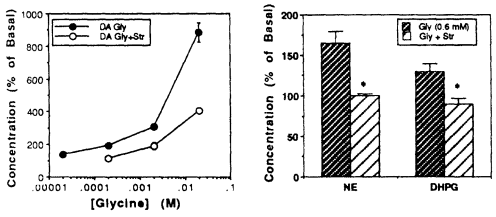
We tested cerebellar Purkinje cells acutely isolated from chick embryos (E16 to P2) for changes in intracellular calcium ($[Ca^{++}]_i$) in response to excitatory amino acids (EAAs) kainate (KA), (±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (ACPD), and n-methyl-D-aspartate (NMDA). Application of each of the amino acids elicited a rise in $[Ca^{++}]_i$ measured in cells loaded with fura-2. Electrophysiology of voltage-clamped cells (without fura) indicated that inward currents were elicited by KA (EC_{50}=118μM) or NMDA (EC_{50}=27μM), whereas no current was elicited by ACPD. The current in response to KA was inhibited by CNQX (a specific antagonist at ionotropic quisqualate receptors); that in response to NMDA was inhibited by AP5 (a specific antagonist at NMDA receptors). Application of caffeine elicited increases in $[Ca^{++}]_i$, indicating a potential contribution to EAA-induced increases in $[Ca^{++}]_i$ from caffeine-sensitive intracellular stores. The results indicate that EAAs can raise the $[Ca^{++}]_i$ in isolated chick Purkinje cells by acting via any of the three main types of EAA receptors, either metabotropic or ionotropic quisqualate receptors or NMDA receptors, and that release from intracellular stores may be induced.

Supported by NIH (NS-11255).

632.11

IN VIVO STIMULATORY EFFECT OF GLYCINE IN STRIATUM AND HIPPOCAMPUS. G. Yadid, K. Pacak, J.D. Harvey-White, I.J. Kopin* and D.S. Goldstein. Clinical Neuroscience Branch, NINDS, 10/5N214, Bethesda, MD 20892.

Glycine (Gly) is an inhibitory neurotransmitter in the spinal cord and medulla. Binding to specific Gly receptors increases transmembrane Cl^- conductance and hyperpolarizes neurons. Strychnine (Str) selectively antagonizes this effect. Gly's role in release of catecholamines in higher brain areas was examined by microdialysis in the striatum and the hippocampal CA3 region of freely-moving rats. After probe insertion, artificial CSF was infused for 20-24 h, and after 3 30-min basal microdialysate samples were obtained, the infusate was changed to CSF containing Gly (0.02-20mM) with or without Str (10 μM). Gly produced dose-related increases in release of dopamine (DA) and its metabolites in striatum and of norepinephrine (NE) and dihydroxyphenylglycol (DHPG) in hippocampus. Str blunted there increases in hippocampus (* = P<0.01) and shifted the dose-response curves to the right by about 1 log unit (Figures). The results indicate a stimulatory effect of Gly on catecholamine release in the striatum and hippocampus, via Str-sensitive receptors.



632.12

THE INTERACTION OF GLUTAMIC ACID AND ASPARTIC ACID WITH EXCITATORY AMINO ACID RECEPTORS IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM. I.O. Adeoshun, E.P. Mtui* Department of Physiological Sciences, Faculty of Health Sciences, Obafemi Awolowo University, Ile-Ife, Oshun State, Nigeria.

The number of amino acid molecules required to react with a receptor to cause its activation was investigated using a 6-barrel iontophoretic electrode assembly filled with glutamate, aspartate and DL-homocysteate.

The experiments were performed on rats anaesthetized with urethane (1.5 g/kg). Extracellular recordings were obtained from the thalamocortical relay cells of the ventrolateral thalamus identified by their response to stimulation of hindlimb nerve and the somatosensory cortex.

Hill plots of log (amino acid currents) vs log $(^y/_{(1-y)})$ where "y" is the ratio of the observed rate of firing to the maximum attainable rate were done on neurones tested with both glutamate and aspartate. The results yeilded average slopes of 2.8 ± 0.2 SE (glutamate, n=17) and 3.4 ± 0.6 SE (aspartate, n=11).

This study suggests that more than one amino acid molecule must react with a receptor to cause its activation.

632.13

TIME DEPENDENCE OF QUISQUALATE INDUCED SENSITIZATION OF HIPPOCAMPAL SLICES TO L-AP5. M.K. Schulte*, R.J. Roon and J.F. Koerner. Dept. of Biochemistry, University of MN, Minneapolis, MN 55455.

A brief exposure of pyramidal and granule cell neurons of the rat hippocampus to the excitatory amino acid agonist L-quisqualic acid (L-QUIS) results in a 30-100 fold increase in sensitivity to depolarization by L-AP4, L-AP5 and related phosphonates (QUIS-effect). We have reported that exposure of slices to L-homocysteine sulfinic acid (L-HCSA), L-serine-O-sulfate (L-SOS) and L-α-amino adipic acid (L-αAA) can "pre-block" induction of this effect and reverse it once it has been induced. The molecular mechanisms involved in induction, pre-blocking and reversal of the QUIS-effect are unknown. One hypothesis suggests that L-QUIS is taken up into cells as a necessary first step in induction. We have suggested that those compounds which pre-block or reverse the effect may also act internally. We have recently shown that active uptake of L-QUIS does occur in hippocampal slices. L-HCSA, L-α-AA and L-SOS are also taken up and treatment of slices with these compounds does not decrease the rate of L-QUIS uptake. The question remains, however, whether or not L-QUIS uptake is required for induction of the QUIS-effect. Using low levels of L-QUIS (<4 μM) we have investigated the time dependence of induction of the QUIS-effect. We observe that the degree of sensitivity to L-AP5 is proportional to the total time the slice is exposed to L-QUIS. The increases in sensitivity to 200 μM L-AP5 as a function of time for 3 different concentrations of L-QUIS are as follows: 2 μM - 3.9 % inhibition/min; 1 μM - 1.9 % inhibition/min; 0.5 μM - 0.8 % inhibition/min. We have also investigated the time dependence of pre-blocking and reversal and found that, like induction, they too are both concentration and time dependent. These results are consistent with a model for induction of the QUIS-effect which requires uptake of L-QUIS and in which compounds pre-block or reverse the effect by either competing with QUIS at an internal binding site or by producing an opposing action at another internal site.

OPIATE RECEPTOR LIGANDS

633.1

ETONITAZENE: A MU RECEPTOR SELECTIVE LIGAND. M.S. Moolten*, J.C. Chen, J.B. Fishman and K.R. Carlson. Pharmacology Dept., U. Massachusetts Medical Center, Worcester, MA 01655.

Etonitazene (ETZ) is a synthetic opiate approximately 1000 times more potent than morphine as an analgesic. Due to its high potency ETZ can be diluted to palatable but behaviorally active concentrations, making its use widespread in oral self-administration studies in rats. Although ETZ competes with high affinity for[^{3}H]-naloxone binding (Pert and Snyder, Mol. Pharm. 10:868-879 1974), its affinity for the various known opiate receptor sites has not been determined. Accordingly, we measured the ability of ETZ, fentanyl, and morphine to compete with [^{3}H]-DADLE (+DPDPE), [^{3}H]-DAGO (+DSLET), [^{3}H]-DPDPE, [^{3}H]-U-69,593, and [^{3}H]-SKF-10,047, in order to label mu_1, mu_2, delta, kappa, and sigma receptors, respectively, in membrane preparations from rat brain. ETZ had a 2500 fold greater affinity than morphine at the mu_1 site (Ki: ETZ=0.00041nM; fentanyl=0.007nM; morphine=1.11 nM). Affinities for the mu2 site were 10-fold greater for ETZ than fentanyl or morphine (Ki=0.4nM, 6.5nM, and 4.5nM, respectively). Affinities for delta, kappa and sigma receptors were similar for all three drugs.

These results demonstrate that ETZ is a high affinity ligand at mu receptor sites. The in vitro binding characteristics of ETZ are consistent with its activity as an analgesic and reinforcer of operant behavior

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633.2

AFFINITY LABELING HUMAN OPIOID RECEPTORS WITH A NOVEL IODINATED DERIVATIVE OF NalBzoH. K.M. Standifer,* G. Ciszewska, J. Cheng, J.Z. Ginos, J.L. Biedler, and G.W. Pasternak, Cotzias Laboratory of Neuro-Oncology and Laboratory of Cellular and Biochemical Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

The BE(2)-C clone of SK-N-BE(2) human neuroblastoma cell line contains high levels of opioid binding sites which were quite similar to levels in brain. Selective binding assays revealed the presence of mu, delta and $kappa_3$ receptors in a 2:1:2 ratio. FPLC of CHAPS-solubilized ^{3}H-NalBzoH affinity-labeled receptors from these cells over a Mono-Q column demonstrates the presence of at least 5 separable peaks of labeling. Substitutions on the benzoylhydrazone moiety of NalBzoH can still be potent opiates. The 3-^{125}I-4-amino- benzoylhydrazone of naloxone (^{125}I-NalAmBzoH) labels opioid binding sites on the BE(2)-C cells with high affinity (K_D 1 nM; B_{max} 190 fmol/mg protein). The sensitivity of the binding towards DADL, DAMGO and DPDPE implies the labeling of mu, delta and $kappa_3$ sites. U50,488H does not compete binding, confirming the absence of $kappa_1$ receptors in these membranes. Like ^{3}H-NalBzoH, ^{125}I-NalAmBzoH can covalently label binding sites following exposure to UV light. SDS PAGE of labeled membranes reveals two major and two minor specific bands between 50,000 and 100,000 daltons which are also competed by low nanomolar concentrations of opioids.

633.3

CHARACTERIZATION OF AN IODINATED ANALOGUE OF THE δ OPIOID ANTAGONIST TYR-TIC-PHE-PHE (TIPP) BY BINDING ASSAY. R. Horvath, M.A. Jarosinski, R.J. Knapp, I. Sora*, V.J. Hruby, and H.I. Yamamura, Univ. of Arizona Pharmacol. & Chemistry Dept's, Tucson, AZ 85724.

TIPP was shown to be a selective δ opioid receptor antagonist using the guinea pig ileum and mouse vas deferens bioassays (Schiller et al., 1992 FASEB abstract #3699). A radiolabeled analogue of this peptide would be valuable for studies of the δ opioid receptor since it is more selective than naltrindole which is the only available δ receptor ligand whose affinity is suitable for radioligand binding assay. One approach to radiolabeling is radioiodination of the Phe^3 ring using a diazonium intermediate (Sharma et al., J. Org. Chem. 56: 4981, 1991). The binding affinities of [4'-I-Phe^3]TIPP were tested at sites labeled by selective opioid receptor radioligands for this reason.

Radioligand	TIPP IC_{50} (nM ± SEM)	[4'-I-Phe^3]TIPP IC_{50} (nM ± SEM)
δ Selective		
naltrindole	29.9	14.4
pCl-DPDPE	6.2 ± 1.4	6.1 ± 0.3
μ Selective		
CTOP	154,600 ± 19,800	53,525 ± 9,375
κ Selective		
U-69593	160,900	not determined

The results show that iodination of the Phe^3 aromatic ring preserves most of the δ opioid receptor binding affinity and selectivity of the parent peptide. Radioiodinated TIPP is being prepared and its binding properties for δ opioid receptors studied. Supported in part by NIDA grants.

633.4

SELECTIVE LABELING AND RESOLUTION OF KAPPA2 RECEPTOR SUBTYPES BY [^{125}I]IOXY. O. Ni[1], J.S. Partilla[1], H. Xu[2], B.R. de Costa[2], K.C. Rice[2*] and R.B. Rothman[1]. [1]NIDA Addiction Research Center, PO Box 5180, Baltimore MD 21224. [2]LMC, NIDDK, NIH, Bethesda, MD 20892.

Previous work demonstrated that, using membranes depleted of μ and δ sites by pretreatment with BIT and FIT, [^{3}H]bremazocine labels two populations of κ_2 binding sites termed κ_{2a} and κ_{2b}, whose anatomical distribution and ligand-selectivity pattern differs from that of μ and δ binding sites. The present study was undertaken to characterize κ_2 binding sites with the novel antagonist ligand, [^{125}I]IOXY. [^{125}I]IOXY (SA=2200 Ci/mmol) was prepared by iodination of BD869, deacetylation, and purification by HPLC. Assays were conducted for 4 to 6 hr at 4^0 C in 50 mM TRIS-HCl, pH 7.4, and 10 mM NaCl. Binding surfaces generated with IOXY, (-)-(1*S*,2*S*)-U50,488, DAMGO and [Leu^5]enkephalin (LE) (274 data points) were fit to one- and two-site binding models. The two site model fit the data considerably better than a one site model ($p<0.001$). The two site fit resolved two sites, present at relative concentrations of 0.64 (κ_{2b}) and 0.44 (κ_{2a}). The Kd/Ki values (nM) for the drugs at the two sites were: IOXY (0.46, 0.73), (-)-(1*S*,2*S*)-U50,488 (3527, 5051), DAMGO (11.9, 1262) and LE (57.7, 12295). Structure-activity studies (in progress) indicate that the two sites labeled by [^{125}I]IOXY are the same as the two sites labeled by [^{3}H]bremazocine. Viewed collectively, these data provide further evidence for the existence of subtypes of the κ_2 binding site.

633.5

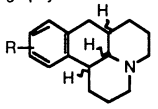
NALTRINDOLE BENZOFURAN IS NOT A δ-OPIOID SELECTIVE ANTAGONIST IN THE RAT SPINAL CORD. P.E. Stewart* & D.L. Hammond. Dept. of Anesthesia and Critical Care, The University of Chicago, Chicago, IL 60637.

Recent studies with the δ-selective opioid antagonist Naltrindole support the presence of δ-mediated antinociception in the spinal cord of the mouse and the rat. Naltrindole benzofuran (NTB) is also reported to be a δ opioid receptor antagonist in the mouse. This study examined the selectivity of NTB in the rat spinal cord using the tail-flick (TF) and hot plate (HP) tests. Sprague-Dawley rats were injected intrathecally (i.t.) with the δ-selective agonist cyclic[D-penicillamine^2, D-penicillamine^5] enkephalin (DPDPE) or the μ-selective agonist [D-Ala^2, $MePhe^4$, Gly-ol^5]enkephalin (DAMGO) and dose-response curves were generated in the presence or absence of NTB. DPDPE and DAMGO each dose-dependently increased TF and HP latencies. NTB (3-10 μg i.t.) dose-dependently antagonized the increase in TF and HP latencies produced by DPDPE. The antagonism did not appear to be competitive. The increase in TF and HP latencies produced by DAMGO was also antagonized by these same doses of NTB. The antagonism was not competitive. Similar results were obtained with s.c. administered NTB (10-30 mg/kg). These data suggest that NTB is not a selective δ-opioid antagonist in the rat spinal cord. Supported by PHS Grant DA 06736.

633.6

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF A PREVIOUSLY UNKNOWN CLASS OF RIGID TETRACYCLIC NEURORECEPTOR LIGANDS, OCTAHYDRONAPHTHOQUINOLIZINES. D.I. Schuster*, B. Cai, Y. Pan, G. Singh, M. Cornebise, A. Fariborzian, G. Stoupakis, J. Dewan and R. B. Murphy, Department of Chemistry and Center for Neural Science, New York University, New York, NY10003.

We have prepared a series of rigid tetracyclic analogues of 3-PPP, dopamine and other neuroreceptor ligands, namely octahydronaphthoquinolizines (OHNQs) (structure shown below). Each OHNQ exists as four diastereomers which are topologically very different. These compounds differ from conformationally flexible analogues which have activity at sigma receptor binding sites (SRBSs), D_2 and other neuroreceptor sites. Binding data show that OHNQs have modest to high affinities to SRBS in bovine cerebellar homogenates labeled with [^{3}H]-haloperidol or [^{3}H]DTG, and have low affinity toward D_2 receptors in bovine striatal preparations. Additional biological activity has been assessed under the NOVASCREEN program. The structure-activity relationships of these compounds toward different neuroreceptors will be presented. These compounds are synthesized in three steps from corresponding 2-tetralones; the key step involves reductive ring closure by high pressure hydrogenation with Raney nickel. All four diastereomers are formed, the structures of which have been assigned by X-ray crystallography and ^{13}C NMR spectroscopy .



633.7

KAPPA OPIOID AGONISTIC AND MU ANTAGONISTIC EFFECTS OF N-CYCLOPROPYLMETHYLNOR-14BETA(BROMOACETAMIDO)-7,8-DIHYDROMORPHINONE IN THE MOUSE. **Q. Jiang[*], A. Seyed-Mozaffari, [1]S. Archer and J.M. Bidlack.** Dept. of Pharmacology, Univ. of Rochester, Rochester, NY 14642 and [1]Dept. of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12181.

Opioid effects of N-cyclopropylmethylnor-14beta(bromoacetamido)-7,8-dihydromorphinone (N-CPM-H_2BAMO) were investigated in the mouse 55 °C warm-water tail flick and acetic acid writhing assays. All opioid agonists and antagonists were given by intracerebroventrical (*i.c.v.*) administration 10 min before antinociceptive tests. In the mouse writhing assay, N-CPM-H_2BAMO produced a dose- and time-dependent antinociception. The antinociceptive effect of N-CPM-H_2BAMO lasted up to 1 hr, with a maximal effect at 10 min after *i.c.v.* administration. The antinociceptive D_{50} value (and 95% C.L.) for *i.c.v.* N-CPM-H_2BAMO was 0.28 (0.19 - 0.39) nmol. This antinociceptive effect of N-CPM-H_2BAMO was antagonized by co-administration of nor-binaltorphimine (nor-BNI), the κ-selective antagonist, in a dose-dependent manner. In the mouse tail flick assay, N-CPM-H_2BAMO failed to produce antinociception. However, when co-administered with morphine, N-CPM-H_2BAMO produced a dose-dependent antagonism of morphine-induced antinociception, but not that of [D-$Pen^{2,5}$]enkephalin, the δ-selective agonist. Nor-BNI (0.3 nmol, which totally antagonized the agonistic effect of N-CPM-H_2BAMO in the writhing assay) failed to prevent the antagonistic effect of N-CPM-H_2BAMO on morphine-induced antinociception. Therefore, these data suggest that N-CPM-H_2BAMO acts at supraspinal κ opioid receptors to produce antinociception in the mouse writhing assay and acts at μ opioid receptors as a μ antagonist in the mouse tail flick assay. (Supported by Grants DA03742 and DA01674).

633.8

DESIGN OF POTENT AND SELECTIVE DYNORPHIN A-RELATED PEPTIDES DEVOID OF MOTOR EFFECTS. S. Lemaire*, R. Michelot, Y. Chen, I. Ibrahim, C. Lapierre and V.K. Shukla. Department of Pharmacology, University of Ottawa, 451 Smyth Rd., Ottawa, Ont. K1H 8M5.

Dynorphin A-(1-13)-Tyr-Leu-Phe-Asn-Gly-Pro, (Dyn Ia), was previously shown to be a highly potent and selective κ opioid peptide. Four analogues of Dyn Ia have been synthesized by the solid-phase procedure: #1, [Ψ-CH_2,$NH^{6,7}$]Dyn Ia; #2. [Ψ-CH_2-$NH^{6,7}$,D-Leu^8]Dyn Ia; #3. [$MeTyr^1$, Ψ-CH_2-$NH^{6,7}$]Dyn Ia and #4. [$MeTyr^1$,Ψ-CH_2-$NH^{6,7}$, D-Leu^8]Dyn Ia. The peptides were purified and compared with Dyn Ia for their ability to compete with the binding of selective κ, μ and δ opioid ligands and to display antinociceptive activity in an acetic acid induced writhing test. All synthetic compounds displayed a high affinity for the κ receptor (Ki against [^{3}H]EKC binding:0.5-1.8 nM) but compounds #3 and 4 were more selective. Compound #2 possessed the highest antinociceptive activity (AD_{50}:1.57 nmol/mouse) and the lowest motor effect (convulsion; CD_{50}:15.4 nmol/mouse). Its κ selectivity ratio (1:5.7:12.9;κ:μ:δ) was comparable to that of Dyn A-(1-13) (1:4.5:40). Compound #2 is thus a potent and selective Dyn A related peptide devoid of motor effect at analgesic doses. Supported by MRC.

633.9

CHRONIC INTRACEREBROVENTRICULAR INFUSION OF THE ANTI-OPIOID PEPTIDE, Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2 (NPFF), DOWN-REGULATES MU OPIOID BINDING SITES IN RAT BRAIN. R.B. Rothman*[1], L.S. Brady[2], H. Xu[1], and J.B. Long[3]. [1]NIDA Addiction Research Center, Baltimore, MD 21224. [2]LNP, NIMH, Bethesda, MD 20892. [3]Dept. of Med. Neurosci., WRAIR, Washington, DC 20307-5100.

NPFF, an endogenous mammalian anti-opioid peptide, has been shown by other laboratories to attenuate 1) the acute antinociceptive effects of morphine, 2) the development of morphine tolerance, and 3) naloxone-induced withdrawal in morphine dependent rats. The present study determined the effect of chronic NPFF on mu opioid receptors, and mRNA for the endogenous opioids dynorphin and enkephalin. Rats received i.c.v. infusions of either saline or NPFF (5 μg/hr) for 13 days via ALZET 2002 osmotic minipumps. Homogenate binding studies, which used whole brain membranes, demonstrated that NPFF decreased the Bmax of mu binding sites (labeled by [^{3}H]DAMGO) from 262±12 to 192±12 fmol/mg protein, and increased the Kd from 1.1 nM to 2.3 nM. Quantitative receptor autoradiography and in situ hybridization experiments were conducted with sections collected at the level of the striatum. The density of mu opioid binding sites labeled by [^{3}H]DAMGO was decreased in all brain areas measured except the corpus callosum, and there was no change in the mRNA for dynorphin or enkephalin in caudate, the N. accumbens or the ventral pallidum. Studies in progress are examining the effect of chronic i.c.v. NPFF on the acute antinociceptive effects of morphine, as well as the development of morphine tolerance and dependence.

633.10

TYR-MIF-1, TYR-W-MIF-1, AND HEMORPHIN HAVE OPIATE AGONIST AS WELL AS ANTAGONIST PROPERTIES IN THE GUINEA PIG ILEUM. J.E. Zadina*, A. J. Kastin, D. Kersh, J. Erchegyi, L.-J. Ge.
VA Med. Ctr. and Tulane Univ. Sch. of Med., New Orleans, LA 70146

Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) is a brain peptide known for its ability to modulate, and particularly antagonize, opiate effects. We have recently isolated a structurally related peptide, Tyr-W-MIF-1 (Tyr-Pro-Trp-Gly-NH_2) from brain. This peptide also has structural similarity to hemorphin (Tyr-Pro-Trp-Thr), a peptide derived from blood digests known (and named) for its opiate agonist activity. These peptides and some analogs and fragments were tested for activity in the guinea pig ileum and opiate receptor binding assays. Each of the peptides showed dose-dependent, opiate agonist activity (inhibition of contraction), that was reversed by naloxone and the mu antagonist CTOP but not by the kappa antagonist nor-BNI. The rank order of agonist potency in the ileum assay and in binding to mu opiate receptors was Tyr-W-MIF-1 > hemorphin > Tyr-MIF-1. In addition, however, the peptides exhibited antagonist activity that was best detected in ileal preparations from animals made tolerant to morphine or treated with the opiate receptor alkylating agent β-CNA to reduce opiate receptor reserve. The peptides reduced the inhibition of contractions induced by morphine and the mu-selective agonist DAMGO. Hemorphin and the free acid of Tyr-W-MIF-1 were particularly potent at inhibiting the actions of opiate agonists. MIF-1 (Pro-Leu-Gly-NH_2) and morphiceptin were ineffective at antagonizing mu agonist activity. The results show that endogenous brain peptides (Tyr-MIF-1 and Tyr-W-MIF-1) and hemorphin can act as both agonists and antagonists. The antagonist action is best observed in the ileum with reduced receptor reserve or from a morphine tolerant animal. The results support the idea that increased sensitivity to the opiate antagonist actions of endogenous peptides occurs in the tolerant state.

633.11

DEVELOPMENT AND VALIDATION OF A FUNCTIONAL ASSAY *IN VITRO* FOR OPIOID ACTION. L.N. Williamson, D.J. Mayer, M.A. Savage*, H. Bakopoulos, B.S. Pitzele, D.W. Hansen, N.S. Chandradumar, and M. Reichman. G.D. Searle, Skokie, IL 60077

Our goal was to develop an automated, high-capacity functional assay *in vitro* that would report on the intrinsic efficacy of opioids. To this end, we characterized the inhibitory effects of opioids on prostaglandin E_1 (PGE_1)-stimulated formation of cyclic adenosine monophosphate (cAMP) in NG108-15 neuroblastoma x glioma (NG) cells. The NG cells were pre-incubated with diverse structural classes of opioids for 15 minutes in assay buffer containing IBMX, a phosphodiesterase inhibitor. We then measured the activities of the opioids for inhibiting prostaglandin E_1-stimulated cAMP formation. The cAMP was measured by RIA methods involving a charcoal-based separation, or the newer scintillation proximity assay. Validation of the assay was accomplished by comparing the EC_{50} values obtained in NG cells to those obtained in the standard *in vitro* assay; opioid-elicited inhibition of electrically-stimulated contractions in mouse *vas deferens*. The results from our automated, high capacity, cell-based assay agree well with the more traditional, labor-intensive, animal-based assay. We are applying these methods to other bioassays.

633.12

EXPRESSION OF c-fos & PREPROENKEPHALIN (PPenk) mRNA IN HUMAN NEUROBLASTOMA SK-N-BE(2) CELLS. J. Cheng*, Y. Zhu, C.E. Inturrisi, K.M. Standifer, J.L. Biedler, G.W. Pasternak. The Cotzias Laboratory of Neuro-Oncology & Laboratory of Cellular and Biochemical Genetics, Memorial Sloan-Kettering Cancer Center and Departments of Neurology & Neuroscience and Pharmacology, Cornell University Medical College, New York, NY 10021

The human neuroblastoma clonal cell line BE(2)-C from the SK-N-BE(2) line expresses functional mu, $kappa_3$ and delta opiate receptors. Binding studies reveal total binding levels similar to brain with a mu:$kappa_3$:delta distribution of 2:2:1. In addition, selective agonists for each receptor subclass inhibit forskolin-stimulated cAMP accumulation. We now demonstrate that this cell line also expresses mRNA for opioid peptides and c-fos. Using solution hybridization, we quantitated the levels of PPenk mRNA and c-fos mRNA in BE(2)-C cells and their regulation by forskolin. The basal level of PPenk mRNA equivalents is 0.14 ± 0.04 pg/μg RNA using a ^{32}P-labeled riboprobe transcribed from a rat cDNA. Preprodynorphin and c-fos mRNA's are also measurable in these cells. Cells exposed to forskolin (100 μM) for 2 hr showed a 4-fold increase in c-fos mRNA levels. This elevation returns to basal levels by 6 hr. This same forskolin treatment also increases PPenk mRNA levels, but far more slowly than c-fos. Thus, BE(2)-C cells contain multiple classes of opioid receptors as well as mRNA's of several opioid peptides and the c-fos proto-oncogene and should prove useful for the study of opioids and their receptors.

633.13

DIFFERENTIAL EFFECT OF CHRONIC MORPHINE TREATMENT ON THE EXPRESSION OF G-PROTEIN AND ENDOGENOUS OPIOID PEPTIDES IN ADULT RAT BRAIN. R.Basheer and A. Tempel*. Lab of Molecular Pharmacology, Hillside Hospital, Long Island Jewish Medical Ctr./Albert Einstein College of Medicine, Glen Oaks, NY 11004.

The molecular mechanism involved in the development of opiate tolerance and dependence is still unclear. The majority of studies have failed to show any correlative alterations in receptor density in adult rats following chronic morphine treatment in vivo. Hence the postreceptor events in the opioid system are gaining importance in elucidating mechanisms involved in opioid tolerance and dependence. The opioid receptor subtypes are linked to G-proteins. Involvement of both pertussis toxin sensitive G subunits (G_i and G_o) and the cholera toxin sensitive G_s subunit in mediating opioid effects has been reported by others. The mechanism of opiate tolerance and dependence may involve a balance between inhibitory and stimulatory G-proteins as well as interactions with different second messenger systems. In order to study the molecular changes in the Central Nervous System following morphine treatment in adult rats the levels of expression of G_s mRNA were measured in hypothalamus, frontal cortex, striatum and hippocampus. In addition, the mRNA levels for endogenous opioid peptides, preproenkephalin (PPE) and dynorphin (Dyn) were also measured. G_s message showed a 97% increase ($p<.02$) in hypothalamus whereas Dyn message levels decreased by 22% ($p<.01$) in hypothalamus following morphine treatment. PPE message levels were decreased by 55% ($p<.01$) in striatum, which is in agreement with earlier reports. These results suggest that chronic morphine treatment differentially alters the expression of G-protein and endogenous opioids in different brain regions (supported by NIDA grant DA-05440).

634.1

TOPOGRAPHICAL ORGANIZATION OF MIDBRAIN DOPAMINE AFFERENTS TO THE MEDIAL PREFRONTAL CORTEX OF THE RAT. A. Bourdelais* and A. Y. Deutch. Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06508, and VA Medical Center, West Haven, CT 06516.

We have previously demonstrated that the responsiveness of the prefrontal cortical dopamine (DA) innervation to mild stress differs across the different cytoarchitectonic fields of the medial prefrontal cortex (PFC). This may be due to either distinct populations of midbrain DA neurons projecting to different parts of the PFC, or alternatively to changes restricted to the axon terminal region (presynaptic regulation of DA release). We therefore examined the distribution of midbrain DA neurons projecting to the PFC by placing small iontophoretic deposits on fluorogold (FG) in the PFC, and dual-labelling for FG and tyrosine hydroxylase (TH).

Retrogradely-labelled (FG-positive) cells were most frequently seen in the rostral VTA (supramammillary region). FG labelled neurons were next most frequently observed in the nuc. parabrachialis pigmentosus (PBP), followed by the caudal linear (CL) and then the rostral linear (RL) nuclei. Only rarely were cells seen in the interfascicularis and paranigral nuclei.

More medial injections of the PFC (i.e., infralimbic and prelimbic) resulted in more double-labelled (FG+TH) cells in the medial VTA (RL and CL). More laterally placed injections resulted in more double-labelled cells in more lateral portions of the VTA (PBP). There also appeared to be a rough anteroposterior gradient. However, our preliminary data suggest only a crude topographical organization, one that may not be consistent with separate populations of midbrain DA neurons innervating different portions of the PFC. Supported by MH-45124 and the West Haven, CT VA.

634.2

IN VIVO SPECT COMPARISON OF THREE HIGH AFFINITY RADIOLIGANDS FOR DOPAMINE D2 RECEPTOR. M.S. Al-Tikriti*, R.M. Baldwin, Y. Zea-Ponce, E. Sybirska, S.S. Zoghbi, M. Laruelle, R.T. Malison, H.F. Kung, R.M. Kessler, T. de Paulis, I. Nakatsuka, H.Saji, E.O.Smith D.S.Charney, P.B. Hoffer, R. B. Innis. Yale University/VA Medical Center,West Haven, CT 06516, University of Pennsylvania, Philadelphia, PA, Vanderbilt University, Nashville, TN, Sumitomo Chemical Co., Osaka, Japan and Kyoto University, Japan.

The regional distribution and pharmacological specificity of iodobenzofuran (IBF), epidepride (EPID) and 2'-iodospiperone (2'-ISP) were measured with serial SPECT scans in non-human primates. A series of 13 IBF, 6 EPID and 8 2'-ISP studies were conducted on 6 ovariectomized female baboons (9-13 kg) under isoflurane anesthesia. Animals were injected with 5-16 mCi ^{123}I-labeled agent i.v. and scanned in the Strichman 810X Imager. All three ligands showed specific striatal uptake which reached peak at 37, 140 and 55 min postinjection (PI) for IBF, EPID and 2'-ISP, respectively, with striatal ratios to nonspecific (cortex or cerebellum) 3, 12 and 2.2, these ratios increased to 10, 19, and 3.4 after 4 hr. *Ex vivo* autoradiographic studies in one animal for each ligand sacrificed at 30, 120 and 80 min PI demonstrated the highest uptake in caudate and putamen, and striatum:cortex ratios were 6, 10 and 5, respectively. From the peak striatal activities, washout rates for those ligands were 32, 8 and 15%/hr. IBF and 2'-ISP plateaued for 30-50 min while EPID for 2 hr, followed by a steady but gradual washout over a period of 3 hr. Raclopride (1 mg/kg) produced complete displacement from striatum of all agents with washout rates increased to 126%, 61% and 38%/hr. Ritanserin and d-amphetamine (1 mg/kg) had no significant effect on striatal washout rates.

634.3

DOPAMINE AUTORECEPTOR ANTAGONIST AJ76 DISRUPTS AUDITORY SENSORY GATING IN RATS. K.E. Stevens*, K.R. Harris, J.C. Lehman, S.L. Stryker and G.M. Rose. Department of Pharmacology, UCHSC and Medical Research, VAMC, Denver, CO 80262

The midlatency auditory evoked potential recorded to the second of a closely-spaced (0.5 sec interval) pair of clicks is reduced as compared to the first, in unmedicated rats and in normal humans. Administration of amphetamine to rats or humans disrupts this "gating", producing a schizophrenia-like pattern of response. Similar alterations in gating have been elicited by elevation of endogenous norepinephrine levels through administration of yohimbine, a presynaptic alpha$_2$-selective adrenergic antagonist. To determine if elevation of endogenous dopamine levels thorough blockade of the autoreceptor would also produce alterations in sensory gating, the dopamine receptor antagonist AJ76 was administered at a dose thought to act only presynaptically (3.5 mg/kg, sc). AJ76 was administered to rats with chronic indwelling recording electrodes located on the brain surface at "vertex". At 20 and 45 min post injection, AJ76-treated rats showed a loss of sensory gating as compared to unmedicated trials. This was due to an increase in the amplitude of the N40 wave in response to the second (test) stimulus with no change in the amplitude of the response to the first (condition) stimulus. There were no significant changes observed at 65 min post injection. Thus, increases in endogenous dopamine levels can impair sensory gating in rats. (Supported by P50 MH44212-03.)

634.4

ANTAGONISM OF COCAINE, AMPHETAMINE AND OTHER DOPAMINERGIC STIMULANTS BY THE PREFERENTIAL DOPAMINE AUTORECEPTOR ANTAGONIST (+)-UH232 IN THE INTRACRANIAL SELF-STIMULATION AND LOCOMOTOR ACTIVITY MODELS. Torben Kling-Petersen*, Elisabeth Ljung and Kjell Svensson. Dept of Pharmacology, Univ. of Göteborg, POB 330 31, 400 33 Göteborg, SWEDEN

The preferential dopamine autoreceptor antagonist (+)-UH232, exerts a weak stimulatory effect when tested in locomotor activity experiments using habituated animals. However, (+)-UH232 also blocks d-amphetamine, cocaine and apomorphine induced hyperactivity. Thus, the behavioral effects of (+)-UH232 appears to be dependent upon the baseline activity of the animal.

Various behavioral models have been utilized in order to investigate the possible positive reinforcing properties of (+)-UH232 . In the intracranial self-stimulation (ICSS) paradigm in the rat, bipolar electrodes aimed at the median forebrain bundle delivers mono-phasic, cathodal current of varying intensities. By establishing a threshold value (called EC50) using a rate/intensity-model, results from different experiments can be statistically compared. (+)-UH232 produced a weak inhibitory effect over a wide dose range (1-16 mg/kg s.c.). Cocaine (1-16 mg/kg i.p.) and d-amphetamine (0.25-1 mg/kg s.c.) on the other hand produced a clear lowering of the EC50 values, indicating stimulatory effects. (+)-UH232 (16 mg/kg s.c.) blocked the stimulatory effects of both cocaine (4-16 mg/kg) and d-amphetamine (0.25-4 mg/kg). It is possible that there is a qualitative difference in the antagonism of these two dopamine stimulants.

We are presently investigating the interactions of (+)-UH232 with the DA reuptake inhibitor GBR-12909 and DA D2 receptor agonist quinpirole in the ICSS paradigm and locomotor activity model. (Supported by The Upjohn Company, Kalamazoo, MI.)

634.5

DESTRUCTION OF MESOLIMBIC DOPAMINE NEURONS BY INTRA-VTA INJECTIONS OF 6-OHDA DOES NOT BLOCK THE LOCOMOTOR ACTIVATING EFFECTS OF NICOTINE.
J-P. Tassin, P. Vézina, T. Jay*, J. Glowinski and D. Hervé. Chaire de Neuropharmacologie, INSERM U.114, Collège de France, Paris, France and Loeb Research Institute, Ottawa Civic Hospital, Ottawa, Canada.

Recently we reported that while acute systemic injections of nicotine substantially increase dopamine (DA) utilization in the nucleus accumbens (N. Acc.), repeating these injections abolishes this effect even though this drug's locomotor effects become enhanced (Vézina et al., J.P.E.T., in press). This suggests that nicotine may elicit at least some of its locomotor effects via non mesolimbic DA systems. In the present study, this possibility was investigated by assessing the locomotor response to nicotine following destruction of the mesolimbic DA system. Rats received bilateral injections of 6-OHDA (4µg/1µl/side) into the ventral tegmental area (VTA). Four weeks later, the locomotor response of these animals to (-)-nicotine bitartrate (0.4 mg/kg, base, s.c.) was compared to that of non-lesioned controls. Depletions of N.Acc. DA of up to 100 % of control concentrations did not block the acute locomotor response to nicotine. Such depletions also did not prevent the progressire enhancement of nicotine's locomotor effects when injections were repeated daily for nine days. These results, together with others demonstrating that such 6-OHDA-induced depletions of N.Acc. DA completely block the locomotor response to amphetamine up to 33 days post-lesion, extend our earlier findings with N.Acc. DA utilization and suggest that mesolimbic DA is not necessary for the elicitation of locomotor activation by nicotine.

634.6

CENTRAL CORTICOSTEROID RECEPTORS AND STRESS RESPONSIVENESS IN TWO PHARMACOGENETICALLY SELECTED RAT LINES. J.A.M. van Eekelen[1], N.Y. Rots[1,2], E.R. de Kloet[1] and A.R. Cools*[2]. [1]Cent.Bio-Pharm. Sci., Leiden Univ., 2300 RA Leiden; [2]Dept. Pharmacol., Nijmegen Univ., 6500 HB Nijmegen, Netherlands.

Two rat lines derived from a normal outbred Wistar population were selected on their susceptibility to the dopamine agonist apomorphine. The drug susceptible (apo-sus) rats are characterized by a fleeing response following defeat, whereas the apo-unsus rats freeze. This difference in behavior suggests a different responsiveness of apo-sus and apo-unsus rats to stress. The hypothalamic-pituitary adrenal (HPA) activity in response to a conditioned emotional stimulus (CER) was determined by blood collection from chronically cannulated rats over 4 hours following CER. In Apo-sus rats, CER-induced plasma ACTH and basal plasma ACTH were elevated. However, this rat line did not show icreased stress-induced or basal plasma B levels. Apo-sus rats also displayed a more pronounced neuroendocrine response to HPA-stimulation with exogenous CRF. Apo-sus and Apo-unsus rats show differences in central MR and GR as studied by in situ hybridization and ligand binding assay. Thus, pharmacogenetically selected rats with different functional dopaminergic activity in the brain reveal enhanced reactivity of the corticosteroid controlled HPA-system.

634.7

NOVELTY OR FAMILIARITY DIFFERENTIALLY AFFECTS IN VIVO STRIATAL/SEPTAL D2 RECEPTOR BINDING IN ISOLATED NEONATAL RATS. K.M.WARD & P.KEHOE * Trinity College, Psychology, Hartford, CT 06106.

Infants separated from their caretaker emit behavioral and physiological responses associated with stress/arousal. Neurochemical mediation of these responses may be affected by the novelty of the isolation environment. Dopamine has been found to increase in young monkeys and guinea pig pups isolated in a novel environment. The present study on neonatal rats examines dopamine receptor occupation following isolation in environments with the degree of novelty varied. In vivo septal /striatal D2 receptor binding was done in Day 10 rats taken from the nest or following 5 min of isolation in a small cup with cage bedding or in a novel 20 x 20cm chamber. Twenty min following an ip injection of 3H-raclopride, a D2 antagonist, the pups were decapitated and brains dissected for striatal, septal and cere-bellar tissue. Compared to nest controls, pups in the cup of bedding demonstrated significantly more septal 3H-raclopride binding while those in the novel chamber had less. These data suggest that isolation in a more familiar environment led to less septal D2 receptor occupation in contrast to novelty which produced the most presumably by the release of dopamine at these terminals.

634.8

METABOLIC MAPPING OF THE "PRIMING" PHENOMENON IN RATS BEARING UNILATERAL 6-HYDROXYDOPAMINE LESION OF THE NIGROSTRIATAL PATHWAY. F.E. Pontieri*, M. Morelli°, R. Terenzi and G. Di Chiara°. Dept. Neurosciences University "La Sapienza", Rome and °Dept. Toxicology, University of Cagliari (Italy).

In rats bearing unilateral 6-hydroxydopamine (6-OHDA) lesion of the dopaminergic nigrostriatal neurons, "priming" with a single administration of the D_2 agonist, LY-171555 (0.2 mg/kg, s.c.), strongly potentiates the contralateral turning induced by the D_1 agonist, SKF-38393 (2.5 mg/kg, s.c.). The 2[^{14}C]-deoxyglucose (2DG) method for the measurement of local cerebral glucose utilization (LCGU) was applied to identify the neural substrates of this phenomenon. Lesioned animals received two injections, three days apart, as follows: Group 1: saline/saline. Group 2: LY-171555/saline. Group 3: saline/SKF-38393. Group 4: LY-171555/SKF-38393. The 2DG experimental procedure was begun 20 minutes after the second administration of drug or vehicle. Unilateral 6-OHDA lesion per-se (Sal-Sal) produced increases in LCGU in the globus pallidus (GP) and in the lateral habenula (LH) of the lesioned hemisphere. Rates of glucose metabolism in LY-Sal treated rats were similar to those measured in the vehicle-treated animals. Single administration of SKF-38393 (Sal-SKF) abolished the lesion-induced metabolic asymmetry in the LH but did not have any effect on the GP; furthermore, it increased LCGU in the substantia nigra pars reticulata (SNr) of the lesioned side. LY-SKF treatment produced marked metabolic asymmetries by increasing LCGU in the SNr and entopeduncular nucleus (EPN), and decreasing it in the LH of the lesioned side. These changes were significant also when compared to the corresponding values of the other experimental groups. Again, LY-SKF failed to modify the lesion-induced metabolic asymmetry in the GP. These results indicate that the behavioral changes observed after SKF-38393 in primed rats are associated with a marked enhancement of the metabolic response in the SNr, EPN and LH and suggest that priming exerts a facilitatory influence on the ability of D_1 receptors to stimulate the activity of the striato-nigral and striato-entopeduncular pathways.

634.9

INTRAPERITONEAL SALINE INJECTION DECREASES DARPP-32 (DOPAMINE- AND CYCLIC AMP-REGULATED PHOSPHOPROTEIN OF M_R=32,000) mRNA LEVELS IN SPECIFIC REGIONS OF MOUSE BRAIN. R. M. Lewis* and R. G. Perez. Dept. of NACS, University of Pittsburgh, Pittsburgh, PA 15261.

DARPP-32 is a cytosolic phosphoprotein phosphatase inhibitor that is phosphorylated by cyclic AMP-dependent protein kinase in response to dopamine. Phosphorylated DARPP-32 down-regulates Na^+,K^+-ATPase. Calcineurin dephosphorylates DARPP-32 in response to NMDA. DARPP-32 is enriched in regions of the brain that have D1 dopamine receptors. Neither expression of DARPP-32 during development of the brain, nor maintenance of DARPP-32 levels in the adult appears to require dopamine. We tested whether excess dopamine could down-regulate the expression of DARPP-32 mRNA. Mice were injected daily with 0.5 ml of 0.9% NaCl (i.p.), or 100 or 200 mg/kg L-DOPA in 0.5 ml of 0.9% NaCl (i.p., 30' after 50 mg/kg Ro 4-4602 in 0.5 ml of 0.9% NaCl, i.p.) for one to five days. Uninjected mice served as controls. Mice were sacrificed six hours after the last injection. Levels of DARPP-32 mRNA were assessed by *in situ* hybridization in sagittal sections of brain. The amount of DARPP-32 mRNA in layer VI of cortex, piriform cortex, and anterior olfactory nucleus of mice injected only with saline was lower than the amount of DARPP-32 mRNA in uninjected mice. No other regions of the brain were affected. Injection of 100 or 200 mg/kg L-DOPA in saline had the same effect as saline alone. We are testing each parameter of the injection protocol to determine the cause of this decrease in DARPP-32 mRNA levels in specific brain regions.

634.10

EVIDENCE FOR COUPLING OF RAT SUBSTANTIA NIGRA (SN) DOPAMINE D1 RECEPTORS TO PHOSPHOINOSITOL (PI) HYDROLYSIS. L.P. Martin* and B.L. Waszczak. Pharmacology Section, Northeastern University, Boston, MA 02115.

We have previously demonstrated that iontophoresis of the D1 agonists SKF 38393 (SKF) and A68930 produce increases in the firing rates of SN pars reticulata (SNpr) neurons. This excitatory response was lost after striatal lesions or injections of the receptor inactivator EEDQ into the SN, suggesting that the effect of SKF on SNpr neurons is mediated through stimulation of D1 receptors on impinging striatonigral terminals. Further studies revealed that adenylate cyclase might not mediate the D1 agonist effect. Specifically, iontophoresis of cAMP analogues did not mimic the agonist effect, whereas intranigral injection of pertussis toxin (PT), an inactivator of Gi and Go proteins, completely abolished the excitatory response to SKF. An ADP ribosylation assay on SN punches taken from PT-treated rats revealed a 64% decrease in the ability of nigral G-proteins to incorporate ^{32}P-NAD, confirming success of the PT injections. These results were surprising since D1 receptors have traditionally been thought to act through Gs to stimulate adenylate cyclase. Consequently, we were prompted to investigate whether PI hydrolysis might provide a second messenger coupling mechanism for the nigral D1 receptor. For these experiments, the SN and striata from 5 rats were pooled, slices were prepared with a tissue chopper, and then incubated in 1.7 μM ^{3}H-myo-inositol for 1 hour. Aliquots of packed slices were incubated for 30 min with or without agonist (500 μM SKF). ^{3}H-Inositol phosphates (^{3}H-IPs) were quantified by anion exchange HPLC. Preliminary results revealed a 4-fold higher basal level of ^{3}H-IPs in SN than striatum (per mg protein). Moreover, SKF appears to stimulate production of total ^{3}H-IPs in both nigra and striatum to a similar extent (56 and 71%, respectively; n=4). These findings, while preliminary, support the possibility that SN receptors involved in the excitatory effect of D1 agonists on SNpr neurons may be coupled to the PI second messenger pathway. (Supported by NS 23541)

634.11

A RAPID, VISUAL, FUNCTIONAL ASSAY FOR THE QUANTITATION OF LIGAND-MEDIATED STIMULATION OF G_i-LINKED RECEPTORS. M. N. Potenza* and M. R. Lerner. Depts. of Cell Bio., Int. Med., & Pharm., & HHMI, Yale Univ. Sch. of Med., New Haven, CT 06510.

The pigment containing cells of certain lower invertebrates have the ability to alter their coloration in response to specific environmental, neuronal, and hormonal stimuli. We have cultured a clonal cell line of melanophores from *Xenopus laevis*. These cells maintain the ability to translocate their melanosomes in a tightly controlled manner. Specifically, decreases in cAMP levels, produced by exposure to low doses of melatonin, induce pigment aggregation, while increases, produced by exposure to low levels of light or doses of MSH, induce pigment dispersion. Employing a cDNA clone encoding a typical G_i-coupled receptor (a human Dopamine $_2$ receptor (D_2R)), we can efficiently introduce and express the D_2R in the melanophores. D_2R-selective-agonist-induced stimulation of the D_2R-transfected, but not wild-type, melanophores produces dose-dependent pigment aggregation, which is blocked in a dose-dependent manner by D_2R-selective agonists. As the pigment translocation process occurs rapidly (essentially complete within 30 minutes), detailed and reproducible dose-response curves for many distinct agonists and/or antagonists can be determined in less than 30 minutes using a standard microtiter plate and plate reader. This system has the potential to rapidly assess drugs for their properties as agonists or antagonists upon any specific G_i-coupled receptor for which a cDNA clone is available. Moreover, the system should allow for the classification of "orphan" G_i-coupled receptors, and for the expression cloning of G_i-coupled receptors (see McClintock, *et. al.*).

634.12

COUPLING OF D_3-LIKE RECEPTORS TO GUANINE NUCLEOTIDE BINDING PROTEINS: A QUANTITATIVE AUTORADIOGRAPHY STUDY M.L. Coco and C.D. Kilts. Depts. of Pharmacology and Psychiatry, Duke Univ. Med. Ctr. Durham, NC 27710.

The recently cloned D_3 receptor has been shown to bind D_2 receptor ligands when expressed in CHO or COS-7 cells (Sokoloff et al., 1990), with the agonist quinpirole demonstrating a 113-fold greater affinity for D_3 vs. D_2 receptors. Additionally, both Sokoloff et al. and Gehlert et al. (1992) report that D_3 receptors, unlike D_1 or D_2, do not appear to shift to a lower agonist affinity state in the presence of GTP or analogues such as Gpp(NH)p. Previously, we sought to examine the D_3-like pharmacology of receptors located in limbic and non-limbic areas of the rat brain using quinpirole and domperidone (a D_2 antagonist with a reported 32-fold greater affinity for D_2 vs. D_3 receptors) to displace [^{125}I]iodosulpiride binding in 10 μm thick coronal brain sections (Soc. Neurosci. Abstr., 17:87,1991). Presently, we are involved in examining the ability of Gpp(NH)p to shift D_3-like receptors to a lower agonist affinity state. Quinpirole (0-1000 μM) displacement of 0.25 nM [^{125}I]iodosulpiride binding in the presence and absence of Gpp(NH)p (10 μM) was measured on serial 10 μM thick coronal brain sections. Gpp(NH)p induced a significant rightward shift in the quinpirole displacement curve for a number ofsubdivisions of the caudate nucleus -- confirming the negative allosteric interaction between G proteins and striatal D_2 receptors. We are presently applying this approach to dopamine projection fields exhibiting high affinity, quinpirole displaceable [^{125}I]iodosulpiride binding (D_3 receptors). (Funded by MH39967)

634.13

DA AUTORECEPTOR MODULATION OF DIFFERENT CALCIUM CURRENTS IN DA NEURONS. L.-X. Liu*, G. Kapatos, and L.A. Chiodo. Cellular & Clinical Neurobiology Prgm., Dept. Psychiatry Wayne State Univ. Sch. Med., Detroit Michigan 48201.

DA neurons normally display a spontaneous activity which is irregular and alternates between a single-spike mode of discharge and one which includes bursts of action potentials. In an attempt to better understand the ionic currents which may be responsible for maintaining these distinct patterns of activity, we have begun to study the inward calcium currents present in the cell body of mesencephalic DA neurons maintained in culture. Voltage-clamp studies were conducted using whole-cell patch-clamp methods. DA neurons were identified and studied in cultures which were 13-21 days old with the following procedure: cultures were incubated for 30-60 minutes in N2 medium containing 0.1% ascorbic acid and 25 μM 5,7-dihydroxytryptamine. DA neurons were found contain three types of calcium currents. A T-type current was observed which exhibited a low threshold for activation (-50 mV), displayed rapid inactivation and was partially blocked by both external application of either amiloride or Ni^{2+}. A classic L-type calcium current was observed which could be activated from a holding potential of -40 mV, slowly and incompletely inactivated and was extremely sensitive to nifedipine. Finally, a N-type current was observed which was activated at same threshold as the L-type current but required prior hyperpolarization of the membrane, was transient and was blocked by external application of omega-conotoxin. DA (50-100 μm) stimulation of the D2 autoreceptors present on these cells resulted in a significant reduction in both L- and N-type calcium currents observed. Studies on the signal transduction pathways involved in mediating this autoreceptor modulation of calcium currents are currently in progress. [MH41557 (LAC), NS26081 (GK)]

634.14

COUPLING OF D2-SHORT RECEPTOR ISOFORM TO ION CHANNELS. M.A. Castellano[1,2], L-X. Liu[2], F.J. Monsma, Jr.[3], D.R. Sibley[3], L.A. Chiodo[2] and G. Kapatos[2]*. [1]Dept. Psychobiology, Univ. La Laguna, Canary Isl. (Spain), [2]Dept. Psychiat. Wayne State Sch. Med. Detroit, MI., [3]NINDS, Bethesda, MD.

Two isoforms of the dopamine (DA) D2 receptor, termed D2-short (D2S) and D2-long (D2L), have been discovered. Because both forms display the same pharmacological characteristics and are present in the same tissues it is impossible to study their individual signal transduction mechanisms. To begin such an analysis, NG108-15 neuroblastoma-glioma hybrid cells transfected to stably express D2S were used to investigate the coupling of D2S with the whole-cell patch technique. Transfected NG108-15 cells maintained in G-418 were found to express inward currents mediated by both T- and L-type Ca^{2+} channels, as defined by voltage-dependence of activation, rate of inactivation and sensitivity to antagonists. Pressure application of DA (100 μM) or the D2 DA agonist quinpirole (QUIN 100 μM) reduced both T- and L-type currents. Application of DA or QUIN also reduced the amplitude of a K^+-dependent outward current. This latter effect was blocked in a concentration-dependent manner by inclusion of the Ca^{2+}-chelator BAPTA in the pipette solution, was not altered by Co^{2+} in the bath solution and could be mimicked by pressure application of thapsigargin (10 μM). These effects were blocked by the D2 receptor antagonist eticlopride and were not observed in nontransfected cells. These results suggest that Ca^{2+}, mobilized from intracellular stores, is involved in the reduction of the K^+ current by D2S receptor stimulation. The inhibitory effect of D2S stimulation on two distinct Ca^{2+}-dependent inward currents and a K^+-dependent outward current suggests that a common mechanism, possibly mediated by mobilization of intracellular Ca^{2+}, may be involved. Similar studies of NG108-15 cells transfected with the D2L form are currently in progress and should determine whether the actions of these two D2 receptor isoforms are mediated by similar transduction mechanisms. [NS26081, MH41557]

634.15

$G_{o\alpha}$ MEDIATES TRANSDUCTION OF DA AUTORECEPTOR MODULATION OF THREE DIFFERENT K^+ CURRENTS IN DA NEURONS. L.A. Chiodo*, L-X. Liu, and G. Kapatos. Cellular & Clinical Neurobiology Prgm., Dept. Psychiatry, Wayne State Univ. Sch. Med., Detroit, Michigan 48201

Dopamine (DA) autoreceptors are known to be critically involved in the regulation of the physiology of mesencephalic DA neurons. Recently, we have observed three different K^+ currents present in these cells, I_A, I_K and I_{ANOM}, are modulated by stimulation of the D2 DA autoreceptor. Because it is known that DA receptors belong to the larger superfamily of G-protein-coupled receptors, we examined the signal transduction pathways involved in mediating this modulation. Whole-cell patch-clamp techniques were used to examine these different currents in DA cells maintained in culture. All three currents were significantly increase by stimulation of DA autoreceptors with either DA or quinpirole (50-100 μM). Preincubation of the DA cells with pertussis toxin (500 ng/ml for 4-5 hrs) completely blocked the DA autoreceptor modulation of these currents. Intracellular application of 100 μM GDPβS also blocked the DA-modulation of these currents while intracellular application of GTPγS mimicked the activation produced by DA. In addition, the intracellular application of a polyclonal antibody that specifically recognizes $G_{o\alpha}$ subunits (antiserum 3, Granneman and Kapatos, J. Neurochem. 54:1995, 1990) completely blocked the ability of DA autoreceptors to modulate these potassium currents while the preimmune serum was without effect. Taken together, these findings demonstrate that I_A, I_K and I_{ANOM} currents are increased by DA autoreceptor activation via a common mechanism which involves modulation by the $G_{o\alpha}$ subunit of the associated K^+ channel proteins. [MH41557 (LAC), NS26081 (GK)]

634.16

DIETARY PROTEIN MODULATES DOPAMINE LEVELS IN THE RAT BRAIN. J. Brock, S. Farooqui*, E. S. Onaivi, A. Hamdi, and C. Prasad. Pennington Biomed.Res.C., Baton Rouge, LA, 70808; Dept. Medicine, LSUMC, New Orleans, LA, 70112.

Rats that consume 50% protein exhibit hyper-activity and hyperresponsiveness to nociceptive stimuli, in which facilitation of dopaminergic activity has been implicated. We studied the regional distribution of dopamine (DA) and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in brains of rats maintained on high-protein (50%), normal-protein (20%), and low-protein (8%) diets for 36 weeks. Brain nuclei which represented different DAergic systems were punch-dissected, analyzed using HPLC, and resulted as follows ($p<0.05$). The 50% protein diet caused elevated DA levels in the substantia nigra, the dentate gyrus, and the striatum. DOPAC:DA ratios covaried with dietary protein in tuberculum olfactorium and amygdala, but increased in the parietal cortex by the 50% and 8% diets. Both diets decreased HVA:DA ratios in frontal cortex, amygdala, striatum, & inter-peduncular nucleus, & were inversely related to dietary protein in the dentate gyrus. These data suggest that the nigrostriatal & mesohippocampal systems were more sensitive than mesocortical and mesolimbic systems to dietary protein.

634.17

CHANGES IN STRIATAL DOPAMINE SYNTHESIS IN PREWEANLING AND ADULT RATS AFTER IRREVERSIBLE RECEPTOR BLOCKADE. C. A. Crawford*, S. A. McDougall, J. K. Rowlett, and M. T. Bardo. Dept. of Psychology, Univ. of Kentucky, Lexington, KY 40506 and Dept. of Psychology, California State Univ., San Bernardino, San Bernardino, CA 92407.

Treatment with the alkylating agent N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) results in presynaptic changes in dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) concentrations,as well as changes in D1 and D2 postsynaptic receptors. The present study was designed to determine if presynaptic changes after EEDQ were the result of changes in DA synthesis rates and if this change was receptor mediated. Preweanling (16-day-old) and adult (89-day-old) rats were treated with EEDQ (7.5 mg/kg) or vehicle, with half the rats in the both age groups receiving a combination of sulpiride (100 mg/kg) and SCH 23390 (1.0 mg/kg) 30 min prior to EEDQ treatment to protect DA receptors. Twenty-four hours after EEDQ treatment, all animals received an injection of the DOPA decarboxylase inhibitor NSD 1015 (100 mg/kg) and were sacrificed 30 min later. The striatum from all animals were removed and DOPA levels were measured by HPLC. Results showed that preweanling rats had significantly lower levels of DOPA than did adult rats and that EEDQ increased DOPA levels for both age groups. Antagonist pretreatment attenuated the DOPA increase by EEDQ, indicating that the increase was receptor mediated. These results suggest that the EEDQ-induced changes in DA and DOPAC levels reported previously were not the result of a change in DA synthesis rates.

634.18

The Effect of Dizocilpine on Basal and Stress-induced Dopamine Metabolism Bret A. Morrow*, Shelly J. Rosenberg, and Robert H. Roth, Yale University School of Medicine, Deptartments of Pharmacology and Psychiatry, New Haven, CT 06510.

Restraint selectivly activates the dopamine (DA) nuerons in the medial prefrontal cortex (mPFC) and nucleus accumbens (NAS) as assessed by postmortem tissue measurements of DA and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) (Roth et al., Ann. N.Y. Acad. Sci. 537:138-147, 1988). This report examines the non-competative N-methyl-D-aspartate (NMDA) antagonist, dizocilpine (MK801) on the stress-induced activation of the mesocortical and mesoaccumbal DA systems. Rats were treated with MK801, 0.01 mg/kg i.p., or saline and, after a 20 min delay, were restrained or left in the home cage for 30 min. The rats were then sacrificed and the brains removed and dissected. Brain samples were homogenized, purified over an alumina column and assayed for DOPAC and DA using high performance liquid chromatography with electrochemical detection. Restraint stress, as expected, significantly increased DA metabolism, as indicated by the DOPAC/DA ratio, in both the mPFC and NAS ($p<0.05$). Pretreatment with 0.01 mg/kg i.p. MK801 prevented this effect in the mPFC ($p<0.05$) but not the NAS ($p>0.05$). MK801 at this dose did not have any significant effect on DA metabolism in the mPFC, NAS or striatum. Previous results indicated that (+)HA-966, an antagonist for the glycine site of the NMDA receptor, prevented cortical stress-induced changes in DA metabolism. These data indicate that the stress induced increase in DA utilization can be prevented by blockade of the NMDA receptor complex.

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634.19

CHRONIC MORPHINE IMPAIRS AXOPLASMIC TRANSPORT IN THE MESOLIMBIC DOPAMINE SYSTEM OF THE RAT BRAIN D. Beitner-Johnson* and E.J. Nestler. Laboratory of Molecular Psychiatry, Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Dopaminergic neurons in the ventral tegmental area (VTA) appear to mediate some of the rewarding properties of opiates, cocaine, and other drugs of abuse. In previous studies, we have demonstrated that chronic morphine and cocaine treatments induce increased protein levels of tyrosine hydroxylase (J Neurochem, 1991, 57:344) and decreased levels of three neurofilament proteins NF-200, NF-160, and NF-68 (J Neurosci, 1992, 12:2165) in the rat VTA. Since NF proteins are associated with axonal transport, it was of interest to determine whether chronic morphine alters axonal transport rates in these neurons. Rats were unilaterally injected with 9 μCi of [^{35}S]-methionine into the VTA, and were then allowed to recover for 3 to 11 days. Serial coronal slices of brain were taken starting from the midbrain and proceeding anteriorly through the medial forebrain bundle (MFB) to the nucleus accumbens (NAc). The VTA, MFB, or NAc was removed from each brain slice, and [^{35}S]-labelled proteins were isolated by TCA precipitation. A time-dependent transport of [^{35}S]-labelled proteins from the VTA to the NAc was observed in control animals. Chronic treatment of rats with subcutaneous morphine pellets impaired this transport by 50% ($p<0.04$, N=10), compared to rats which had received chronic placebo pellets.

Long-term exposure to morphine may impair the brain's endogenous reward system by reducing the ability of these cells to properly transmit dopaminergic and other signals to neuronal elements in the NAc.

REGULATION OF SEROTONIN RECEPTORS

635.1

5-HT2 RECEPTOR LEVELS IN A7r5 CELLS ARE INVERSELY REGULATED BY RECEPTOR STIMULATION.

Wu Yang*, Timothy Gallaher and Jean C. Shih. Dept. Mol. Pharmacol. Toxicol. University of Southern California, School of Pharmacy. Los Angeles, CA 90033

Preliminary studies of the 5-HT2 receptor in a rat aorta cell line (A7r5) indicate that the number of receptors and receptor stimulated PI formation can be regulated by growth in the presence of agonists or antagonists. Growth of the cells in Modified Eagle's Medium supplemented with 0.5% Fetal Bovine serum (FBS) for 24 or 48 hours results in a two-fold increase in the specific binding activity of [3H]ketanserin compared to cells grown for the same time in 10% FBS supplemented media. However, growth for 48 hours in the 0.5% supplemented media in the presence of 1 μM DOI or 5-HT results in decreased [3H]ketanserin binding, comparable to that grown in 10% supplemented media. Conversely, growth for 48 hours with 10% supplemented media in the presence of 1 μM ketanserin results in a two-fold increase in [3H]ketanserin binding, comparable to that grown in 0.5% FBS supplemented media. Cells grown in 10% FBS media in the presence of 1 μM ketanserin also show a similar increase in the level of PI formation relative to cells grown in 10% media but not treated with ketanserin. FBS contains many hormones including 5-HT. These results suggest that the activity of 5-HT2 receptors is regulated by a mechanism which is inversely sensitive to its stimulation. (Supported by NIMH grants R37 MN39085, K05 MH00796, R01 MH37020).

635.2

SIGNALLING VIA THE SEROTONIN-RECEPTOR OF C6BU-1 CELLS H. Sommermeyer and T. Glaser*. Inst. of Neurobiology, Troponwerke GmbH & Co.KG., Berliner Str. 156, 5000 Köln 80, Germany

The serotonin-stimulated accumulation of inositol-phosphates in the rat glioma cell line C6BU-1 was characterized using a simple and rapid experimental procedure. Addition of serotonin (5-HT) to C6BU-1 cells prelabeled with [^{3}H]-inositol increased the inositol-phosphates content 2.5 to 3.5-fold with an EC_{50} value of 0.8 μM. The stimulatory effect of 5-HT was highly dependent on the presence of LiCl which was half-maximal effective at a concentration of 4 mM. The maximal effect of LiCl was achieved at 10 mM.

The 5-HT-stimulated PI-response was inhibited by 5-HT_2-/5-HT_{1C}-receptor antagonists. The order of potency was spiperone = risperidone > ritanserin > pelanserin > ketanserin = mianserin > pipamperone = metitepine. Several well known neuroleptics, like cis-flupentixol, haloperidol and clozapine antagonized the 5-HT-induced PI-response. Cis-flupentixol was about 10 times more potent than haloperidol or clozapine. The latter two exhibited their antagonistic properties only at relative high concentrations in the μM-range. Neither the 5-HT-uptake inhibitor citalopram, the muscarinic cholinergic antagonist pirenzepine, the α_1-adrenoceptor antagonist prazosin, nor the D_2-dopaminergic antagonist sulpiride inhibited the 5-HT-induced PI-response in C6BU-1 cells.

635.3

LACK OF EFFECT OF LONG-TERM ESTROGEN TREATMENT ON SEROTONIN-INDUCED OUTWARD CURRENT IN HIPPOCAMPAL PYRAMIDAL CELLS OF MALE CASTRATED RATS.

F.A. Dijcks, J.H. Couvée and G.S.F. Ruigt*. Organon Int., Dept. of Neuropharmacology, POB 20, 5340 BH Oss, The Netherlands.

Several lines of evidence suggest an interaction between sex hormones and serotonergic neurotransmission in the central nervous system. In an electrophysiological study using current-clamp techniques Beck et al. (*Neurosc. Lett. 106:181-187; 1989*) showed that chronic estrogen treatment (E2 for 3-6 days) restored the blunted 5-HT_{1A}-mediated hyperpolarization in CA1 pyramidal cells of ovariectomized rats. Given the fact that the central effects of testosteron are mainly mediated through estrogen receptors (testosteron is aromatized to E2 in the CNS) we were interested to know whether the serotonergic response in male rat hippocampus would be affected by castration and steroid suppletion.

In the present experiment we examined the influence of castration and long-term E2 treatment (2-3 wks) on serotonine-induced currents in CA1 hippocampal pyramidal cells of the male rat. Rats were either castrated or sham-operated. Sham-operated animals (SHAM) received a subcutaneous implant containing cholesterol, whereas castrated animals received either cholesterol (CAS) or cholesterol/estrogen (CAS/E2) implants. Intracellular and extracellular (population spike) recordings were performed in the CA1 pyramidal cell layer. Neurons showing a resting membrane potential (RMP) ≤ -55 mV, a spike amplitude (SA) ≥ 80 mV and an input resistance (R_{in}) ≥ 25 MΩ were included in the analysis. We recorded 44 cells from 24 animals with characteristics as shown in the next table (data as avg±sem):

treatm	n	RMP (mV)	Rin (MΩ)	$I_{5\text{-}HT}$ (nA)	E2 (pg/ml)	weight (g)
SHAM	14	-65±2	56±4	0.30±0.04	6±1	278±17
CAS	16	-66±1	59±3	0.26±0.03	7±1	233±12
CAS/E2	14	-69±1	61±3	0.27±0.03	33±4	181±8

From these results and the fact that 3 μM spiperone fully antagonized the serotonin-induced currents we conclude that chronic physiological levels of sex steroids do not affect the serotonin (5-HT_{1A})-induced outward current in hippocampal pyramidal cells of the male rat.

635.4

5-HT1-LIKE RECEPTORS ARE LINKED TO INHIBITION OF ADENYLYL CYCLASE AND INCREASE IN INTRACELLULAR CALCIUM CONCENTRATION IN VASCULAR SMOOTH MUSCLE (VSM) CELLS FROM BOVINE BASILAR ARTERY.

B.J. Ebersole[1]*, C.A. Diglio[2], D. Wosner[1], and K.A. Berg[1]. [1]Dept. of Anesthesiology , Mt. Sinai School of Medicine of CUNY, NY, NY 10029 and [2]Dept. of Pathology, Wayne State University School of Medicine, Detroit, MI 48201.

VSM cells derived from bovine basilar arteries by the explant method were grown in culture for up to 20 passages. In the presence of 1 μM forskolin and the phosphodiesterase inhibitor rolipram, 5-HT1-like agonists inhibited by 85-95% the accumulation of cAMP in intact cells with the rank order of potency 5-carboxamidotryptamine (5-CT, EC50=1.2 nM) > 5-HT (3.2 nM) > 5-benzyloxytryptamine (25 nM) > RU24969 (63 nM) > 8-OH-DPAT (205 nM). In suspensions of cells loaded with the calcium-sensitive probe fura-2, 5-CT and 5-HT caused transient increases in $[Ca^{2+}]i$ of 2-3 times resting levels. Both the inhibition of cAMP formation and increase in $[Ca^{2+}]i$ were blocked by the antagonist methiothepin (50-100 nM), but not by 100 nM ketanserin or spiperone. 5-CT-mediated inhibition of cAMP accumulation was not blocked by 10 μM pindolol. Both responses were blocked by pretreatment with pertussis toxin. The rank order of agonist potency, as well as the antagonist sensitivity, indicates responses mediated by 5-HT1-like (perhaps 5-HT1D) receptors. Because these cells constituitively express 5-HT1-like receptors, they may serve as a useful model for the study of 5-HT1-like receptor-mediated signal transduction mechanisms in VSM. Supported by GM34852 and AHA Grant-in-Aid 890750.

635.5

PHARMACOLOGICAL CHARACTERIZATION OF 5-HYDROXYTRYPTAMINE (5-HT_3) RECEPTOR SUBTYPES IN SPRAGUE-DAWLEY RAT CORTEX, C57BL/6 MOUSE CORTEX, CD-1 (ICR) MOUSE CORTEX AND CD-1(ICR) MOUSE ILEUM. E. Stefanich, D.W. Bonhaus, R.M. Eglen and E.H.F. Wong*. Dept. of Neurosciences, Inst. of Pharmacology, Syntex Research, Palo Alto, CA 94304.

5-HT_3 receptors are widely distributed in the central and peripheral nervous systems; thus 5-HT_3 receptor antagonists may have clinical applications in both central and G.I. disorders. Previous studies have demonstrated species-dependent variants of 5-HT_3 receptors. The current study characterizes 5-HT_3 bindings sites in two strains mouse and in two tissues (cortex and ileum) within a single strain (CD-1) of mouse.

Membranes were prepared from sprague-dawley rat cortex, C57BL/6 mouse cortex, CD-1 (ICR) mouse cortex and CD-1(ICR) mouse ileum. 5-HT_3 receptors were labeled with [^{3}H](-)-(S)-N-(1-azabicyclo [2.2.2.] oct-3-yl)-2,4,5,6-tetrahydro-1-H-benzo [de] isoquinolin-1-one hydrochloride mono ethanolate (RS 42358-197) a selective high affinity 5-HT_3 receptor antagonist (Wong et al., Br. J. Pharmacol. 105:33P; 1992).

The radioligand bound with similar affinities (0.08 to 0.20 nM) to homogeneous populations of saturable binding sites (Bmax values of 30 to 44 fmol/mg protein) in each of the tissues. However, affinities of specific antagonists differed by more than 10 fold both between strains of mice and within a single strain of mouse (when comparing 5-HT_3 receptors in brain cortex to those in ileum). These results demonstrate, for the first time, subtypes of 5-HT_3 binding sites both between strains of mouse and between tissues within a single strain of mouse.

635.6

ALLOSTERIC INTERACTIONS OF AGONISTS AND ANTAGONISTS AT 5-HYDROXYTRYPTAMINE (5-HT_3) RECEPTORS D.W. Bonhaus*, R.M. Eglen and E.H.F. Wong. Dept. of Neurosciences, Inst. of Pharmacology, Syntex Research, Palo Alto, CA 94304.

5-HT_3 receptors are ligand-gated ion channels and, as such, may be subject to allosteric regulatory mechanisms. To investigate the nature of ligand interactions at the 5-HT_3 receptor the effects 5-HT_3 receptor agonists and antagonists on the dissociation of a selective high affinity 5-HT_3 antagonist, ([^{3}H](-)-(S)-N-(1-azabicyclo[2.2.2.]oct-3-yl)-2,4,5,6-tetrahydro-1-H-benzo[de] isoquinolin-1-one hydrochloride mono ethanolate; [^{3}H]RS 42358-197 (Wong et al., Br. J. Pharmacol. 105:33P; 1992), was examined.

The dissociation of [^{3}H]RS 42358-197 from NG108 cell membranes was significantly slower in the presence of saturating concentrations of 5-HT_3 (0.06 $\pm$ 0.01 min^{-1}) or other agonists than in the presence of unlabelled RS 42358-197 (0.16 $\pm$ 0.01 min^{-1}) or other 5-HT_3 antagonists. One explanation for these findings is that 5-HT_3 receptor agonists bind to sites distinct from the antagonist binding site to decrease [^{3}H]RS 42358-197 binding by an allosteric interaction. Alternatively agonists may allosterically **increase** the affinity of the binding site to which the antagonist is bound thereby slowing [^{3}H]RS 42358-197 dissociation. This latter interpretation is consistent with the finding that Hill slopes of competition curves for antagonists at the 5-HT_3 receptor are not different from unity whereas agonists produce Hill slopes greater than 1.0. Regardless of mechanism, these findings indicate that the dissociation rate constants for [^{3}H]RS 42358-197 are determined by the nature of the displacing ligand, a finding inconsistent with a competitive interaction. This, in turn, raises the possibility that there are multiple sites on the 5-HT_3 receptor-gated channel towards which ligands can be directed.

635.7

DECREASED SEROTONIN 5HT_2 RECEPTORS IN THE FRONTAL CORTEX OF BRAINS SPECIFIC TO NEUROLEPTIC TREATED PATIENTS WITH CHRONIC SCHIZOPHRENIA R.R. Conley, G.N. Pandey, F.J. Peretti, W.T. Carpenter*, C.A. Tamminga, and R.C. Roberts. Univ. of Maryland, Baltimore, MD 21201.

Changes in serotonin 5HT_2 receptor number in the brains of chronic schizophrenic patients was investigated using ^{3}H-LSD and ^{3}H-ketanserin as ligands. Thirteen subjects were studied: eight had taken neuroleptics up to their death; five had been off neuroleptics, by clinical case review, for more than one year prior to death. Ketanserin binding was saturatable with a Kd of 1.3 nM and a Bmax of 195 fmol/mg protein in drug free cases and a Kd of 1.6 nM and Bmax of 148 fmol/mg protein for on drug cases (p=NS). LSD binding was saturatable with a Kd of 0.75 nM and a Bmax of 379 fmol/mg protein in drug free subjects and a Kd of 0.75 nM and Bmax of 162 fmol/mg protein in on drug cases (p>.01). An analysis of six matched normal controls yielded a Bmax of 153 fmol/mg protein for ketanserin (Kd 1.4 nM) and Bmax of 302 fmol/mg protein for LSD (p<.02 vs. on drug cases)(Kd 0.63 nM). Neuroleptic treatment is associated with a reversible decrease in serotonin receptors in neocortical tissue from chronic schizophrenic subjects.

635.8

OVEREXPRESSION OF THE THIRD INTRACELLULAR LOOP PROTEINS OF RAT 5-HT RECEPTORS: DEVELOPMENT OF SUBTYPE-SPECIFIC ANTIBODIES AND NUCLEOPROBES. **M.-C. Miquel, J.A. Gingrich, E. Doucet, E.J. Kidd, C. Gérard, H. Gozlan, B. Berger* and M. Hamon, INSERM U.288, CHU Pitié Salpêtrière, 91 Boulevard de l'Hôpital, 75013 PARIS, FRANCE.**

In order to develop a systematic series of tools to permit the localization of 5-hydroxytryptamine (5-HT) receptors in the central nervous system, we have used the technique of polymerase chain reaction (PCR) to amplify and clone regions corresponding to the putative third intracellular loop of these receptor proteins. These PCR fragments when cloned into pBluescript were either used to generate labelled riboprobes (sense and antisense) or cDNA probes. The same fragments were also cloned into the plasmid pGEX2-KG, and were used to generate in-frame fusion proteins with the glutathione-S-transferase enzyme. These proteins were then overexpressed, purified, and used as antigens to immunize rabbits. The protein was either fixed with formaldehyde or left in its native form in order to obtain antibodies which can be used for electron microscopy on fixed tissues and for immunoautoradiography on unfixed tissue. We first applied this method to the recently sequenced 5-HT_{1A} receptor (Albert et al., 1990). Our results show that both the "fixed" and "native" antibodies are able to immunoprecipitate CHAPS-solubilized 5-HT_{1A} receptors labelled with [^{3}H]-8-OH-DPAT. In addition, both sets of antibodies recognize the same protein as our antipeptide antibodies (El Mestikawy et al., 1990), and their regional distributions are well correlated with that of the mRNA for the 5-HT_{1A} receptor (Miquel et al., 1991). Fusion proteins from the same regions of the other G-protein coupled 5-HT receptors (5-HT_{1B}, 5-HT_{1D}, 5-HT_2) have been obtained in our laboratory and are currently being injected into rabbits. Albert et al. (1990) *J. Biol. Chem.*, **265**, 5825-5832; El Mestikawy et al. (1990) *Neurosci. Lett.*, **118**, 189-192; Miquel et al. (1991) *Neurochem. Int.*, **19**,453-465.

635.9

CHARACTERIZATION OF A FUNCTIONAL 5-HT_2 RECEPTOR IN THE HUMAN NEUROBLASTOMA CELL LINE IMR-32 A. Cholewinski, M. Elliott, T. Flanigan, N. Newberry, R. Newton, S. Phipps, A. Reavley, D. Grahame-Smith, and R. Leslie.* Oxford Univ.-SmithKline Beecham Centre for Applied Neuropsychobiology, Oxford OX2 6HE, U.K.

Scatchard transformation of saturation binding to IMR-32 cell membranes with [^{3}H]ketanserin revealed a linear, single-site with a K_d of 0.57nM and a B_{max} of 147 $\pm$ 18fmol/mg protein. Displacement studies with 5-HT antagonists demonstrated a 5-HT_2 receptor subtype rather than a 5-HT_{1C} (spiperone = ketanserin < mesulergine < 5-HT). Northern blot analysis of poly(A)$^+$ mRNA from IMR-32 cells identified two transcripts of 5.6 and 6.0kb which hybridised with a rat 5-HT_2 cDNA probe. Measurements of phosphoinositide turnover during 5-HT stimulation showed a concentration-dependent increase with an EC_{50} = 1.9μM and a maximal response of three times over basal. The effect was inhibited by spiperone (1μM) and ketanserin (1μM). Brief applications of 5-HT stimulated increases in $[Ca^{2+}]_i$ in a concentration-dependent manner (EC_{50} = 0.5μM). These increases occurred in calcium-containing and calcium-free media, implying release of calcium from intracellular stores. Responses were blocked by ketanserin (10nM), spiperone (10nM), mesulergine (1μM) and DOI (1μM), but not by pindolol (1μM) and granisetron (1μM). 5-HT (10μM) hyperpolarised 34/50 IMR-32 cells (median -7mV). This response was associated with an input resistance reduction and was ketanserin(30nM)-sensitive. These data demonstrate a functional 5-HT_2 receptor on IMR-32 cells.

635.10

HOMOLOGOUS DESENSITIZATION OF SEROTONIN-2 RECEPTORS IN RAT GLIOMA C6BU-1 CELLS. A.Kagaya*, M.Mikuni, S.Muraoka, H.Shinno, K.Saitoh, T.Ogawa, S.Yamawaki and K.Takahashi. Dept. Neurol. Psychiat. Hiroshima Univ. Schl. Med., Hiroshima and Div. Mental Disorder Res. Natl. Inst. Neurosci. NCNP, Tokyo Japan.

It has been characterized that serotonin-2 receptors are responsible for serotonin-induced intracellular calcium mobilization in rat glioma C6BU-1 cells. As several studies suggest that serotonin-2 receptors can be desensitized and down regulated in various tissues, investigators have directed their attention to the function of these receptors. However, the precise mechanism of the desensitization of serotonin-2 receptors is so far not understood. We have now investigated the desensitization of serotonin-2 receptor-mediated intracellular calcium mobilization in C6 cells. The receptors were desensitized after pretreatment of the cells with serotonin in dose and time dependent manner. The desensitization was reversed by W-7, a calmodulin antagonist, which was co-pretreated with serotonin. Isoproterenol or thrombin did not affect the calcium response to serotonin when they were pretreated. These results suggest that serotonin-2 receptor-mediated signalling system was desensitized homologously and that the desensitization was mediated at lease in part by calmodulin dependent pathway.

635.11

5-HT AGONIST REGULATION OF THE RAT 5-HT_{1C} RECEPTOR . A. Sharma*,and K.C.F.Fone. Dept. Physiol. & Pharmacol., Queen's Medical Centre, Nottingham, NG7 2UH, U.K. (SPON: BRAIN RESEARCH ASSOCIATION).

5-HT_2 receptors are downregulated by both agonist and antagonists (Leysen et al., 1989) but similar regulation of the 5-HT_{1C} receptor has yet to be studied. To explore this further, the behavioural response and 5-HT_{1C} receptor protein-like immunoreactivity (5-HT_{1C}-LI) were measured in selected rat brain and cord regions following repeated treatment with the 5-HT_2/5-HT_{1C} agonist (DOM) and the 5-HT_{1C}/5-HT_{1B} agonist (m-CPP).

Adult male Wistar rats (300-335g) received twice daily injections of either DOM (2.5mg/kg i.p), m-CPP (5mg/kg i.p), or saline (0.154M 1ml/kg i.p., n=6 each) for 5 days. Following the first and alternate injections, rats were placed in a behavioural chamber, and a series of motor behaviours (wet-dog shakes, back-muscles contractions, rears and 90° turns) and yawns were measured separately but continuously for 30 mins. After (30mins) the final injection, rats were decapitated and the 5-HT_{1C}-LI measured using a polyclonal antiserum raised against the rat 5-HT_{1C} receptor protein (Sharma et al.,1992). Results were analysed by ANOVA followed by Dunnett t-test (behaviour) or Student's t-test (5-HT_{1C}-LI). Repeated DOM injection reduced back muscle contractions ($P<0.05$) and wet-dog shakes (n.s), whilst 5-HT_{1C}-LI was elevated in the dorsal spinal cord ($P<0.01$) and reduced in both the hypothalamus ($P<0.05$) and ventral spinal cord (n.s). With repeated injection m-CPP continued to attenuate turns and rears compared with saline ($P<0.01$ and $P<0.001$ respectively) while yawns increased progressively ($P<0.05$) and 5-HT_{1C}-LI was unchanged in any brain or cord region.

A DOM-induced motor behavioural tolerance was observed confirming previously reported rapid agonist-induced downregulation of 5-HT_2 receptors (Leysen et al., 1989) with selected changes in 5-HT-LI. In contrast, no downregulation of either the hypolocomotor effect (reported to be 5-HT_{1C}-mediated, Kennett & Curzon, 1988) or 5-HT_{1C}-LI was observed despite repeated m-CPP administration.

635.12

DOWNREGULATION AND TURNOVER OF CORTICAL 5-HT2 RECEPTORS MEDIATED BY ACTIVATION OF 5-HT1A RECEPTORS AND LESION: SELECTIVE REDUCTIONS IN 5-HT2 RECEPTOR PRODUCTION RATES

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These studies investigated the effects of serotonergic lesion and 5-HT_{1A} receptor activation on the regulation of cortical 5-HT_2 receptors. Rats received a single i.p. injection of either vehicle (50% ETOH/H_2O), or the irreversible receptor inactivator EEDQ (10 mg/kg), and were sacrificed at 0.25-14 days to determine 5-HT_2 receptor recovery. Another group of rats were similarly treated, but also received daily 1 mg/kg s.c. injections of the selective 5-HT_{1A} agonist, 8-hydroxydipropylaminotetralin (DPAT), from day 1 throughout the recovery time-course. To investigate the influence of presynaptic input and postsynaptic 5-HT_{1A} activation on 5-HT_2 receptor turnover, treatment paradigms identical to those above were also carried out in 5,7-DHT-lesioned rats. Decreases in ^{3}H-paroxetine-labeled 5-HT uptake sites confirmed a 95% lesion throughout the recovery. DPAT or lesion alone was found to reduce the density of 5-HT_2 receptors by 17% and 23%, respectively. These effects appeared to be additive as DPAT, in lesioned rats, downregulated 5-HT_2 receptors by 36%. Receptor turnover studies indicated that in either lesioned or DPAT-treated rats, the 5-HT_2 downregulation was due to selective decreases (28-32%) in receptor production rates. Likewise, 5-HT_2 receptor production rates were decreased by 34% in lesioned DPAT-treated rats compared with lesioned saline-treated controls. NO significant changes in 5-HT_2 receptor degradation rate constants were observed in any of the groups. These data demonstrate that removal of 5-HT presynaptic input, as well as, the direct activation of postsynaptic 5-HT_{1A} receptors can downregulate cortical 5-HT_2 receptors. Downregulation by lesion or 5-HT_{1A} receptor activation is mediated by selective decreases in 5-HT_2 receptor production rates, presumably via decreased 5-HT_2 mRNA levels.

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SEROTONIN RECEPTORS: PHARMACOLOGIC CHARACTERIZATION

636.1

IN VIVO ACTIVITY OF CP-94,253, A SELECTIVE SEROTONIN 5-HT_{1B} RECEPTOR AGONIST. F.D. Tingley, A.W. Schmidt, J.E. Macor, and D.W. Schulz*. Pfizer Central Research, Groton, CT 06340.

Characterization of the functional properties of 5-HT_{1B} receptors *in vivo* has been hampered by a lack of agonists having adequate selectivity for this receptor. CP-94253, (3-[1,2,5,6-tetrahydro-pyrid-4-yl]-5-propoxypyrrolo[3,2-b]pyridine) binds with high affinity to 5-HT_{1B} receptors (K_i= 2 nM) and is 45-fold selective for 5-HT_{1B} vs 5-HT_{1A} receptors (Koe *et al.*, Drug Dev. Res., in press). Moreover, it causes anorexia in rats (Koe *et al.*), a behavior attributed to activation of 5-HT_{1B} receptors (Kennett *et al.*, Eur. J. Pharmacol. 141:429). We have characterized the functional activity of CP-94,253 *in vitro*, and have examined its effects *in vivo* by measuring serotonin utilization in rat and guinea pig brain.

CP-94,253 behaved as a full agonist at 5-HT_1 receptors, inhibiting forskolin-stimulated adenylate cyclase activity in a receptor-selective manner (EC_{50}: 5-HT_{1A} 1800 nM, 5-HT_{1B} 10 nM, 5-HT_{1D} 190 nM). One hour following ip injection, serotonin turnover in rat hypothalamus and cortex was inhibited dose-dependently (ED_{50} = 2 mg/kg ip). While the selective 5-HT_{1A} agonist 8-OH-DPAT was approximately equipotent in both species, a dose of 32 mg/kg CP-94,253 was required in order to diminish 5-HT turnover in guinea pigs, a species lacking 5-HT_{1B} receptors. These data suggest that CP-94,253 acts selectively at release-modulating 5-HT_{1B} receptors, rather than somatodendritic 5-HT_{1A} autoreceptors.

636.2

PRECLINICAL PHARMACOLOGICAL CHARACTERIZATION OF LY228729, A SELECTIVE 5-HT_{1A} AGONIST. M.M. FOREMAN*, R.W. FULLER, D.T. WONG, D.L. NELSON, D.O. CALLIGARO, J.D.LEANDER AND M.E. FLAUGH , Lilly Research Laboratories, Eli Lilly Corporate Center, Indianapolis, IN, USA

LY228729 (6-carboxamido-4-dipropylaminotetrahydrobenzindole) is a rigid tryptamine derivative with a carboxamide serving as a protophilic group to mimic the hydroxyl in 5-HT. In radioligand diplacement assays, LY228729 had a Ki of 0.19 nM for the 5-HT_{1A} receptor and much lower affinities for other monoaminergic receptors with Ki values at least 1000 times higher. LY228729 significantly reduced hypothalamic 5-HIAA levels (0.03 mg/kg s.c.) and increased serum corticosterone levels (0.3 mg/kg s.c.) in rats. LY228729 (0.03 mg/kg s.c.) significantly reduced hypothalamic 5-HTP accumulation after decarboxylase inhibition. LY228729 (0.01 mg/kg s.c.) decreased ejaculatory latency in rats. Maximal 5-HT_{1A} syndrome responses in rats, e.g. lower lip retraction and flat posture, were observed between 0.1 and 1 mg/kg s.c., and p.o. doses were 10 times higher for equivalent responses. LY228729 increased punished responding at 0.08 mg/kg s.c. and lowered unpunished responding at 0.32 mg/kg s.c. in rats. These results indicate that LY228729 is a selective 5-HT_{1A} agonist with appropriate potency and bioavailability properties for clinical evaluation.

636.3

[^{3}H]5-CARBOXAMIDOTRYPTAMINE LABELS MULTIPLE HIGH AFFINITY BINDING SITES IN VERTEBRATE BRAIN. C.D. Mahle*, H.P. Nowak, D.J. Bucci, R. B. Carter, and F. D. Yocca. Dept. Neuropharmacology, Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492.

Binding sites displaying high (5-HT_{1D}) and low (5-HT_{1E}) affinity for 5-carboxamidotryptamine (5-CT) have been revealed using [^{3}H]5-HT and selectively masking 5-HT_{1A} and 5-HT_{1C} receptors. To probe the possible heterogeneity in 5-HT_{1D} binding sites, we have eliminated a portion of the complex nature of [^{3}H]5-HT binding by utilizing [^{3}H]5-CT. In corticolimbic regions of guinea pig brain, when using a low concentration of [^{3}H]-5-CT selectively labelling 5-HT_{1D} sites, 5-CT competed monophasically in striatum (K_i=0.68±0.04 nM), frontal cortex (K_i=1.85±0.35 nM), and hippocampus (K_i=3.89±0.42 nM). Sumatriptan further differentiated amongst high affinity [^{3}H]5-CT binding sites however, in both guinea pig and bovine striatum, frontal cortex and hippocampus, where biphasic displacement curves yielded a high affinity 5-HT_{1D} site, as well as a low affinity sumatriptan-insensitive site. Although pigeon brain contains a high density of 5-HT_{1D} receptors, 5-HT, 5-CT and sumatriptan competition for [^{3}H]5-CT binding in optic tectum, brain stem, and telencephalon yielded apparently monophasic displacement curves. These results have been confirmed autoradiographically, demonstrating lack of heterogeneity in [^{3}H]-5-CT binding in pigeon brain, yet localization of multiple [^{3}H]-5-CT binding sites in corticolimbic regions of guinea pig brain. Preliminary reults suggest multiplicity of [^{3}H]5-CT binding sites in corticolimbic regions of human brain. These results suggest species differences exist with regard to the heterogeneity of 5-HT_{1D}-like binding sites in vertebrate brain.

636.4

SEROTONIN AND SEROTONIN$_3$ RECEPTOR AGONISTS POTENTIATE THE INHIBITORY ACTION OF DOPAMINE ON MEDIAL PREFRONTAL CORTICAL CELLS IN RATS. Jian Yu Zhang* and Rex Y. Wang. Dept. of Psychiatry and Behavioral Sciences, SUNY at Stony Brook, Stony Brook, NY 11794.

The effects of serotonin (5-HT) and selective 5-HT_3 receptor agonists 2-methylserotonin and 5-HTQ on the suppressant action of dopamine (DA) on medial prefrontal cortical (mPFc) cells was studied using the techniques of single cell recording and microiontophoresis. The microiontophoretic application of DA (5-80 nA) produced a current-dependent suppression of mPFc cell firing. When subthreshold currents of 5-HT, 2-methyl-5HT or 5-HTQ were administered concurrently with DA, they potentiated the inhibition produced by DA but not that of GABA. Similarly, the electrical stimulation of the ascending 5-HT fiber pathway via the caudal linear raphe nucleus also potentiated the inhibition induced by DA. The effects of potentiation were blocked by the selective 5-HT_3 receptor antagonist granisetron. The potentiated effects were not altered in rats pretreated with either parachlorophenylalanine (PCPA) or α-methyl-p-tyrosine. However, the pretreatment with either PCPA or 5,7-dihydroxytryptamine markedly attenuated the inhibitory action of DA on mPFc cells. Taken together, our preliminary results show that the inhibitory action of DA on mPFc cells depends upon 5-HT input, i.e. 5-HT may have a permissive role. Furthermore, the modulatory effect of 5-HT on DA's action is primarily mediated by 5-HT_3-like receptors. If our results can be extended to other brain regions, the blockade of the potentiating effect of 5-HT and 5-HT_3 receptor agonists on the inhibitory action of DA by 5-HT_3 antagonists may partially account for their antipsychotic potential and potential for treating drug addicts.

636.5

EFFECTS OF 5-HT_3 RECEPTOR ANTAGONISTS IN ETHANOL- AND DIAZEPAM-WITHDRAWN RATS. A.K. Mehta* and M.K. Ticku. Univ. TX Hlth. Sci. Ctrs., Dept. of Pharmacology, San Antonio TX 78284-7764

The effect of 5-HT_3 receptor antagonists such as ICS 205-930, MDL 72222, metoclopramide and zacopride was investigated on ethanol as well as diazepam withdrawal phenomena in the present study. There was a significant increase in locomotor activity in the ethanol as well as diazepam withdrawn rats. The treatment of rats with 5-HT_3 receptor antagonists during withdrawal phase did not modify the effect in spite of the fact these agents had a slight (10-20%) depressant effect per se on locomotor activity in control rats. The ethanol-withdrawn rats were more sensitive to pentylenetetrazole (PTZ)-induced convulsions as compared to control animals. 5-HT_3 receptor antagonists did not attenuate the increased sensitivity of ethanol-withdrawn rats to PTZ. Furthermore, 5-HT_3 receptor antagonists did not elicit any significant effect per se on PTZ-induced convulsions in control rats. These observations indicated that 5-HT_3 receptor antagonists are ineffective in attenuating hyperlocomotor-activity following abrupt termination of chronic administration of ethanol or diazepam, and increased sensitivity to PTZ in the ethanol-withdrawn rats.

636.6

5-HT_2 RECEPTOR-MEDIATED ACTIVATION OF INTERNEURONS IN PIRIFORM CORTEX IS POTENTLY ANTAGONIZED BY RISPERIDONE, A NEW ATYPICAL ANTIPSYCHOTIC DRUG. R.L. Gellman* and G.K. Aghajanian. Depts. of Pharmacology and Psychiatry; Yale University, New Haven, CT 06510.

Potent 5-HT_2 receptor antagonism is hypothesized to underly the therapeutic action of new, clinically effective, atypical neuroleptic drugs such as risperidone (Leysen, et. al., 1992). Using electrophysiological techniques, we have recently shown that 5-HT activates, via 5-HT_2 receptors, a subpopulation of GABAergic interneurons located on the layer II/III border of piriform cortex (Sheldon & Aghajanian, 1991). Activation of these interneurons induces IPSPs in layer II pyramidal cells. In the present study we investigated the ability of several antipsychotic drugs, including risperidone, clozapine and haloperidol, to antagonize the 5-HT_2-mediated activation of interneurons and 5-HT_2-elicited IPSPs in pyramidal cells.

In brain slices, 5-HT-activated interneurons were identified by extracellular recording using previously established criteria (Sheldon & Aghajanian, 1991). The response to a bath application of 5-HT (100 μM, 1-2 minutes) was measured at baseline and in the presence of increasing concentrations of the antipsychotic drug. Risperidone dose-dependently blocked the excitatory 5-HT response with an IC_{50} of ~9 nM. Clozapine also dose-dependently blocked the 5-HT response with an IC_{50} of ~2 μM. In contrast to the complete antagonism of the 5-HT response by risperidone and clozapine, haloperidol did not completely block the excitatory 5-HT response even at the highest concentration used (10 μM). Parallel results were found for 5-HT-elicited IPSPs recorded intracellularly from layer II pyramidal cells. Antagonism of excitatory 5-HT_2 receptors on a specific subpopulation of interneurons in cortical regions may be one site at which atypical antipsychotic drugs such as risperidone exert their therapeutic effects.

636.7

SUMATRIPTAN: LACK OF EFFECT ON MEMBRANE POTENTIAL OF GUINEA-PIG ISOLATED TRIGEMINAL GANGLION. H.E. Connor and C.T. O'Shaughnessy*, Department of Neuropharmacology, Glaxo Group Research Ltd, Ware, Herts, SG12 0DP, UK. (SPON: Brain Research Association)

The aim of this study was to investigate the effect of sumatriptan, a selective 5-HT_1 receptor agonist, on membrane potential of guinea-pig isolated trigeminal ganglion (TG). TGs were divided into 3 longitudinally and placed in 2-compartment baths. The d.c. potential between compartments was recorded extracellularly. Drugs were applied to the Krebs superfusion fluid of one compartment. KCl (3mM) and GABA (0.1mM) caused depolarisations of the TG (0.30 ± 0.05 and 0.55 ± 0.08mV respectively n=11-19). 5-HT (1-10μM) caused small depolarisations (0.06 ± 0.02mV). Responses to each of these agents was enhanced by pretreatment of the TG with collagenase to enhance desheathing. Sumatriptan (0.1-10μM) had no effect on TG membrane potential: collagenase pretreatment or changing the composition of the Krebs solution failed to reveal any effect of sumatriptan. These data provide no evidence to suggest that sumatriptan inhibits neurotransmission in trigeminal ganglion. Further studies are required to investigate the possiblity that the anti-migraine action of sumatriptan results from a 5-HT_1 receptor mediated inhibition of sensory neurotransmission in trigeminal craniovascular sensory nerves.

636.8

EFFECTS OF RENZAPRIDE AND CISAPRIDE ON FAST SYNAPTIC TRANSMISSION IN GUINEA PIG ILEUM MYENTERIC PLEXUS. H. Pan* and J.J. Galligan. Dept. of Pharmacol./Toxicol., Michigan State University, E. Lansing, MI 48824

The effects of renzapride (Renz) and cisapride (Cis) on fast nicotinic excitatory postsynaptic potentials (epsps) were studied using conventional techniques in vitro. Epsps were evoked by single focal stimuli applied to interganglionic nerve strands. Drugs were applied by superfusion or by ejection from a pipette (ACh, 1 mM). Renz (n=16) at .01, .03, 0.1 and 0.3 μM potentiated epsps by 22±7%, 52±14%, 79±21% and 85±20% respectively. The 5-HT_3/5-HT_4 antagonist ICS 205-930 (1 μM, n=4) shifted Renz dose-response curve to the right ~30-fold. ICS alone did not affect epsp amplitude. Renz had no effect in 3 cells. Cis (n=8) at .01, 0.1 and 1 μM potentiated epsps by 20±11%, 69±25% and 88±23% respectively; this effect was blocked by ICS (1 μM). Cis had no effect in 9 cells. Renz and Cis did not affect ACh responses and resting membrane potential or resistance. These data indicate Renz and Cis can act as agonists at presynaptic 5-HT_4 receptors on some myenteric neurons. Stimulation of presynaptic 5-HT_4 receptors enhances ACh release. (Supported by DK 40210)

636.9

HETEROGENEOUS NEURONOTROPHIC RESPONSES OF EMBRYONIC GLIA TO STIMULATION BY 5-HT RECEPTOR AGONISTS. J.Liu*, M.B.Wilkie, E.Weiss and J.M. Lauder. Univ. N.C. Sch. Med., Chapel Hill, NC 27599-7090.

These studies were designed to test the hypothesis that glial 5-HT receptors mediate trophic effects of 5-HT. We found 5-HT receptor mRNAs and proteins were expressed by embryonic day 14 raphe (RR) or substantia nigra (SN) glia in vitro. RR & SN glia conditioned media with different neuronotrophic activities for 5-HT and TH neurons following treatment with 5-HT receptor agonists (10 nM 5-HT, 8-OH-DPAT or DOI). When 5-HT or TH neurons were co-cultured with homotypic or heterotypic glia, different neuronotrophic effects of 5-HT agonists were seen. Synthesis of S-100β and IGFs in glial cultures seemed to be differentially stimulated in response to selective 5-HT agonists, and 5-HT and TH neurons had typical responses to S-100β and IGF-II. RR and SN glia exhibited specific cAMP responses to 5-HT agonists, suggesting that embryonic glial 5-HT receptors are functional. These results indicate that during embryogenesis 5-HT receptors may mediate release of glial-derived factors which have neuronotrophic activity for 5-HT neurons, as well as other cells in the vicinity.

636.10

CHARACTERIZATION OF NOVEL SEROTONIN 5HT_2 RECEPTOR AGONISTS. **C.G. Johnson, J.E. Macor, C.B. Fox, L.A. Lebel, B.K. Koe, and S.H. Zorn*, Central Research Div., Department of Neuroscience, Pfizer Inc, Groton, CT 06340.**

A series of rotationally restricted phenolic analogs of the neurotransmitter serotonin (5HT) have been synthesized in which the 5-hydroxyindole portion of 5HT is replaced by a dihydropyano[3,2-e]indole. CP-118,952, CP-123,479, CP-123,028, and CP-132,484 possess lower affinity for 5HT_{1A} receptors than 5HT ([^{3}H]8-OH-DPAT, IC_{50} nM: 1900, 610, 5400, 5300, 5.2 respectively) and comparable affinity to 5HT at 5HT_2 receptors ([^{125}I]DOI, IC_{50} nM: 63, 14, 81, 14, 20, respectively). In the rat brain cerebral cortical slice preparation where agonist stimulation of 5HT_2 receptors results in the hydrolysis of phosphatidyl inositol, 100 μM concentrations of 5HT, the synthetic 5HT_2 agonist DOI, CP-118,952, CP-123,479, CP-123,028, and CP-132,484 stimulated the accumulation of inositol phosphates (IP) that is 76%, 66%, 32%, 42%, 54% and 155% above basal levels, respectively. The response to each agonist was concentration dependent, and CP-132,484 produced a maximal response that was 2-3 fold greater than that produced by 5HT. The response to CP-132,484 is selectively antagonized by 5HT_2 receptor antagonists, but not by antagonists of muscarinic or α_1 adrenergic receptors. The results indicate that a new class of compounds exemplified by CP-132,484 are 5HT_2 agonists, and as such will be useful tools in the further study of 5HT receptors.

636.11

COMPARATIVE ACTIONS OF NOVEL ANTAGONISTS AND HIGH EFFICACY AGONISTS AT 5-HT_{1A} AUTORECEPTORS: AN ELECTROPHYSIOLOGICAL AND NEUROCHEMICAL ANALYSIS. J.-M. Rivet, F. Lejeune, A. Gobert, H. Canton*, G. Lavielle, J.-L. Peglion and M. J. Millan. I.D.R.S., 7 rue Ampère, 92800 Puteaux, Paris, France.

Inhibitory serotonin (5-HT)$_{1A}$ autoreceptors are localized on neurones of the dorsal raphe nucleus (DRN), the origin of ascending serotoninergic projections in the brain. Several ligands which act as antagonists of *post*-synaptic 5-HT_{1A} receptors behave as agonists at *pre*-synaptic sites owing to the greater receptor reserve of the latter. Here, we examined actions of novel 5-HT_{1A} ligands at DRN-localized autoreceptors by 1/, recording of their electrical activity in chloral hydrate-anaesthetized rats and 2/, evaluation of 5-HT turnover (5-hydroxytryptophan accumulation in NSD-1015-treated-rats) in the striatum. The high efficacy 5-HT_{1A} agonists, S 14671, S 14506, 8-OH-DPAT and WY 50,324, the partial agonists, buspirone and LY 165,163 and the 'antagonist'/partial agonists, BMY 7378 and NAN 190 all inhibited DRN firing and 5-HT turnover. S 14671 was exceptionally potent; ID_{50} = 0.16 µg/kg, i.v. for electrical activity and 0.63 µg/kg, s.c. for turnover. In contrast, WAY 100,135, (-)-tertatolol and spiperone failed to modify DRN activity alone and reversed S 14671-effected inhibition of DRN firing. The novel and pure post-synaptic 5-HT_{1A} antagonists, S 15535 and S 15931 each inhibited DRN electrical activity and 5-HT turnover at doses close to those at which they block post-synaptic 5-HT_{1A} receptors (see adjacent presentation). Further, in distinction to BMY 7378, NAN-190, WAY 100,135 and spiperone, they failed to enhance striatal accumulation of di-hydroxyphenylalanine suggesting a lack of D_2-antagonist properties. In conclusion, 5-HT_{1A} ligands can act either as autoreceptor and post-synaptic agonists (e.g., S 14671 and 8-OH-DPAT), as autoreceptor agonists and post-synaptic partial agonists (e.g., BMY 7378 and NAN 190) or as autoreceptor and post-synaptic antagonists (WAY 100,135, spiperone and (-)-tertatolol). In contrast, the novel ligands, S 15535 and S 15931 act as autoreceptor agonists and pure post-synaptic antagonists; these properties may confer an original therapeutic profile

636.12

S 15535 AND S 15931: NOVEL BENZODIOXOPIPERAZINE ANTAGONISTS OF POST-SYNAPTIC 5-HT_{1A} RECEPTORS DISPLAYING HIGH POTENCY, PURITY AND SELECTIVITY M. J. Millan*, H. Canton, A. Gobert, J.-M. Rivet, F. Lejeune, K. Bervoets, M. Brocco and J.-L. Peglion. I.D.R.S., 7 rue Ampère, 92800 Puteaux, Paris, France.

Of proposed antagonists of post-synaptic 5-HT_{1A} receptors, several are also 5-HT_{1B} /β-adrenoceptor antagonists ((-)-tertatolol), D_2 antagonists (BMY 7378, spiperone and WAY 100,135) or α_1-adrenoceptor antagonists (NAN 190). Further, certain are partial agonists (BMY 7378 and NAN 190). We have, thus, synthesized benzodioxopiperazine derivatives as novel 5-HT_{1A} antagonists. Affinity at 5-HT_{1A} sites (pKi) and *in vivo* potency for inhibition of 8-OH-DPAT-induced flat-body posture, corticosterone secretion and hypothermia in rats were determined. S 15535 and S 15931 yielded values of 8.7/2.3 mg/kg (mean ID_{50}, s.c., across all tests) and 8.8/1.4 mg/kg, respectively. Maximal antagonism of 8-OH-DPAT-induced hypothermia was 95% and 97% for S 15535 and S 15931, respectively. These values were superior to those for BMY 7378 and NAN 190; 66% and 71%, respectively. Further, ≥ 50-fold higher doses of S 15535 and S 15931 than required for inhibition of 8-OH-DPAT-induced hypothermia failed to reduce core temperature when given alone. They were, thus, pure antagonists. Affinities of S 15535 and S 15931 for other 5-HT receptor types, α_1-, α_2- and β-adrenoceptors, D_1- and D_2-receptors were ≥ 100-fold lower than for 5-HT_{1A} receptors. Further, *in vivo*, whereas BMY 7378 acted as a D_2 antagonist (e.g., inhibition of dopaminergic stereotypies) at similar doses as for 5-HT_{1A} antagonism, S 15535 and S 15931 showed ≥ 25-fold separation. In addition, while NAN-190 induced ptosis (reflecting α_1-antagonism) at doses 20-fold *lower* than for 5-HT_{1A} antagonism, S 15535 and S 15931 were active only at 25- and 7.5-fold *higher* doses, respectively. In conclusion, S 15535 and S 15931 are potent, pure and selective antagonists at post-synaptic 5-HT_{1A} receptors. Apart from their utility as pharmacological tools, they may have therapeutic potential as, for example, promnesics or anxiolytics devoid of the secondary actions of post-synaptic 5-HT_{1A} agonists.

636.13

STEREOSELECTIVE BLOCKADE OF THE GUINEA PIG 5-HT TERMINAL AUTORECEPTOR AND THE 5-HT_{1D} BINDING SITE BY THE OPTICAL ISOMERS OF METITEPINE. L.M. Hawkins, L.O. Wilkinson,* M.S. Beer, M. Hibert§ and D.N. Middlemiss. Merck, Sharp and Dohme, Terlings Park, Harlow, Essex, U.K. and §Marion Merrell-Dow, Strasbourg, France

These experiments used the chiral 5-HT antagonist metitepine to examine the stereoselectivity of the 5-HT_{1D} binding site and the 5-HT terminal autoreceptor in guinea pig, a proposed model of 5-HT_{1D} receptor activation. The 5-HT_{1D} binding site (labelled by [^{125}I] GTI using 1 µM 5-HT to define non-specific) and K^+ stimulated [^{3}H]-5-HT release (25 mM) were measured in guinea pig frontal cortex.

	Apparent pA_2 against 5-HT	Sumatriptan	pIC_{50}
(+) Metitepine	6.7	7.1	7.2
(-) Metitepine	7.8	7.8	7.7

These data support the identification of the terminal 5-HT autoreceptor in guinea pig frontal cortex as a 5-HT_{1D} receptor and reinforce similarities between the 5-HT_{1D} and the 5-HT_{1B} receptor, which has similar stereoselectivity for the isomers of metitepine.

636.14

SPECIES DIFFERENCES IN THE RECOGNITION OF TRYPTAMINES BY THE ANTAGONIST-LABELLED 5-HT_2 RECEPTOR. D.B. Wainscott, J.E. Audia, J.S. Nissen and D.L. Nelson*. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

This laboratory has reported that selected ergolines displayed species differences in their affinity for the antagonist-labelled 5-HT_2 receptor (Nelson et al., *Soc. Neurosci. Abstr.* **17**: Abstr. #168.1, 1991). Substitutions at the N1-position were responsible for the species selectivity seen. Since ergolines contain the tryptamine pharmacophore, the present work was undertaken to determine if simple molecules, such as the tryptamines, also show a similar structure-activity relationship. Selected tryptamines were therefore examined at the rat and human [^{3}H]ketanserin-labelled 5-HT_2 receptor with the following results.

	IC_{50} Rat, (nM)	IC_{50} Human, (nM)
Tryptamine	10929 ± 315	3000 ± 240
N1-Isopropyltryptamine	3858 ± 602	7034 ± 532
5-Methoxytryptamine	3791 ± 143	943 ± 109
N1-Isopropyl-5-MeO-tryptamine	3561 ± 266	6327 ± 133

The tested N1-unsubstituted tryptamines had higher affinity for the human versus the rat 5-HT_2 receptor, while the N1-isopropyltryptamines had higher affinity for the rat versus the human 5-HT_2 receptor. Addition of an N1-isopropyl group decreased affinity at the human 5-HT_2 receptor and either increased affinity at the rat 5-HT_2 receptor or had no effect. These results show that the tryptamines have a similar species selectivity as the ergolines and provide evidence that the ergolines bind to the antagonist-labelled 5-HT_2 receptor in an orientation similar to that of the tryptamines.

636.15

SPECIES SELECTIVITY OF CERTAIN SUBSTITUTED ERGOLINES AND TRYPTAMINES FOR THE AGONIST LABELLED 5-HT_2 RECEPTOR. M. P. Johnson*, D. L. Nelson and M. Baez. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

Previously it had been reported (Nelson et al., *Soc. Neurosci. Abstr.* **17**: Abstr. #168.1, 1991) that a simple structural modification at the N(1) position of selected substituted ergolines showed differences in affinity for the antagonist [^{3}H]ketanserin labelled 5-HT_2 receptor depending upon the species examined. The present study was undertaken to determine whether the same structure-activity relationship exists for the agonist [^{125}I]1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) labelled 5-HT_2 receptor. Several standard compounds including 5-HT, spiperone and DOI itself were found to have equal affinity for the agonist-labelled 5-HT_2 receptor in rat and squirrel monkey homogenates. As seen with the antagonist-labelled receptor, N(1) alkyl substituted ergolines, such as mesulergine, LY 53857 and LY 237733, showed a 4-10 fold higher affinity for the rat versus monkey 5-HT_2 receptor. In contrast, N(1) unsubstituted ergolines, such as ergonovine, LY 86057 and LY 193525, showed 7-10 fold higher affinity for the monkey versus rat agonist-labelled 5-HT_2 receptor. Tryptamine and 5-methoxytryptamine (5-MT) were also found to have slightly higher affinity for the monkey rather than rat 5-HT_2 receptor. Similarly, N(1) isopropyl substitution of tryptamine and 5-MT resulted in a 6-16 fold higher affinity for the rat 5-HT_2 receptor. Interestingly, 5-MT was found to have 100-fold higher affinity than N(1) isopropyl 5-MT for the agonist-labelled monkey 5-HT_2 receptor. The present results confirm that the same structure-activity relationship is seen for the antagonist- and agonist-labelled 5-HT_2 receptor. Also, the present results suggest that certain substituted ergolines bind in a 'tryptamine-like' conformation to the 5-HT_2 receptor.

Preliminary experiments using molecular biology techniques suggest that there are relatively few transmembrane changes between species variants of the 5-HT_2 receptor. Studies designed to investigate which amino acid(s) changes are responsible for the ergoline and tryptamine structure-activity relationships seen are currently underway.

636.16

UPREGULATION OF 5-HT_2 RECEPTORS IN RODENT BRAIN BY REPEATED ORAL ADMINISTRATION OF SR 46349B A SELECTIVE 5-HT_2 ANTAGONIST. M. Rinaldi-Carmona, J. Simiand+, F. Oury-Donat, P. Soubrié, J.C. Brellière, J.P. Chambon* and G. Le Fur. Sanofi Recherche, 371 rue du Professeur J. Blayac, 34184 Montpellier Cédex 04, France. + Sanofi Recherche, 195 route d'Espagne 31036 Toulouse Cédex France.

Adaptive changes in 5-HT_2 receptor were investigated in rodents after repeated administration of SR 46349B, a potent, selective and competitive 5-HT_2 receptor antagonist (K_i = 0.72 ± 0.05nM). Chronic administration (twice a day for 3 days and on the morning of the 4th day) of SR 46349B (10mg/kg, orally) causes 24h later a marked increase of the maximum binding capacity (B_{max}) of ketanserin, measured "*ex vivo*" in brain cortical membranes, without any change in its affinity constant.

5-HT_2 receptor number is increased by 31% in rats and by 100% in mice. Further, 5-HT_2 agonist administration (1mg/kg, i.p. ± DOI) produced in chronic SR 46349B (10mg/kg, orally) treated mice a significant increase (+ 41%) of the amount of (^{3}H) inositol phosphate compared to corresponding controls. In addition, subacute administration of SR 46349B (10mg/kg, orally) causes a 4 or 2 fold increase of the head-twitch responses to 5-HTP (200mg/kg, i.p. ; in mice) or to (±) DOI (0.5mg/kg, i.p. ; in rats), respectively.

These results show that chronic administration of SR 46349B produced a parallel enhancement in 5-HT_2 receptor number, in 5-HT_2 receptor-linked signal transduction and in 5-HT_2 receptor-mediated behavioural responses, in rodents. These findings suggest for the first time that an upregulation of 5-HT_2 receptors occurs following repeated treatment with a selective antagonist.

636.17

IDENTIFICATION AND CHARACTERIZATION OF BINDING SITES FOR ^{3}H-SERTINDOLE.

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An important new advance in the pharmacotherapy of schizophrenia has been the introduction of drugs which have anti-psychotic activity without extrapyramidal side effects (e.g., dystonia, parkinsonism, and akathisia) usually seen with classical neuroleptic drugs. The exact molecular mechanism of action of atypical neuroleptic drugs is still unknown. The apparent lack of a common receptor binding profile (Sanchez et al., 1991; Drug Devel. Res. **22**, 239) that is correlated with the anti-psychotic effects of atypical neuroleptics suggests that a yet unknown receptor mechanism may be involved. To pursue this, the binding of the tritiated form of a new atypical neuroleptic, sertindole (Skarsfeldt & Perregaard, Eur. J. Pharmacol. **182**, 1990, 613) to rat brain homogenate or membrane preparations, has been studied.

^{3}H-sertindole binding is trypsin sensitive with very high affinity for sertindole (7.1 x 10^{10} M^{-1}). Sertindole binding is found throughout the brain in densities between 15 and 22 fmol/mg_{tissue} with the highest densities found in the superficial layer of the frontal cortex. The affinity of a large number of standard compounds reveals that barbiturates, opiates (including sigma compounds), benzodiazepines, phencyclidines, Ca-antagonists as well as GABAergic, glutamatergic, cholinergic, histaminergic, peptidergic, and dopaminergic compounds have low or no affinity for the sertindole binding sites. However, compounds with a 5-HT_2 and α_1 component show high affinity for sertindole binding sites especially neuroleptics characterized as atypical.

636.18

TYPICAL TRICYCLIC ANTIDEPRESSANTS POSSESS POTENT 5-HT_{1c} RECEPTOR ACTIVITY.

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For several years, we have known that many typical tricyclic antidepressants possess potent 5-HT_2 antagonist activity and induce a down-regulation of 5-HT_2 receptors. Whether tricyclic antidepressants bind to other 5-hydroxytryptamine (5-HT) receptors has been relatively unexplored. With the recent cloning of several types of 5-HT receptors, we have begun to systematically re-examine the affinities and agonist-antagonists profiles of clinically-useful compounds in an effort to clarify their mechanisms of action. Using 5-HT_{1c} receptors transiently expressed in COS-7 cells or stable cell lines, we discovered that typical tricyclic antidepressants possessed high affinities for the cloned 5-HT_{1c} receptor. Nortryptyline, amoxapine and amitriptyline had the highest affinities (Kd's<5 nM) while clomipramine, desipramine, imipramine, doxepin, maprotiline and iprindole had intermediate affinities (Kd's 20-100 nM). Zimelidine, sertraline, nomifensine, clorgyline and fluoxetine all had weak affinities (Kd's>>1000 nM) for the cloned 5-HT_{1c} receptors. These results suggest that 5-HT_{1c} receptor blockade could contribute to the unique action of many antidepressants (supported by the PMA Foundation).

SEROTONIN RECEPTORS: ACTION ON NEUROTRANSMISSION

637.1

IN VITRO BINDING PROFILE OF COMPOUNDS HAVING AFFINITY FOR BOTH DOPAMINE AND SEROTONIN RECEPTORS

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In recent years efforts have been devoted to the development of novel antipsychotic drugs because of the several unwanted side effects of classical neuroleptics among which extrapiramidal symptoms are prominent. It appears that the severity of side effects is reduced with drugs which are also 5HT-2 antagonists. As a consequence much effort has been oriented towards obtaining drugs simultaneously affecting both systems. Within this context we have adopted a dual approach investigating the pharmacological activity of compounds in which a sulpiride like moiety was combined to a functional group having affinity for the serotonin receptor and for a series of p-dimethoxybenzoquinoline isosters, where the carbon atom in position 4 was replaced by an heteroatom. The pharmacological profile of the above mentioned compounds was investigated by means of radioreceptor binding techniques. In particular activity on D1 and D2 receptors was evaluated using rat striatum membrane preparations. Tritiated SCH 23390 (D1) and spiroperidol (sulpiride displaceable, D2) were used as selective ligands. Cortical membranes and tritiated ketanserin were used to determine the activity on 5HT-2 receptors. In some occasion the activity on 5HT-1 and 5HT-3 receptors was also assayed using the appropriate ligand and tissue preparation. The binding studies indicate that p-dimethoxy-4-naphtho-oxazine and the p-dimethoxy-4-naphtho-thiazine compounds had the dual activity on dopaminergic and serotoninergic receptors with IC_{50} ranging between1.8x10^{-7} and 1.5x10^{-6}M. It appears that in this series of compounds the p-dimethoxybenzene moiety confers the affinity for both dopamine and serotonin receptors.

637.2

ENHANCED PLATELET INTRACELLULAR CALCIUM RESPONSE TO SEROTONIN IN BIPOLAR DISORDER AND MELANCHOLIC MAJOR DEPRESSION.

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There has been extensive interest in central serotonergic dysfunction as an important factor in the etiology of affective disorders. The readily accessible human platelet possesses serotonin-2(5-HT2) receptors and has been suggested as a possible model for the central serotonergic neuron. In this study, 5-HT-stimulated intracellular calcium(Ca) mobilization was measured in the platelets of depressed patients to assess 5-HT2 receptor function, using the Ca-sensitive fluorescent probe fura-2. Informed consent was obtained from all patients and normal subjects. The 5-HT-induced Ca response was significantly higher in unmedicated patients with bipolar disorder and melancholic major depression than in those with non-melancholic major depression and normal controls. The enhanced Ca response to 5-HT failed to correlate with severity of depressive symptoms. In patients with bipolar disorder and melancholic major depression, there was no significant difference in 5-HT-stimulated Ca response between unmedicated group and euthymic-treated group. These results suggest that 5-HT2 receptor function is increased in some type of depression and that the enhanced Ca response to 5-HT may be trait dependent rather than state dependent.

637.3

ENDOGENOUS SEROTONIN UPTAKE INHIBITORS ISOLATED BY CALMODULIN-SEPHAROSE AFFINITY CHROMATOGRAPHY

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In our previous attempts to isolate endogenous serotonin uptake inhibitors, we discovered endogenous compounds which were recognized by rabbit anti-imipramine antibodies. The specificity of our antisera indicated that they possess high affinity for drugs sharing a common structural component - N,N-dimethylaminopropyl aliphatic chain linked to the 5-nitrogen atom of the 10,11-dihydroazepine ring. Many tricyclic antidepressants contain such a backbone structure. The structurally related compound chlorpromazine or its derivatives immobilized on various gels were previously used for affinity chromatography purification of calmodulin. We therefore examined the use of Sepharose-immobilized calmodulin to isolate the endogenous serotonin uptake inhibitors, assuming that they possess the common structural component as described above. Calf brain and human plasma extracts and human urine were chromatographed on Calmodulin-Sepharose affinity columns. Multiple substances which inhibit platelet serotonin uptake, ^{3}H-imipramine binding and or ^{3}H-paroxetine binding were isolated and appeared to be confined to the fractions displaced by EGTA. Further purification by Bio-Gel P-2 exclusion chromatography yielded substances which were recognized by anti-imipramine and or anti-paroxetine antibodies. The interaction of these endogenous compounds with calmodulin implicates the Ca^{++} dependent/calmodulin regulatory mechanism of serotonin uptake.

637.4

ANTIBODY RECOGNITION OF ENDOGENOUS SEROTONIN UPTAKE INHIBITORS

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Radioimmunoassay (RIA) enables the quantification of very low concentration of hormones or drugs in human serum or tissue extracts. In our previous attempts to isolate endogenous serotonin uptake inhibitors, we identified substances in calf brain and human plasma extracts which reacted positively with anti-imipramine antibodies (Psychiatry Research, 34:205, 1990). In order to further characterize the RIA positive substances, we characterized several polyclonal antibody preparations against other common psychotropic drugs: a neuroleptic (haloperidol), a potent non-tricyclic uptake inhibitor (paroxetine), a tricyclic antidepressant with potent norepinephrine but weaker serotonin uptake inhibition effect (desipramine) and chlorimipramine, an imipramine analogue. The affinity of these antibodies for a variety of serotonergic and related compounds as expressed in IC_{50s} differ profoundly. Calf brain and human plasma extracts after Bio-Gel P-2 chromatography yielded fractions which differed in their antibody reactive profile. The relationship of these antibody-reactive substances to fractions which also demonstrated inhibition of serotonin uptake and ^{3}H-imipramine binding is presented.

637.5

EFFECTS OF GLUTAMATE AND/OR DEXAMETHASONE ON SEROTONIN-INDUCED INTRACELLULAR CALUCIUM MOBILIZATION IN C6 GLIOMA CELLS. H.Shinno, M.Mikuni*, A.Kagaya, K.Saitoh, S.Yamawaki and K.Takahashi. Div. Mental Disorder Res., Natl. Inst. Neurosci., NCNP, Tokyo, 187, Japan and Dep. Neurol. Psychiat., Hiroshima Univ. Sch. Med., Hiroshima, 734, Japan.

We have investigated 5-HT-induced Ca mobilization in rat glioma C6 cells. As we reported in this society last year, treatment of C6 cells cultured in DMEM(Dulbecco's Modified Eagle's Medium) with 100nM dexamethasone for 24-48 hours significantly potentiated the ability of 5-HT to cause intracellular Ca mobilization in a dose and time dependent manner without alteration of the unstimulated levels of the intracellular Ca.

On the other hand, the treatment of C6 cells cultured in RPMI(Roswell Park Memorial Institute)-1640 which contains excitatory amino acids with dexamethasone lowered 5-HT-induced Ca mobilization. In addition, pretreatment of C6 cells in DMEM with glutamate, which was added 3 min prior to the 5-HT stimulation, prevented the enhancement of 5-HT-stimulated Ca mobilization induced by 100nM dexamethasone, while other excitatory amino acids such as aspartate did not.

These results are suggesting that there is an interaction between glutamate and dexamethasone-induced enhancement of 5-HT-2 receptor function in C6 cells.

637.6

ANTAGONISM OF MK212-INDUCED INCREASE IN NOREPINEPHRINE (NE) TURNOVER IN RAT HYPOTHALAMUS BY $5HT_{1C/2}$ RECEPTOR ANTAGONISTS SERGOLEXOLE (SE, LY281067), AMESERGIDE (AM, LY237733) AND LY53857 (LY). D.T.Wong*, D.A.Mayle, R.D.Marsh and P.G.Threlkeld. Lilly Research Labs., Lilly Corp. Center, Indianapolis, IN 46285

SE, AM and LY, ergoline $5HT_2$ antagonists (Cohen et al, JPET 235:319, 1985; 251:1006, 1989; Misner et al, J.Med.Chem. 33: 652, 1990), exhibited higher affinities for $5HT_{1C}$ receptors labeled by [^{3}H]-mesulergine in membranes of bovine choroid plexus (pKi = 8.19, 8.57, 8.47) than for $5HT_2$ receptors labeled by [^{3}H]-ketanserin in membranes of rat cerebral cortex (pKi = 7.97, 8.11, 7.77). Thus, SE, AM and LY had 1.7, 3 and 5 ($p < 0.001$) times higher affinity for $5HT_{1C}$ sites than for $5HT_2$ sites. MK212, a $5HT_{1C/2}$ agonist, at 5 mg/kg i.p., increased hypothalamic levels of catecholamine metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxy-4-hydroxy-phenylglycol sulfate (MHPG-SO4) (Mayle et al, 21st Meeting Soc. Neurosci. 1991, p. 90), and that increase in DOPAC was antagonized most potently with LY, followed by AM and SE (ED_{50}s: 0.7, 1, 3 mg/kg). The MK212-induced increases of HVA and MHPG-SO_4 were also antagonized , while the ergolines alone had no effect. These data show that the antagonism of MK212-induced increase of NE turnover in hypothalamus by the ergolines correlates better with their affinity of $5HT_{1C}$ receptors than of $5HT_2$ receptors.

637.7

CHARACTERIZATION OF POTASSIUM CONDUCTANCE INCREASED BY SEROTONIN IN AREA CA3 OF HIPPOCAMPAL SLICES D. Okuhara*, K. Choi and S.G. Beck, Department of Pharmacology, Loyola University Chicago Stritch School of Medicine, Maywood, Il 60153.

The hippocampus receives extensive 5-hydroxytryptamine (5-HT) innervation from the median and dorsal raphe. 5-HT elicits a pronounced hyperpolarization in area CA3 hippocampal pyramidal cells through activation of a $5\text{-}HT_{1A}$ receptor. The characteristics of the $5\text{-}HT_{1A}$ mediated hyperpolarization in areas CA1 and CA3 differ in many respects. This study was designed to compare the potassium conductance increased by $5\text{-}HT_{1A}$ activation in areas CA1 and CA3. Standard intracellular recording techniques for current and voltage clamp were used. The hyperpolarization in area CA3 was not altered when either potassium chloride or potassium methylsulphate electrodes were used, but was blocked with cesium chloride filled electrodes. Under voltage clamp 5-HT elicited an outward current with a reversal potential of approximately -105 mV in 3 mM KCl artificial cerebrospinal fluid. The reversal potential shifted when the extracellular potassium concentration was changed to 5 or 10 mM KCl. The maximal response in area CA3 (approximately 600 pA) was three times larger than the amount of current elicited by $5\text{-}HT_{1A}$ activation in area CA1. The voltage dependency of this potassium conductance is currently under investigation. These results indicate that $5\text{-}HT_{1A}$ receptor activation increases potassium conductance in both CA1 and CA3. Supported by KO2 MH00880 and NS28512.

637.8

ANALYSIS OF THE ROLE OF 5-HT AND 5-HT RECEPTOR SUBTYPES IN MEDIATION OF THE PERISTALTIC REFLEX OF THE GUINEA PIG DISTAL COLON P. R. Wade*, G. Joseph, R. Chouinard, and M. D. Gershon Dept. of Anatomy and Cell Biology, Columbia Univ. P & S, New York, NY.

The peristaltic reflex, a coordinated propulsive wave of intestinal motility, is evoked in the guinea pig distal colon *in vitro* by insertion of an artificial fecal pellet. This activity occurs at reproducible rate that is maintained for hours. We tested the hypothesis that 5-HT plays an essential role in mediating this reflex. Propulsion of artifical fecal pellets in the distal colon *in vitro* was found to be blocked by tetrodotoxin (0.1 μM) and diminished, but not abolished, by hexamethonium (0.1 mM). The reflex is thus nerve-mediated and involves nicotinic cholinergic synapses; however, a non-cholinergic parallel pathway also exists. Propulsion was eliminated by desensitization of 5-HT receptors with 5-HT (1-10 μM), and by antagonists at $5\text{-}HT_{1P}$ (N-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide [50 μM]) and $5\text{-}HT_4$ receptors (tropisetron [>1 μM]). In contrast, $5\text{-}HT_3$ antagonists (granisetron [10 μM] or tropisetron [< 1 μM]) had no effect. Other compounds that affected the $5\text{-}HT_{1P}$-mediated slow EPSPs evoked in ileal myenteric neurons and/or $5\text{-}HT_4$ receptors (including substituted benzamides such as renzapride [> 1μM] and zacopride [> 1μM]) all inhibited propulsion. These data show that 5-HT participates in the peristaltic reflex in the guinea pig distal colon and that both $5\text{-}HT_{1P}$ and $5\text{-}HT_4$, but not $5\text{-}HT_3$ receptors are involved. Supported by NIH grant NS 12969.

637.9

5-HT INCREASES cAMP IN GANGLIA ISOLATED FROM THE GUINEA PIG SMALL INTESTINE: MEDIATION BY A NOVEL 5-HT RECEPTOR. E. Fiorica-Howells and M.D. Gershon*. Dept. of Anat. & Cell Biol., Columbia Univ. P & S, New York, N.Y. 10032.

The slow depolarization evoked in enteric neurons by 5-HT is mimicked by elevation of intracellular cAMP. We therefore tested the hypothesis that 5-HT increases cAMP in neurons of the myenteric plexus of the guinea pig small intestine. Ganglia were isolated by differential filtration from collagenase digests of dissected preparations of longitudinal muscle with adherent myenteric plexus. The isolated ganglia (trapped on filters) were incubated with putative agonists for 10 min at 37° C in the presence of isobutylmethyl xanthine (10 μM; IBMX) and/or preincubated with putative antagonists for 10 min. cAMP was measured by radioimmunoassay. 5-HT induced a concentration-dependent increase in cAMP (ED_{50} = 0.3 μM). This effect was not shared by the $5\text{-}HT_{1P}$ agonist, 5-hydroxyindalpine (10 μM), or blocked by the $5\text{-}HT_{1P}$ antagonist, N-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide (5-HTP-DP; 0.1 mM), which mimic or antagonize the 5-HT-evoked slow depolarization of myenteric neurons that is also elicited by raising cAMP. It is thus unlikely that the slow depolarization evoked by 5-HT is mediated by cAMP. Compounds that raised cAMP in isolated ganglia included: (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), 5-carboxyamidotryptamine, 5-methoxytryptamine, and renzapride. Antagonists (at 10 μM) at $5\text{-}HT_1$, $5\text{-}HT_2$, $5\text{-}HT_3$, and $5\text{-}HT_4$ receptors, including methiothepin, methysergide, spiperone, tropisetron, DAU 6285, R58639, and R88226 all failed to prevent the rise in cAMP in response to 5-HT or other agonists. The response was inhibited by a high concentration of ketanserin (10 μM); however, mRNA encoding the $5\text{-}HT_2$ receptor could not be detected in isolated ganglia by Northern analysis. The elevation of cAMP induced in isolated myenteric ganglia by 5-HT thus cannot be attributed to mediation by a known subtype of 5-HT receptor and thus must be mediated by a novel 5-HT receptor subtype. Supported by NIH: NS 12969 and NS22637.

637.10

CHARACTERIZATION OF THE SEROTONIN UPTAKE SITE AND THE 5-HT1C RECEPTOR IN THE FAWN-HOODED RAT. B.A.Hulihan-Giblin*, Y.D. Park, C.S.Aulakh and D. Goldman. Laboratory of Neurogenetics, NIAAA Bethesda, MD 20892

The Fawn-Hooded (FH) rat strain possesses a platelet storage pool deficiency. In addition to the reduced peripheral accumulation of serotonin (5-HT), there is considerable evidence that central serotonergic function is altered in the FH rat. There is substantial behavioral and pharmacological data to indicate that $5\text{-}HT_{1C}$ receptors are altered in the FH strain relative to Sprague-Dawley (SD) and Wistar rats. Earlier reports have suggested diminished [^{3}H]imipramine binding to platelets and in brain tissue of FH rats, but these results have not been replicated. In the study presented here, three rat strains, FH, SD and Wistar were compared for differences in both the $5\text{-}HT_{1C}$ receptor and the serotonin (5-HT) uptake site. [^{3}H]Mesulergine was used to label $5\text{-}HT_{1C}$ brain receptors in four brain regions. In the hippocampus, hypothalamus and striatum there were no significant differences in either the Bmax or kD values among the three strains. However, the Bmax values for [^{3}H]mesulergine binding in the cortex were significantly greater in FH as compared to SD and Wistar, while affinity constants (kD) were significantly lower. [^{3}H]Paroxetine, was used because of its selectivity in labelling the 5-HT uptake site. In the cortex, the Bmax values for the FH strain were significantly greater than SD or Wistar, while in the hippocampus the FH Bmax values were significantly greater than SD, but not Wistar. However, the Bmax values for [^{3}H]paroxetine binding in the hypothalamus were significantly less than those of the SD and Wistar rats. Preliminary studies did not reveal any kD differences in the 5-HT uptake site among the three strains. The regional serotonergic differences found in the FH strain, relative to SD and Wistar, provide some support for the use of the FH rat as a genetic model for disorders such as alcohol abuse, anxiety and depression; which have been linked to serotonin dysfunction.

637.11

SEROTONIN-INDUCED POTASSIUM INCREASE IN THE RAT CEREBROSPINAL FLUID AS MEASURED WITH AN ION-SELECTIVE ELECTRODE. T. R. Yu and L. Yu*. Dept. of Medical & Molecular Genetics, Indiana Univ. School of Medicine, Indianapolis, IN 46202.

Serotonin (5-HT) exerts diverse physiological effects in the central and peripheral nervous systems and in smooth muscle by interacting with pharmacologically distinct 5-HT receptor subtypes. The 5-HT_{1C} receptor is found in many brain regions, and is particularly enriched on the epithelial cells of the choroid plexus. To investigate physiological functions that the 5-HT_{1C} receptor may mediate, the level of potassium in the cerebrospinal fluid (CSF) was measured by an ion-selective electrode upon serotonin stimulation of the rat choroid plexus. Anesthetized rats were placed on a stereotaxic unit, and a potassium-selective electrode was positioned in the right lateral ventricle. Infusion of 10 μl of serotonin solution into the left lateral ventricle consistently produced a small but detectable increase in the CSF potassium level. The increase could be detected within 1-3 min of serotonin infusion, and lasted between 5-10 min. These data suggest that the effect may be a result of the activation of the 5-HT_{1C} receptor on the choroid plexus upon its exposure to CSF-borne serotonin, which produces a modulation in the rate of potassium filtration across the choroid plexus.

637.12

MODULATION OF THE ELECTRICALLY-EVOKED RELEASE OF [^{3}H]5-HT IN THE GUINEA PIG BRAIN BY 5-HT_3 RECEPTORS. P. Blier* and C. Bouchard. Neurobiological Psychiatry Unit, Dept. of Psychiatry, McGill University, Montréal, Québec, Canada H3A 1A1.

5-HT_3 receptors have been shown to modulate the release of noradrenaline, acetylcholine, dopamine, and cholecystokinin in the brain. We have recently reported on the capacity of 5-HT_3 receptors to modulate the electrically-evoked release of [^{3}H]5-HT in preloaded slices of the guinea pig hypothalamus (Eur. J. Pharmacol. 211:365,1992). The present study was undertaken to determine whether these 5-HT_3 receptors are also present in other regions of the limbic system and to assess their physiological relevance. The selective 5-HT_3 agonist 2-methyl-5-HT (0.1-3 μM) enhanced in a concentration-dependent manner the electrically-evoked release of [^{3}H]5-HT from preloaded slices of frontal cortex, hippocampus, and hypothalamus when added to the superfusate 8 min before the stimulation, without affecting the basal outflow of radioactivity. At 10 μM, 2-methyl-5-HT increased the basal outflow of radioactivity but this effect did not occur when using a calcium-free superfusate. The enhancing effect of 2-methyl-5-HT (1 and 3 μM) on the evoked release of [^{3}H]5-HT was blocked by the 5-HT_3 antagonists zacopride, MDL 72222, BRL 24924, ICS 205-930, ondansetron, BRL 46470A, quipazine, and mCPP. TFMPP and (+)tubocurarine were ineffective. When 2-methyl-5-HT was introduced 20 min before the stimulation, it did not alter the evoked release of [^{3}H]5-HT, suggesting that these 5-HT_3 receptors desensitize rapidly. The enhancing effect of 2-methyl-5-HT was not present when a calcium-independent release of [^{3}H]5-HT was elicited with fenfluramine. The 5-HT reuptake blocker paroxetine enhanced the overflow of [^{3}H]5-HT when introduced 8 min before the stimulation; this effect of paroxetine was blocked by ICS 205-930. These 5-HT_3 receptors can therefore be activated by synaptic 5-HT.

637.13

α_2 MEDIATED CONTROL OF 5-HT NEURONAL FIRING IN GUINEA-PIG DORSAL RAPHE NUCLEUS. M.K.Mundey*, C.A.Marsden* & A.Fletcher[1]* (SPON: Brain Research Association). Dept. Physiol. & Pharmacol, Q.M.C. Nottingham. NG7 2UH, U.K. [1]Wyeth Research Ltd. Huntercombe Lane South, Taplow, Maidenhead, Berkshire. SL6 OPH, U.K.

The selective 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT) produces a reversible inhibition of the firing of serotonergic neurones in the rat dorsal raphe nucleus (DRN) an effect also demonstrated in the guinea-pig DRN. Previous studies in the rat have demonstrated that the α_2 antagonist idazoxan increases the firing of identified 5-HT units in the DRN. The present study investigates the effects of idazoxan on 5-HT neuronal firing in the guinea-pig DRN. Male Dunkin-Hartley guinea-pigs (280-350g) were anaesthetised with urethane (1.3g/kg i.p.) and the jugular vein cannulated for i.v. administration of drugs. Single barrelled glass electrodes, filled with 2M NaCl containing 2% pontamine sky blue, were implanted into the DRN using stereotaxic coordinates taken from lambda (A +0.7-1.0; L 0.0; V -6.5-7.5mm). 5-HT neurones were identified by their slow, regular, firing pattern (0.5-4.0 spikes/sec) and their reversible inhibition by 8-OHDPAT (10μg/kg). The α_2 antagonist idazoxan (10μg/kg) significantly increased the firing rate of neurones in the DRN sensitive to 8-OHDPAT and revealed that these neurones exibited one of two types of firing pattern. The first type consisted of a phasic bursting pattern; each cycle lasting between 60 and 130 secs with the rate oscillating from 4-8 spikes/sec at the beginning, to 20-32 spikes/sec at the end (n=7). This effect was not attenuated by 8-OHDPAT but the α_2 agonist clonidine (15μg/kg) significantly decreased the bursting activity with normal spontaneous activity returning within 8-20 mins. With the second type idazoxan also increased the firing rate (82±5%, n=16) of 8-OHDPAT sensitive cells but without producing a phasic firing pattern. These results suggest that there may be two types of serotonergic cells in the guinea-pig DRN responsive to idazoxan. Furthermore the activity of the 5-HT neurones in the DRN of the guinea pig are under an α_2 adrenergic inhibitory tone as in the rat DRN.

M.K.M. is a SERC CASE student in conjunction with Wyeth Research (U.K.) Ltd.

637.14

CHARACTERIZATION OF SEROTONIN (5-HT) RECEPTORS ON NEURONS OF THE DIAGONAL BAND OF BROCA OF THE RAT. Wai Ling Lee* and J.P.Gallagher, Dept. of Pharmacology & Toxicology, University of Texas Medical Branch, Galveston, TX 77550.

5-HT has different actions at neurons in the vertical versus horizontal limb of the diagonal band of Broca (vnDBB, hnDBB). The principle effect of 5-HT on neurons in the vnDBB was membrane depolarization (10 out of 14 neurons, 72%); two neurons (14%) did not respond, while two were hyperpolarized. On the other hand, in the hnDBB (n=46) the majority (70%) of neurons were hyperpolarized by 5-HT, 10% did not respond, 10% exhibited a transient or biphasic membrane potential change, and 10% were depolarized by 5-HT.

In an attempt to classify the sub-type of 5-HT receptor responsible for membrane hyperpolarization of neurons in the hnDBB, agonist activity and antagonist efficacy were determined. 5-HT (1-30 μM) induced primarily a concentration dependent membrane potential hyperpolarization which was associated with a reduction of cell input resistance. This hyperpolarizing action was mimicked by the following 5-HT_{1A} agonists: 5-CT (nM), ipsapirone (μM), and 8-OH-DPAT (μM). The putative 5-HT_{1A} antagonist, BMY 7378 (2-10 μM) was effective, while NAN-190 (10-100 μM) was ineffective.

Our results demonstrate the presence of different 5-HT receptors on vnDD vs. hnDBB. Furthermore, our data with antagonists suggest that a different sub-type of inhibitory 5-HT_{1A} receptor is present on hnDBB neurons.

(Supported by NSF-BNS 9245634)

SEROTONIN: PHARMACOLOGY I

638.1

BEHAVIORAL, NEUROCHEMICAL AND ANATOMICAL EFFECTS OF NEONATAL 5,7-DIHYDROXYTRYPTAMINE (5,7-DHT) TREATMENT IN RATS. M. Mercugliano *, H.Q. Nguyen, S. Djali and I. Lucki. Children's Seashore House and Depts. of Pediatrics, Psychiatry and Pharmacology, University of Pennsylvania, Philadelphia, PA. 19104.

The consequences of the loss of serotonin (5-HT) neurons during early postnatal development were examined in rats after administration of the neurotoxin 5,7-DHT (100 μg i.c.v.) on postnatal day 1. Analysis of 5-HT content in separate groups of 4- and 8-week old rats indicated extensive depletion of 5-HT in the striatum and cortex, but not in the brainstem. Immunohistochemistry for 5-HT in 8-week old littermates revealed a 73% reduction in number of neurons in the dorsal raphe nucleus with relative sparing (27% depletion) of 5-HT neurons in the median raphe nucleus. Neonatal lesions of the 5-HT system were associated with residual changes in behavior in the forced swimming test at 4 and 8 weeks of age. Rats treated with 5,7-DHT showed decreased mobility during a 15-min forced swimming test as compared with vehicle-treated controls, which may relate to an altered response to stress. These results suggest a regionally heterogenous effect of the lesion on 5-HT content, with decreases in terminal regions and preservation in the brainstem, in spite of significant neuronal loss in the dorsal raphe. Furthermore, lesions of the 5-HT system induced during the neonatal period were associated with long-term behavioral changes which may be associated with the role of 5-HT in psychiatric conditions.

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638.2

p-CHLOROAMPHETAMINE (PCA)-INDUCED MONOAMINERGIC NEUROPATHOLOGY IN MICE: PROTECTIVE EFFECT OF MK-801. P.K. Sonsalla* and L. Manzino. Neurology Dept., UMDNJ-RWJ Med.Sch., Piscataway, N.J. 08854.

The neurotoxic effect of various amphetamines in rats differ in selectivity for dopaminergic (DA) or serotonergic (5HT) neurons. Furthermore, the neurotoxic profile of the various amphetamines differ among species. For example, methamphetamine, which produces toxicity to both DA and 5HT neurons in rats has little effect on 5HT neurons in mice. PCA, which is a potent and selective 5HT neurotoxin in rats, has been reported to be neurotoxic to 5HT neurons in mice at high doses and with continuous infusion. One goal of the present study was to investigate if other dosing paradigms for PCA would produce neurotoxicity in mice and to determine whether this toxicity was selective for 5HT neurons. Furthermore, it has been demonstrated that the NMDA receptor antagonist MK 801 attenuates or prevents both the DA and the 5HT neuropathology produced by methamphetamine in rats but fails to prevent PCA-induced 5HT toxicity in rats. A second goal was to evaluate the protective effect of MK 801 in PCA-induced neurotoxicity in mice. Two injections of PCA (50 mg/kg, i.p.) administered 6 h apart produced a 50% depletion in neostriatal serotonin and 5-hydroxyindoleacetic acid 5 days after treatment. Surprisingly, neostriatal tyrosine hydroxylase activity and dopamine content in these mice were reduced by 38% and 62%, respectively. MK 801 (2.5 mg/kg, i.p., administered 30 min before each PCA injection) completely prevented the PCA-induced changes. These results indicate that PCA is not a selective serotonergic neurotoxin in mice and that NMDA receptors appear to be involved in mediating its neurotoxic actions, at least within the mouse neostriatum.

638.3

PARA-METHYLTHIOAMPHETAMINE (MTA), A New *p*-CHLOROAMPHETAMINE (PCA) ANALOGUE, DEVOID OF SEROTONIN NEUROTOXICITY. Xuemei Huang, Danuta Marona-Lewicka and David E. Nichols*. Depts. of Pharmacology and Toxicology, and Medicinal Chemistry and Pharmacognosy, Purdue University, W. Lafayette, IN 47907.

Para-Methylthioamphetamine (MTA), an analogue of *p*-chloroamphetamine (PCA), was compared to PCA in a number of pharmacological assays. MTA was about 2-fold more potent than PCA at inhibiting synaptosomal uptake of [^{3}H]-5-HT, and about 7-fold and 10-fold less potent than PCA at inhibiting synaptosomal uptake of [^{3}H]-DA and [^{3}H]-NE. In drug discrimination assays, MTA was nearly equipotent to PCA in animals trained to discriminate saline from 3,4-methylenedioxymethamphetamine (MDMA), or two related analogues *S*-MBDB or MMAI. Superfusion experiments in brain slices show that MTA causes dose-dependent increases of tritium efflux from rat frontal cortex slices preloaded with [^{3}H]5-HT. The degree of tritium efflux induced by 10 μM MTA was comparable to that induced by an equal molar concentration of PCA. The potential neurotoxicity of MTA was examined by measuring monoamine and metabolite levels at one week following an acute dose. A 10 mg/kg dose of PCA caused a 70-90% decrease of cortical, hippocampal and striatal 5-HT and 5-HIAA levels, while twice the molar dose of MTA (21.3 mg/kg) had no effect on 5-HT and 5-HIAA levels. Thus, substitution of the *p*-chlorine atom of PCA with a methylthio yielded a potent, selective, serotonin releaser, apparently lacking serotonin neurotoxic effects. This work also supports the idea that catecholamine systems may play a critical role in the neurotoxicity of PCA-like compounds.

638.4

CHRONIC FLUOXETINE ALTERS SEROTONIN-MEDIATED HORMONE SECRETION Q. Li*, M.S. Brownfield†, G. Battaglia, T.M. Cabrera, A.D. Levy, P.A. Rittenhouse, L.D.Van de Kar. Dept. Pharmacol. Loyola Univ. Chicago, Sch. Med. Maywood, IL 60153, †Univ. Wisconsin, Sch. Vet. Med., Madison, WI 53706.

The endocrine responses to serotonin (5-HT) agonists were used to assess the state of serotonergic function after chronic treatment with antidepressants. Fluoxetine (10 mg/kg), a 5-HT uptake blocker, was compared with the norepinephrine uptake blocker, desipramine (DMI 5 mg/kg). Both were injected (i.p) once a day for 21 days. MK-212 (5-HT$_{1-2}$ agonist 0-20 mg/kg i.p), RU 24969 (5-HT$_{1A/1B}$ agonist 0-10 mg/kg i.p) and DOI (5-HT$_{1C/2}$ agonist 0-5 mg/kg i.p) were administered 18 hrs after the final antidepressant injection and 30 min before decapitation. Fluoxetine potentiated the MK-212 and DOI-induced increase of plasma corticosterone, and the MK-212-induced increase of plasma ACTH level. DMI only potentiated the effect of MK-212 on plasma ACTH and corticosterone concentration. Both fluoxetine and DMI increased the effect of RU 24969 and DOI on plasma prolactin levels. Both fluoxetine and DMI potentiated the effect of DOI and the high dose of MK-212 on plasma oxytocin levels. Only MK-212 increased vasopressin levels and this effect was potentiated by fluoxetine. Fluoxetine decreased, but DMI increased, the effect of high dose MK-212 on plasma renin concentration. Neither fluoxetine nor DMI influenced the B_{max} or K_d of 5-HT$_1$ or 5-HT$_2$ receptors measured. The results suggest that fluoxetine influences 5-HT$_{1C/2}$ mediated ACTH and corticosterone release, but DMI does not. Also, both fluoxetine and DMI might influence 5-HT$_{1B}$ and/or 5-HT$_{1C/2}$ mediated prolactin secretion and 5-HT$_{1C/2}$ mediated oxytocin release. It is difficult to asses the influence of antidepressants on vasopressin secretion, because only MK-212 increased its secretion. Overall, the antidepressants potentiated most hormonal responses to 5-HT agonists. Since none of the 5-HT receptors measured were changed by treatment with fluoxetine or DMI, the influence of fluoxetine or DMI on the hormone responses to 5-HT agonists may not happen at the receptor level, but possibly at the second messenger level. (Supported by MH 45812).

638.5

ALTERATIONS IN RAPHE AND FRONTAL CORTEX SEROTONIN OVERFLOW FOLLOWING FOCAL AND SYSTEMIC ADMINISTRATION OF FLUOXETINE. K. Wozniak*, A. Pert and M. Linnoila. LCS/NIAAA and BPB/NIMH, Bld. 10, Rm 3C102, Bethesda, MD. 20892

Fluoxetine is an antidepressant drug that is a potent inhibitor of 5-hydroxytryptamine (5-HT) reuptake. This study describes in vivo assessment of this compound on serotonergic transmission in two brain regions. Male Sprague-Dawley rats were anaesthetized with chloral hydrate and stereotaxically implanted with concentric dialysis probes into frontal cortex and raphe nuclei. Microdialysis samples were collected and assayed for 5-HT content. After basal levels of 5-HT were attained in both areas, fluoxetine was applied either focally into one region or administered systemically. Focal fluoxetine (100μM) significantly increased local extracellular levels of 5-HT by approximately 400%. Both the frontal cortex and the raphe nuclei displayed similar sensitivity to fluoxetine perfusion. A concurrent decrease of 20% in 5-HT occurred in each normally perfused region following focally applied fluoxetine at the other site. Systemic fluoxetine (15mg/kg, i.p.) also significantly increased 5-HT by about 300% in the raphe nuclei, but in contrast there was a concurrent decrease in 5-HT (50%) in frontal cortex. This is an in vivo demonstration of opposite effects of systemic fluoxetine in two brain regions. Since reuptake blockade has similar effects on 5-HT in terminal as well as somatodendritic regions, it appears that the uptake sites have similar characteristics in both areas. A decrease in 5-HT overflow in the frontal cortex following application of fluoxetine to the raphe nuclei presumably reflects the activation of somatodendritic autoreceptors. The elevation of cortical 5-HT by focally applied fluoxetine on the other hand, probably activates feedback inhibitory pathways. The net effect of systemic fluoxetine appears to be determined predominantly by increased 5-HT in the somatodendritic regions which dramatically inhibits raphe neuron firing, resulting in a decrease in cortical release.

638.6

RECOVERY OF CENTRAL 5-HT AXONAL PROJECTIONS AFTER MDMA INJURY: OBSERVATIONS IN RODENTS C. Scanzello, G. Hatzidimitriou, A. Martello*, J. Katz, G. Ricaurte. Dept. of Neurology, Johns Hopkins School of Medicine and NIDA Addiction Res. Ctr., Baltimore, MD 21224.

Although the neurotoxic potential of the recreational drug, (±) 3,4-methylenedioxymethamphetamine (MDMA), has been well established, the fate of the damaged serotonin (5-HT) neurons remains uncertain. In MDMA-treated primates, there is evidence that 5-HT neuronal damage is persistent, and possibly permanent. The purpose of the present study was to determine if this was also true in MDMA-treated rodents. Rats were given MDMA (10 mg/kg; i.p) four times at 2 hour intervals. Two, 8, 16, 32 and 52 weeks later, groups (n = 8) of MDMA-treated rats, along with age-matched controls (n = 8), were analyzed for regional brain 5-HT, 5-HIAA, and [^{3}H]paroxetine-labelled 5-HT uptake sites. In addition, some animals were studied immunohistochemically using an antibody directed at 5-HT. Two weeks after MDMA treatment, there was a marked (42-82%) reduction in all presynaptic 5-HT neuronal markers, and in the density of 5-HT axons. By 16 weeks, 5-HT neuronal markers had partially recovered, and by 32 weeks, serotonergic recovery was largely complete. One year after MDMA treatment, there was evidence of sustained recovery in all brain regions. Detailed analysis of the group data revealed that while most MDMA-treated rats recovered, a few developed persistent 5-HT deficits. Immunocytochemical studies yielded results which corroborated the neurochemical findings. These results indicate that 5-HT axons in most (but not all) rats recover from MDMA injury, and argue against the notion that there is accelerated aging of brain 5-HT neurons following MDMA exposure. (Supported by NIDA grant DA05707)

638.7

DEXFENFLURAMINE NEUROTOXICITY: FURTHER PRECLINICAL STUDIES IN MICE AND MONKEYS. A. Ridenour, M. Martello, J. Katz and G. Ricaurte*. Dept. of Neurology, Johns Hopkins School of Medicine and NIDA Addiction Res. Ctr., Baltimore, MD 21224.

Recent findings indicate that dexfenfluramine, a drug prescribed for appetite suppression, damages central serotonin (5-HT) neurons in nonhuman primates (squirrel monkeys). Combined with similar observations in rodents (rats), these findings have raised concern that dexfenfluramine may damage 5-HT neurons in the human brain. The significance of the findings in squirrel monkeys and rats has been questioned on the grounds that: 1) the loss of presynaptic 5-HT neuronal markers has not been demonstrated to be permanent and 2) the only animal to have a metabolic ratio (ratio of plasma areas between dexfenfluramine and dexnorfenfluramine) similar to man is the mouse. We now report that serotonergic deficits in the dexfenfluramine-treated squirrel monkey last for at least one year after drug treatment. In addition, we report that 5-HT neurons in the mouse, like those in the rat and squirrel monkey, are damaged by dexfenfluramine. Notably, doses of dexfenfluramine which induced 5-HT deficits in the mouse did not produce significant weight loss. These results suggest that concerns over the possible health hazards of dexfenfluramine in man are well justified. Further, findings in the mouse suggest that the neurotoxic actions of dexfenfluramine are not mediated by its metabolite, dexnorfenfluramine, since only low levels of dexnorfenfluramine are found in the mouse brain. (Supported by NIDA grant DA06275)

638.8

PERSISTENT EFFECTS OF FENFLURAMINE ON DEVELOPING SEROTONIN (5-HT) AND DOPAMINE (DA) NEURONS IN REAGGREGATE TISSUE CULTURE. L. Woff, P.C. Hoffmann and A. Heller. Dept. of Pharmacol. and Physiol. Sciences, University of Chicago, Chicago, IL 60637.

Fenfluramine, an anorexic amphetamine derivative, depletes central 5-HT and DA levels in adult rats. Only the reductions in 5-HT however, are long-lasting. To study whether developing neurons would be similarly affected, reaggregate tissue cultures were prepared from neurons of mesencephalic tegmentum and corpus striatum of embryonic mice. Reaggregates were exposed to (±)-fenfluramine (10^{-7} to 10^{-4}M) between 15-22 days of culture and analyzed for monoamines. Fenfluramine decreased monoamine levels in a dose-dependent manner. Maximal reductions at 10^{-4}M fenfluramine were 79% for 5-HT, and 77% for DA. To determine whether the reductions in monoamine levels would persist, reaggregates were exposed to 10^{-5}M fenfluramine during days 15 to 22 of culture. Cultures were then allowed to recover in drug-free media for an additional 21 days. During the recovery period, samples of reaggregates were collected for analysis of monoamines. Following the 15-22d treatment period, DA and 5-HT levels in drug-treated cultures were significantly reduced from control levels. During the recovery period, an increase in monoamine levels was seen in both control and drug-treated cultures. However, the increase in monoamines in the treated groups was not sufficient to overcome the initial neurochemical insult produced by 7 days of fenfluramine exposure. The results indicate that, unlike in adult neurons, fenfluramine has marked and persistent effects on developing DA and 5-HT neurons. Supported by MH42134.

638.9

FENFLURAMINE INDUCED SEROTONIN DEPLETIONS ARE ENHANCED BY MK-801. K.E. Sabol*, J.B. Richards, C.S. Brent, L.S. Seiden. Dept. Pharm./Phys. Sci., University of Chicago, Chicago, IL

The effects of MK-801 (the non-competitive NMDA receptor antagonist) on dl-fenfluramine (FEN) induced serotonin depletions and *in vivo* release of serotonin were studied. Serotonin tissue concentrations were measured 2 wks after treatment with FEN or FEN+MK-801. FEN treated rats received either 1, 2, or 4 injections of 12.5 mg/kg DL-FEN at 1 hr intervals; FEN + MK-801 treated rats received the same FEN regimens with 2.5 mg/kg MK-801 administered 15 min before and 90 min after the first FEN injection (N=8 each gp). MK-801 did not alter FEN-induced serotonin depletions in frontal cortex and septum. MK-801 significantly enhanced FEN-induced serotonin depletions in striatum (1, 2, and 4 FEN injections), amygdala (1 and 4 FEN injections), somatosensory cortex (1 FEN injection), hippocampus (1 FEN injection), and hypothalamus (4 FEN injections). MK-801 alone significantly decreased serotonin depletions in striatum and amygdala. Serotonin release was measured in the striatum of awake rats using *in vivo* dialysis. FEN-induced serotonin release (a single injection of 12.5 mg/kg) was not altered by pretreatment (15 min) with 2.5 mg/kg MK-801.

MK-801 enhanced FEN-induced serotonin depletions in all regions except frontal cortex and septum. This result is in contrast to reports indicating that MK-801 blocks dopamine and serotonin depletions induced by methamphetamine. MK-801 did not enhance the FEN-induced serotonin release *in vivo*. This result indicates that the enhanced serotonin depletions with MK-801+FEN are not due to an enhanced increase in serotonin release. (Supported by NIDA DA-00085 & RSA10562 (L.S.Seiden).

638.10

TIANEPTINE (A 5-HT UPTAKE ENHANCER) INCREASES THE SECRETION OF CORTICOSTERONE AND RENIN BUT NOT PROLACTIN. L.D. Van de Kar*, Q. Li, A.D. Levy, P.A. Rittenhouse, and T.M. Cabrera. Dept. Pharmacology. Loyola Univ. Chicago, Stritch Sch. Medicine., Maywood, IL 60153.

Tianeptine is considered a selective serotonin (5-HT) uptake enhancer with antidepressant potential. We determined the acute neuroendocrine responses to injections of tianeptine (0-20 mg/kg, ip). Plasma prolactin was not altered by injections of tianeptine. In contrast, plasma corticosterone and renin concentrations were dose-dependently elevated. The maximal increase in plasma corticosterone was observed at a dose of 10 mg/kg, 15 minutes post-injection. Plasma corticosterone returned to normal levels within 30-60 minutes post-injection. Plasma renin concentration was elevated for a longer duration. At 2 hours post-injection, plasma renin concentration was still higher than saline injected rats. To determine whether 5-HT uptake sites mediate the neuroendocrine effects of tianeptine, rats were pretreated with the 5-HT uptake blocker fluoxetine (10 mg/kg ip) 3 hours before the injection of tianeptine (2-20 mg/kg ip). The rats were sacrificed 15 minutes after the tianeptine injection. Fluoxetine did not alter the effect of tianeptine on either plasma corticosterone or renin concentrations. The data suggest that the endocrine effects of tianeptine are mediated by a mechanism that is independent of the 5-HT uptake sites. Supported in part by MH45812 and DA04865.

638.11

UPTAKE INHIBITOR-INDUCED INCREASE IN SEROTONIN IN RAT HYPOTHALAMUS. J.J. Rutter* and S.B. Auerbach. Nelson Biol. Lab., Rutgers Univ., Piscataway, NJ 08855.

In vivo microdialysis was used to examine the effects of peripheral uptake inhibition on extracellular 5-HT and 5-HIAA in the hypothalamus of unanesthetized rats. Dialysis probes were perfused with aCSF, and samples were collected at 30 min intervals during the experiment. After stable baseline 5-HT was obtained, fluoxetine (10 mg/kg, i.p., n=5) was injected. Extracellular 5-HT was increased by 145 ± 24% within 30 min. Conversely, 5-HIAA was decreased by 20 ± 4% within 1.5 h. These alterations in 5-HT and 5-HIAA persisted for the entire 24 h observation period. To confirm the neuronal origin of the dialysate 5-HT, 8-OH-DPAT (500 ug/kg, s.c.) was administered 26 h after fluoxetine. This resulted in a 75% decrease in dialysate 5-HT.

In a similar experiment, sertraline (10 mg/kg, i.p.,n=3) resulted in a 110 ± 28% increase in 5-HT, and a 40 ± 15% decrease in 5-HIAA. These effects also lasted for 24 h post-injection. The 5-HT releaser fenfluramine (10 mg/kg, i.p.) was injected 26 h after sertraline to determine if the uptake site was still effectively blocked. This would appear to be the case, since fenfluramine only resulted in a 75% increase in 5-HT 1 day after sertraline, as compared to an 800% increase in 5-HT in control animals injected with fenfluramine.

In conclusion, selective uptake inhibitors produced a long-lasting change in 5-HT neurotransmission upon acute peripheral injection. Supported by NSF grant 9109662.

638.12

ARE THERE TWO SEROTONIN UPTAKE CARRIERS IN RAT BRAIN? S. Kongsamut*, C. Smith, J. Roehr & M. Szewczak. Neuroscience Strategic Business Unit, Hoechst-Roussel Pharmaceuticals, Somerville, NJ 08876

Molliver (see *J Clin Psychopharm* 7:3S;1987) proposed the idea of multiple serotonin (5HT) uptake carriers based on: (1) histochemical evidence that fine nerve endings from dorsal raphe serotonergic neurons (but not beaded endings from median raphe) are destroyed by p-Cl amphetamine (PCA) (2) PCA only enters these endings through a 5HT uptake carrier. We explored this using a pharmacological approach.

We first examined PCA selectivity for dorsal raphe neurons by measuring PCA-depletion of 5HT (measured by HPLC) in various brain regions. PCA (10 mg/kg) depleted 5HT equally well in the striatum (dorsal raphe input) and hippocampus (both dorsal and medial raphe).

We next examined inhibition of PCA-induced 5HT depletion by 5HT uptake blockers and found (as previously reported) that clomipramine had a shorter duration than expected. This discrepancy suggested that clomipramine might be selective for another uptake carrier. To test this, we treated rats with PCA (10 mg/kg, twice 24h apart) and allowed the PCA-sensitive neurons to degenerate over 7-10 days. The remaining [^{3}H]5HT uptake into synaptosomes from these rats (whole brain or selected brain regions) was still inhibited by the uptake blockers.

Finally, we injected rats i.p. with 5HT uptake blockers to examine any differential activity in various brain regions. Each drug inhibited [^{3}H]5HT uptake, more so in the hippocampus than in the striatum.

In summary, we find no conclusive evidence for the existence of multiple 5HT uptake carriers in the different brain regions.

638.13

EFFECT OF SERTRALINE ON MONOAMINE UPTAKE IN RATS AND MICE. Susan K. Hemrick-Luecke and Ray W. Fuller*. Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285

Sertraline (1S,4S-N-methyl-4-[3,4-dichlorophenyl]-1,2,3,4-tetra-hydro-1-naphthylamine) is a selective inhibitor of the serotonin (5HT) uptake carrier (Koe, et al., Eur. J. Pharmacol. 141:187-194, 1987). Sertraline blocked the depletion of brain serotonin by p-chloro-amphetamine (PCA), being more potent in rats (ED50 1.0 mg/kg, i.p.) than in mice (ED50 4.0 mg/kg, i.p.) when injected 1 hour before PCA. In rats, a 10 mg/kg i.p. dose of sertraline decreased brain 5-hydroxy-indoleacetic acid concentrations for as long as 24 hours, suggesting a long duration of uptake inhibition. Sertraline is metabolized to desmethylsertraline (DMS). At 8 hrs and longer times after sertraline injection into rats (10 mg/kg, i.p.), brain concentrations of DMS were higher than those of sertraline and may have contributed to 5HT uptake inhibition. Sixteen hours after sertraline injection, a time at which sertraline levels were only one-third those of DMS, the PCA-induced depletion of rat brain 5HT was antagonized with an ED50 of 7.0 mg/kg. In mice, 1-8 hour pretreatments with sertraline at 32 mg/kg had no effect on the 6-hydroxydopamine (6-OHDA)-induced depletion of heart norepinephrine (NE). Sertraline pretreatments shown to induce a decrease in the number of β-adrenoreceptors in rat brain (19.2 mg/kg, i.p., b.i.d. for 4 days), antagonized the 6-OHDA-induced depletion of rat hypothalamic NE and epinephrine (EPI). One hour after the last dose, brain levels of sertraline were 2 times and levels of DMS were 13 times those observed 1 hour after a single dose (19.2 mg/kg, i.p.) of sertraline, which had no effect on NE or EPI uptake in rat hypothalamus after 6-OHDA injection.

638.14

IN VIVO LABELING OF SEROTONIN UPTAKE SITES WITH [^{11}C]McN-5652. U.Scheffel*, M. Suehiro, R.F.Dannals, G.A.Ricaurte, H.T.Ravert, C. Steinert and M. Stathis. Departments of Radiology and Neurology, The Johns Hopkins Medical Institutions, Baltimore, MD 21205.

McN-5652 (1,2,3,5,6,10b-hexahydro-6-[4-(methyl thio) phenyl] pyrrolo-[2,1-a]-isoquinoline), a potent serotonin (5-HT) uptake blocker, was labeled with ^{11}C and evaluated in mice as a potential PET imaging agent for central serotonin (5-HT) uptake sites. Two resolved stereoisomers of [^{11}C] McN-5652, (+)McN-5652 and (-)McN-5652, and the racemate, (±)[^{11}C]McN-5652, were synthesized and studied *in vivo*. After i.v. injection, the (+) and (±) tracers accumulated rapidly and to a high degree in the mouse brain in 5-HT transporter-rich areas, whereas the inactive (-) isomer was cleared rapidly. At 60 min, hypothalamus/ cerebellar ratios were 3.9 for the (±) isomer, 5.9 for the (+) isomer and 1.3 for the (-) isomer. *In vivo* binding of the racemate was blocked in a dose-dependent manner by paroxetine and (+)McN-5652, but not by (-)McN-5652, GBR 12,909, desipramine or ketanserin. The data indicate that the *in vivo* binding of (+) and (±)[^{11}C]McN-5652 is saturable, selective and stereospecific and suggest that they may be promising radioligands for PET studies of 5-HT transporters. The labeled inactive isomer may permit an estimate of non-specific binding.

638.15

DISTRIBUTION OF SEROTONIN AXONS IN THE HYPOTHALAMUS AND HIPPOCAMPUS OF THE MOUSE. C.F. Phelix*, C. Cantu, R. Kaakaji, and M.J. Wayner, Division of Life Sciences, The University of Texas at San Antonio, TX 7249-0662

Serotonergic projections to the hypothalamus and hippocampus influence several known functions, i.e., neuroendocrine regulation, nociception, memory and anxiety. Coronal sections of the mouse forebrain were stained with the use of a rabbit anti-serotonin antibody using the unlabeled antibody peroxidase-antiperoxidase method, including a silver postintesification method. The stained tissue sections were projected onto white paper for cartography and semiquantitation. The regional distributions and relative density of serotonin axons for the mouse were compared with reports in the rat. The hypothalamus contained many, widely distributed axons with a density in the medial hypothalamus greater than the lateral hypothalamus. This pattern is reversed from the rat. The ventromedial hypothalamic nucleus displayed a reversed pattern compared to the rat. Other hypothalamic areas, e.g. paraventricular nucleus, contained moderate amounts of axons similar to the rat. In the hippocampus two major patterns were observed. A very high density of axons were present in the stratum lacunosum moleculare of the Ammon's horn. The lowest density was found in the dentate gyrus. The only pyramidal cells contacted by serotonin terminals were found in CA3. This distribution is consistent with reports in the rat but varies from reports of serotonin receptor densities in the hippocampus. Supported by UTSA-Faculty Research Award

638.16

SEROTONERGIC INNERVATION OF THE CORE AND SHELL OF THE NUCLEUS ACCUMBENS (ACB): NORMAL ULTRASTRUCTURE AND RELATIONSHIP TO CATECHOLAMINERGIC AFFERENTS. E. J. Van Bockstaele* and V. Pickel, Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, N.Y. 10021.

We examined the ultrastructural basis for known functional interactions between serotonin immunoreactive (5HT-ir) terminals, local neurons and catecholamine afferents in the core and shell of the Acb. These monoamines were identified in the same sections of tissue using the avidin-biotin immunoperoxidase method for a rabbit 5HT antiserum and silver intensified gold labeling for a mouse antibody against the catecholamine synthesizing enzyme, tyrosine hydroxylase (TH). By light microscopy, 5HT-ir processes appeared less dense and topographically more heterogeneous than the TH-ir varicosities throughout the Acb. Using electron microscopy, 5HT and TH-ir profiles included unmyelinated and a few myelinated axons and terminals. In the core, 5HT-ir terminals rarely formed synaptic junctions with neuronal targets but were more often found in apposition with other terminals most of which were not TH-ir. In fact, TH and 5HT-ir axons were usually separated by a distance of >0.5 μm in fields containing both labels. In the shell, 5HT-ir terminals (i) were larger in size (0.3-0.4 μm for shell vs. 0.1-0.2 μm for core), (ii) contained relatively more dense core vesicles, (iii) frequently formed symmetric and asymmetric contacts with dendrites and spines and (iv) were not often in contact with TH-ir axon terminals. The morphological differences suggest that 5HT terminals in the shell vs. core may be functionally distinct and may arise from different 5HT neurons. Moreover, the sparsity of direct appositions between TH and 5HT terminals suggest that release of dopamine by 5HT may occur through diffusion in extracellular space or may be indirectly mediated by targets of 5HT terminals. Supp. NS09100-01, MH40342, 00078.

638.17

NICOTINE AND COCAINE INTERACT WITH SEROTONERGIC NEURONS. H. Tamir*, K.P. Liu, M. Adlersberg, H.S. Hsiung, P.R Wade, E.A. Nunez and M.D. Gershon, N.Y. State Psych. Inst. and Columbia U. N.Y.,N.Y. 10032.

The hypothesis was tested that synaptic vesicles, which are acidic, are targets for the action of nicotine and cocaine, each of which is a weak base. Secretion of 5-HT from synaptosomes and thyroid parafollicular (PF) cells was studied in the presence or absence of nicotine (50 μM) or cocaine (50 μM). In addition, the effects of these compounds on the actions of 5-HT on receptors in the enteric nervous system was also investigated. Cocaine, but not nicotine, increased the basal release of ^{3}H-5-HT from synaptosomes. Both cocaine and nicotine enhanced the veratridine- and K^+-stimulated release of ^{3}H-5-HT from synaptosomes, an affect that persisted when $[Ca^{2+}]_e$ was lowered. Neither cocaine, nor nicotine affected the basal release of 5-HT from PF cells; however both drugs inhibited the secretion of 5-HT from PF cells in response to TSH. Trapping of acridine orange confirmed that the acidity of the 5-HT-storing PF granules increased in response to stimulation of the cells with TSH; however, both cocaine and nicotine alkalinized these granules. Intracellular records from enteric neurons indicated that nicotine (5 μM) strongly potentiated the rapid depolarization mediated by 5-HT3 receptors; this action was manifest after the depolarizing phase of the response to nicotine itself. These data show that cocaine and nicotine can interact with serotonergic elements in a complex manner that may have both pre- and postsynaptic components. Supported in part by grants NIMH 37575, NS12969, DK19743.

638.18

WITHDRAWN

638.19

Differential serotonergic innervation of spinal somatic and parasympathetic motoneurons in rats. W. Wu*, M.W. Wessendorf and R. Elde Dept. Cell Biol. & Neuroanat., Univ. Minnesota, Minneapolis, MN 55455.

This study examined whether there might be differences in peptide coexistence between the serotonergic neurons innervating somatic and parasympathetic motoneurons. Somatic and parasympathetic motoneurons were retrogradely labeled in the spinal cord with hydroxystilbamidine (Fluoro-Gold) applied to either the sciatic or pelvic nerve, respectively. Ten μm sections containing labeled neurons were stained for either serotonin (5HT) and substance P (SP), or for 5HT and thyrotropin-releasing hormone (TRH) using 2-color immunofluorescence. When sections were examined, it was found that most retrogradely labeled profiles (99% of sciatic profiles and 91% of pelvic profiles) were apposed by 5HT varicosities. In tissue stained for 5HT and TRH, 104 out of the 106 sciatic profiles that were apposed by 5HT varicosities were apposed by 5HT/TRH varicosities, as were 111 out of 113 pelvic profiles. Similarly, in tissue stained for 5HT and SP, all sciatic nerve profiles that were apposed by 5HT varicosities were apposed by 5HT/SP varicosities (319 out of 319). However, almost none of the profiles labeled from the pelvic nerve were apposed by 5HT/SP varicosities (3 out of 168). These findings suggest that about 98% of somatic motoneuron profiles were innervated by 5HT varicosities containing both SP and TRH. In contrast, at most 2% of the profiles of parasympathetic preganglionic motoneurons were apposed by 5HT/SP/TRH varicosities with the great majority being apposed by 5HT varicosities containing TRH but not SP. Thus, we conclude that different populations of raphespinal serotonergic neurons modulate the activities of sciatic and pelvic neurons. Our earlier studies have demonstrated the expression of TRH and SP receptor mRNA in the spinal cord, suggesting that these peptides could exert direct actions on spinal neurons. Supported by DA 05466 and DA 06299.

639.1

IMMOBILIZATION OF TRYPTOPHAN HYDROXYLASE BY IMMUNE ADSORPTION: A METHOD TO STUDY REGULATION BY PROTEIN KINASES. P.A. Johansen*, I. Jennings[1], R.G.H. Cotton[1], and D.M. Kuhn. Lafayette Clinic & CCN Program, Wayne State Univ., Detroit, MI, and [1]The Murdoch Institute, Royal Children's Hosp., Melbourne, Australia.

Tryptophan hydroxylase (TPH) is the initial, rate-limiting enzyme in the biosynthesis of serotonin. TPH is an unstable enzyme, and detailed studies of its regulation have been limited by the lack of highly purified, active enzyme. We have utilized immunoprecipitation as a rapid, effective method to affinity purify TPH from brain. TPH was immobilized by adsorption to Pansorbin following binding to the monoclonal antibody PH8, and resuspended pellets were assayed for TPH activity. TPH could be completely immunoprecipitated from tegmental extracts in 1.5 hours, and the recovery of TPH activity using this method was 40%. Immobilized TPH (Im-TPH) displayed an apparent K_m for BH_4 of 84 ± 13 μM, and an apparent K_m for tryptophan of 44 ± 19 μM. The V_{max} values for Im-TPH were 33-50% of those observed for crude TPH. The thermo-stability of Im-TPH was nearly identical to that of soluble TPH. Like soluble TPH, Im-TPH was completely inhibited by the catechol compound apomorphine (IC_{50}=1.56 μM), while dopamine caused partial inhibition (54%). Im-TPH was much more sensitive to activation by phosphatidylserine than soluble TPH. Purified calcium/calmodulin-dependent protein kinase produced a 2-fold activation of Im-TPH, while purified PKA failed to produce activation. Using this method it was also demonstrated that PKA phosphorylates Im-TPH, supporting our previous results with soluble TPH that phosphorylation of TPH by PKA does not activate the enzyme. These results indicate that Im-TPH retains the characteristics of the soluble enzyme and can be used to study regulation by protein kinases.

639.2

MORPHINE-INDUCED INCREASE IN SEROTONIN IN RAT DIENCEPHALON. R. Tao, S.M. Grauer, and S.B. Auerbach*. Nelson Biol. Lab., Rutgers Univ., Piscataway, NJ 08855.

Increased 5-HT release may mediate some effects of opioids. Thus, we have studied the effect of morphine on 5-HT and 5-HIAA in the diencephalon of unanesthetized rats. Dialysis probes were perfused with aCSF (140 mM NaCl, 3 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 1.2 mM Na_2HPO_4, 0.27 mM NaH_2PO_4). After stable baseline 5-HT was obtained (1.38±0.18 pg n=13), morphine (20 mg/kg, s.c.) was injected. Extracellular 5-HT was increased about 30% between 30 to 60 min, and returned to baseline 2 hr after morphine. This increase represents an average of values from 5 rats that showed no increase in 5-HT and 8 rats with increases between 30 to 100%. In contrast, for all rats, 5-HIAA levels increased steadily to a maximum of about 60% above baseline, 3 hr after injection. Intracellular breakdown of 5-HT to 5-HIAA may have limited the magnitude and time course of the effect of morphine. To test this, 1.0 mM pargyline was added to aCSF for 12 h before morphine injection. With MAO inhibited, basal 5-HT was 6.78±1.38 pg (n=5), and morphine then led to a reliable increase in 5-HT of about 65% lasting more than 3 hr. In conclusion, morphine produced a short-lasting increase in extracellular 5-HT that appeared to be limited in time and magnitude by intracellular breakdown of 5-HT to 5-HIAA. Regulation of MAO activity or access of intracellular pools of 5-HT to MAO could play a role in the extent of opioid-induced 5-HT release. Supported by NSF grant 9109662.

639.3

EFFECTS OF PROLONGED EXERCISE TO FATIGUE ON BRAIN MONOAMINES IN THE RAT. S.P. Bailey, J.M. Davis*, S.J. Kelly and E.N. Alborn. Depts. of Exercise Science and Psychology. Univ. of South Carolina, Columbia, SC 29208

The effects of prolonged exercise to fatigue on brain monoamines has yet to be described. The purpose of this experiment was to describe changes in brain monoamine concentrations in discrete brain areas following prolonged moderate intensity exercise to fatigue. Twenty-four (3 groups;n=8 each) treadmill accommodated male Wistar rats were sacrificed by decapitation at rest, following 1 hr of treadmill running, and after exhaustive treadmill running (20 $m \cdot min^{-1}$ & 5% grade). After sacrifice, brains were removed and midbrain (MB), striatal (ST), hippocampal (HI), and hypothalamic (HY) tissue were dissected, sonicated in 0.2 M PCA, and stored at -80 °C until analysis. In each brain area, norepinephrine (NE), dopamine (DA), DOPAC, 5-hydroxytryptamine (5-HT), and 5-HIAA were measured by HPLC-EC. 5-HT and 5-HIAA were elevated ($p<.05$) in all brain regions following 1 HR of exercise and at exhaustion as compared to rest. 5-HIAA was greater at exhaustion than following 1 HR of exercise in MB and ST. NE, DA, and DOPAC concentrations were elevated approximately 2-fold ($p<.05$) in MB, ST, and HY following 1 HR of exercise versus rest. However, at exhaustion NE, DA, and DOPAC concentrations in MB, ST, and HY were reduced to levels similar to rest. No differences in NE, DA, and DOPAC were found in HI. The results of this study indicate that prolonged exercise is associated with enhanced brain 5-HT and dopamine metabolism during the initial 1 HR; whereas at fatigue 5-HT metabolism is further heightened and dopamine metabolism is attenuated.

639.4

SEROTONIN RELEASE IN THE HYPOGLOSSAL (XII) NUCLEUS REGION UNDER CONDITIONS OF SUPPRESSED RAPHE SYSTEM ACTIVITY. L. Kubin*, H. Tojima, O. Taguchi, C. Reignier, A.I. Pack and R.O. Davies. University of Pennsylvania, Philadelphia, PA. 19104-4283.

Serotonin (5HT) may play an important role in controlling the excitability of XII motoneurons as both 5HT terminals and receptors are present within the XII nucleus. Previously, we found (Neurosci.Lett.136,1992) that methysergide, a non-selective 5HT receptor antagonist, depressed and 5HT increased the spontaneous activity of XII motoneurons when microinjected into the XII nucleus in decerebrate cats. In the present experiments, we studied the endogenous 5HT release in the XII nucleus region under experimental conditions associated with a suppression of activity in 5HT-containing raphe neurons. In decerebrate, paralyzed and artificially ventilated cats, the activities of the phrenic, XII and cervical motor nerves were recorded. The procedures used were: pontine microinjections of carbachol which produce a depression of cervical, XII and raphe neuronal activity similar to that occurring during REM sleep (5 cats); and injections of 8-OH-DPAT, a $5HT_{1A}$ receptor agonist, which also powerfully depresses the activity of raphe neurons (0.5 mg,s.c., 4 cats). A microdialysis probe was placed in the region of XII nucleus and perfused with artificial CSF. 5HT was electrochemically detected in successive 20 min/10 μl samples with a detection limit of 0.25 pg 5HT/5 μl. After a 3 h stabilization period, the resting 5HT level was 1.3±0.5(SE) pg/5μl for probes having *in vitro* recovery rates of 9.5-12.5%. During the carbachol-induced motor suppression, 5HT was reduced to 78±5%(SE) of control; after the recovery from the motor suppression, it increased to 109±12%(SE). Subsequent injection of 8-OH-DPAT reduced 5HT to 47±7%(SE). These data are consistent with the presence of a serotonergic drive to XII motoneurons that originates in the raphe system. During REM sleep this drive is likely to be reduced, thus contributing to the reduced tone in upper airway muscles. Supported by NIH SCOR grant HL-42236.

639.5

EFFECT OF DORSAL RAPHE NUCLEUS STIMULATION ON SOMATOSENSORY EVOKED RESPONSES IN RAT. W. Liu*, F.M. Sessler and B.D. Waterhouse. Dept. Physiol & Biophys. Hahnemann U., Phila., PA 19102

The neocortex is a major target of serotonergic axons emanating from the brainstem dorsal raphe nucleus (DRN). Despite the well documented anatomy of these projections, the potential impact of serotonin (5-HT) on the function of neocortical circuits remains unclear. In previous in vitro studies, we have observed a prominent depressant action of 5-HT on synaptically evoked excitatory postsynaptic potentials (EPSPs) and spiking activity in neurons recorded from the barrelfield region of the rat somatosensory cortex. In order to compare these actions of 5-HT with those produced by endogenous 5-HT release within a sensory cortical circuit, we examined the effects of DRN stimulation on somatosensory neuronal responses to activation of afferent sensory pathways in anesthetized rats. Recordings were made from neurons in the barrelfield region of the somatosensory cortex. Single unit responses were evoked by mechanical displacement of individual vibrissa. The spontaneous firing rate of recorded neurons ranged from 0.4 to 30.4Hz with an average of 9.1±6.5Hz. Whisker stimulation produced excitatory responses with instantaneous firing frequency of 47.1±25.8Hz (ranging from 14.0 to 122.5Hz). The effects of DRN stimulation (0.5-4Hz, 0.2ms) on these two parameters varied with stimulus intensities. Evoked responses were reduced at stimulus currents between 50-100uA and increased at currents above 200uA. Spontaneous discharge of cortical neurons was decreased at current 50uA and increased at stimulus currents above 100uA. Our results indicate that DRN stimulation with low current causes suppression of spontaneous discharge as well as unit responses to whisker stimulation. The depressant actions of DRN could be mediated by 5-HT, the major transmitter of this nucleus. While the transmitter responsible for such depressant effects cannot be identified on the basis of these experiments, the data are consistent with our in vitro observation that synaptically evoked activity of somatosensory cortical neurons can be supressed by 5-HT. Our in vitro and in vivo data support the hypothesis that serotonergic afferents to the neocortex may play a role in regulating the flow of sensory information through local sensory circuits by way of an action on synaptic transmission. (NIDA DA 05117 & AFOSR 870138 to BDW)

639.6

ORGANIZATION OF THE RAT DORSAL RAPHE(DR): INPUT/OUTPUT RELATIONSHIPS OF SELECTED SUBREGIONS WITHIN THE NUCLEUS. M.L. Kirifides*, M.A.L. Nicolelis, R.C.S. Lin and B.D. Waterhouse, Dept. of Physiol. and Biophys. Hahnemann Univ., Phila., PA 19102.

Previous studies have demonstrated the existence of a crude topographic organization of the dorsal raphe (DR) nucleus with respect to selected efferent targets in the forebrain and brainstem. Furthermore, other investigators have identified the lateral habenula, cortex, substantia nigra and the hypothalamus as major sources of input to the DR. The relationship between DR afferents and subregions of the nucleus which have specific efferent targets has not been the subject of a detailed investigation. The goal of the present study was to use retrograde fluorescent tracers to determine the sources of inputs to different subregions of the DR. Adult Long Evans hooded rats (300gms) received injections of fluorogold (.5 ul) or rhodamine microspheres (.3 ul) in the midline (p 7.0 - 9.0, l 0.0, d 6.1) or "lateral wing" (p 8.0, l .5, d 5.5) regions of the DR nucleus. Coronal sections (80 um) through the DR were examined using fluorescence microscopy. Prominent inputs to the rostral portion of the midline DR originate in the lateral habenula, lateral hypothalamus, prefrontal cortex, and substantia nigra. In contrast to the midline DR, the lateral wing region of the nucleus receives inputs from a more restricted medial zone of the lateral habenula. In addition, the lateral wings receive inputs from the dorsomedial hypothalamus and neocortex but not the substantia nigra. Overall, these data suggest that activity within medial and lateral portions of the DR may be subject to regulation by different regions of the forebrain. (Supported by NIDA DA 05117 to BDW)

639.7

SEROTONIN (5-H T) DECREASES INSPIRATORY-MODULATED SYNAPTIC CURRENT IN NEONATAL RAT PHRENIC MOTONEURONS. A.D. Lindsay* & J.L. Feldman. Systems Neurobiology Lab., Dept. of Physiological Science, UCLA, Los Angeles, CA, 90024-1527.

Spinal respiratory motoneurons typically respond to exogenously applied 5-HT with an increase in inspiratory-modulated firing and little or no increase in firing during expiration. The effect on the underlying inspiratory drive is not known. We studied the effect of 5-HT on phrenic motoneurones (PMNs) in the isolated brainstem and spinal cord preparation from neonatal rats. PMN activity was recorded intracellularly under current and voltage clamp conditions. 5-HT was applied to the phrenic nucleus via pressure ejection from micropipettes positioned over the PMN pool. The effect of 5-HT on exogenously applied glutamate was also studied. Local application of 1 mM 5-HT (pH 7.4) decreased peak inspiratory-modulated synaptic current (I_{insp}) by 23 ± 6%. As observed by others, there was a concurrent increase in PMN excitability. This included a depolarization accompanied by a tonic inward current and increased input resistance, and a leftward shift in the *fI* relationship. The 5-HT_2 antagonist ketanserin selectively blocked the increase in excitability without blocking the decrease in I_{insp} produced by 5-HT. The 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl mimicked the increase in excitability but did not decrease I_{insp}. Under current-clamp conditions firing produced by local application of 10 mM glutamate was enhanced by 5-HT, but the underlying membrane current produced by glutamate was unaffected. Therefore, a presynaptic mechanism via 5-HT_1 receptors is suggested for the 5-HT-induced decrease in I_{insp}. Supported by NIH Grant NS24742 and a Muscular Dystrophy Fellowship to ADL.

639.8

ACUTE EFFECT OF 2,4,5-TRIHYDROXYMETHAMPHETAMINE ON THE HIPPOCAMPAL SEROTONERGIC SYSTEM. M. Johnson*, H.-K. Lim, R.L. Foltz, G.R. Hanson and J.W. Gibb. Dept. Pharmacology and Toxicology and Center for Human Toxicology, University of Utah, Salt Lake City, UT 84112.

3,4-Methylenedioxymethamphetamine (MDMA) decreases tryptophan hydroxylase (TPH) activity of the rat brain shortly after administration. TPH activity is recovered after incubation under nitrogen gas and dithiothreitol, indicating that oxidation of sulfhydryl sites on the enzyme causes the inhibition. 2,4,5-Trihydroxymethamphetamine (THM) was identified as a product of MDMA metabolism and induces a long-term decline in central tyrosine hydroxylase (TH) and TPH. The purpose of this study was to determine if THM can produce a rapid decline in TPH activity similar to MDMA. Male Sprague-Dawley rats (180-250 g) were injected i.c.v. with 1 µmole THM and killed 3 h later. In vitro TPH activity was measured using HPLC-EC; TH activity was determined with a radioisotopic method. THM failed to alter cortical TPH activity but reduced striatal and hippocampal TPH activity to 86% and 54% of control, respectively. Striatal TH activity remained unaltered. 6-Hydroxydopamine (1 µmole), a structural analogue of THM, failed to reduce hippocampal TPH activity but 1 µmole of 5,6-dihydroxytryptamine (5,6-DHT), a serotonergic neurotoxin, reduced TPH activity to 5% of control. This suggests that THM cyclizes to a 5,6-DHT-like compound to induce a rapid decrease in TPH activity. Since in vitro reducing conditions failed to reverse the effects of THM and 5,6-DHT, the loss in TPH activity may differ from the changes induced by MDMA. (Supported by USPHS grants DA 00869, DA 04222 and DA 05860)

639.9

2,4,5-TRIHYDROXYAMPHETAMINE, A METABOLITE OF MDMA, DECREASES TRYPTOPHAN HYDROXYLASE ACTIVITY *IN VITRO*. I.M. Elayan, M. Johnson, H.K. Lim, G.R. Hanson, R.L. Foltz and J.W. Gibb*. Dept. Pharmacology and Toxicology and Center for Human Toxicology, University of Utah, Salt Lake City, UT 84112.

3,4-Methylenedioxymethamphetamine (MDMA) induces a rapid decline in brain tryptophan hydroxylase (TPH) activity that is reversed by incubating TPH under nitrogen gas and dithiothreitol. Intracerebroventricular injection of 2,4,5-trihydroxyamphetamine (THA), a metabolite of MDMA, also induces a rapid decline in TPH activity suggesting that the metabolite may be responsible for the MDMA-induced changes. The purpose of this study was to determine if this rapid decline in TPH activity can be reproduced by THA *in vitro*. The hippocampus and striatum from male Sprague-Dawley rats were incubated in different concentrations of THA. Contralateral tissues were used as controls. After a 1-h incubation at 37 °C under a flow of 95% O_2 and 5% CO_2, hippocampal TPH activity was decreased to 3%, 47%, 68%, 71% and 83% of control after exposure to 5, 0.5, 0.1, 0.01 and 0.001 mM THA, respectively. Striatal TPH activity was reduced to 17%, 54%, 70%, 95% and 98% of control, respectively. Incubation of TPH under nitrogen gas and dithiothreitol failed to return the enzymatic activity to control levels. In contrast to MDMA, these results demonstrate that THA decreases TPH activity *in vitro*. However, this loss in enzymatic activity is different than the rapid decline in TPH activity induced *in vivo* by MDMA since this change in activity is irreversible. (Supported by USPHS grants DA 00869, DA 04222 and DA 05860)

639.10

***IN VITRO* ELECTROPHYSIOLOGIC ASSESSMENT OF AGE-RELATED SEROTONIN AUTORECEPTOR FUNCTION.**

H. Zheng* and J.M. Lakoski. Dept. Pharmacology and Toxicology, Univ. Texas Med. Br., Galveston, TX 77555-1031.

The alteration of hormonal and neuronal receptor function have emerged as an etiology common to many age-related diseases, including decline of the female reproductive axis. Aging of serotonin (5-HT) systems has been identified to include changes in cellular physiological responses mediated by a 5-HT_{1A} autoreceptor in the dorsal raphe nucleus (DRN) as recorded *in vivo* in the reproductively middle-aged and senescent rat. Using an *in vitro* preparation of the DRN, we have addressed the pattern of age-related decline in 5-HT receptor function in this brain region.

Electrophysiologic recordings were conducted in 400µm thick slices containing the DRN from female Fischer 344 rats (6, 12, 18, 26 mo); all animals were at diestrous or constant diestrous at time of sacrifice. In slices continually perfused with 10 µM phenylephrine to activate cell firing, no significant decline in baseline spontaneous activity was observed between 12 and 26 mo groups. However, a marked age-related decline in sensitivity to 5-HT was apparent; 30 µM 5-HT produced an average of 40% vs 3% decline in cell firing in middle-aged vs old groups, respectively. Application of selective 5-HT_{1A} antagonists NAN-190 or BMY-7378 did not attenuate 5-HT responses but, rather, produced only agonist-like inhibition of cell firing (2.5-10 µM). In summary, marked age-related decline in 5-HT receptor function is apparent in the *in vitro* DRN of the female rat.

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639.11

CHANGES IN MONOAMINE LEVELS AND TURNOVER INDUCED BY SHORT-TERM FLUOXETINE TREATMENT. C.R. McKittrick*, V. Luine†, J. Marrast-Host†, and M. Frankfurt. Lab. of Neuroendocrinology, The Rockefeller University, New York, NY 10021 and †Dept. of Psychology, Hunter College, New York, NY 10021.

Fluoxetine, a clinically active antidepressant, is believed to act by inhibiting serotonin (5-HT) uptake in the brain, thus increasing 5-HT availability at the synapse. We tested the effects of short-term fluoxetine administration on monoamine levels and turnover in regions of the brain involved in affective states. Fluoxetine (10mg/kg) was administered i.p. to male rats daily for 4 days. The monoamine oxidase inhibitor, pargyline, was used to assess the rate of 5-HT accumulation. Monoamine levels in discrete brain regions were determined by HPLC. In the ventromedial hypothalamic nucleus (VMN), fluoxetine treatment significantly increased 5-HT (↑46%) while decreasing levels of 5-HIAA (↓44%), the major metabolite of 5-HT. 5-HT turnover was also decreased in the VMN (↓52%). Fluoxetine reduced 5-HIAA in the CA1 region of hippocampus (↓49%). Norepinephrine (NE) content in the dorsal raphe was significantly increased (↑99%), although 5-HT and 5-HIAA remained unchanged. No changes in monoamine content were observed in the medial preoptic area. This study shows regional variation in the effects of fluoxetine on the brain 5-HT system. Furthermore, our data indicate that, following its initial effects on 5-HT uptake, the mechanism of action of fluoxetine may involve alterations in the metabolism of both 5-HT and NE neurons.

Supported by HD12011.

639.12

SEROTONIN (5-HT) INHIBITS GASTRIC ACID SECRETION BY NONLUMINAL, VAGAL INDEPENDENT MECHANISMS. K.J. LePard and R.L. Stephens, Jr.*, Department of Physiology, The Ohio State University, Columbus, Ohio 43210.

Vagal stimulation increases luminal and portal 5-HT release. Previous studies suggest that released 5-HT produces an inhibitory tone on stimulated acid secretion. The mechanism of 5-HT-induced inhibition on stimulated acid secretion was investigated. In urethan-anesthetized rats with gastric and portal cannula, luminal and whole blood 5-HT levels were measured in response to the vagal stimulant RX77368. Basal luminal and portal 5-HT levels were elevated 1100% and 25%, respectively after intracisternal RX77368. Luminal perfusion of 5-HT (10, 30, 370 ng/10 min) had no effect on pentagastrin-stimulated acid secretion. However, systemic 5-HT (5 mg/kg,i.p.) inhibited pentagastrin-stimulated acid secretion by 67%. Bilateral cervical vagotomy did not reverse 5-HT-induced inhibition of carbachol (1 mg/kg)-stimulated acid secretion. The results suggest that 5-HT acts either through local mechanisms or splanchnic afferents to produce an inhibition in stimulated acid secretion. Supported by NIH DK 42880.

639.13

GENDER AND ESTROUS CYCLE EFFECTS OF 8-OH-DPAT ON HYPOTHALAMIC SEROTONIN. S. Maswood*, G. Stewart and L. Uphouse. Department of Biology and Department of Chemistry, Texas Woman's University, Denton, Texas, 76204

The effects of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) on the synthesis, utilization and turnover of 5-HT has been studied primarily in males. However gender as well as estrous cycle differences in 8-OH DPAT-induced behaviors have been recently reported. The present research was initiated to study the effects of 8-OH DPAT on the levels of 5-HT and 5-HIAA in female rodents and to compare the findings to males. A dose of 0.25 mg/kg of 8-OH-DPAT was injected i.p. to estrous or diestrous females and age-matched males. Thirty minutes later they were decapitated and the hypothalamus collected for HPLC analysis. Males showed the greatest change in 5-HT, 5-HIAA and the 5-HIAA/5-HT ratio. In females the effect of 8-OH-DPAT was greater in diestrous than in estrous females. These findings are consistent with previous gender and estrous cycle differences in the behavioral effects of 8-OH-DPAT.

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639.14

CO-EXPRESSION OF SEROTONERGIC AND GLUTAMATERGIC PROPERTIES BY SINGLE RAPHE NEURONS IN VITRO. M.D. Johnson*. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Immunocytochemical studies suggest that serotonergic neurons of the pontomesencephalic raphe nuclei may contain other transmitters, including glutamate, GABA, and neuropeptides. However, direct electrophysiological evidence for co-release of these transmitters has been lacking. To investigate this matter further, dissociated pontomesencephalic raphe neurons from neonatal Long Evans rats were grown in microcultures and their synaptic interactions were studied using intracellular recording techniques. Serotonergic neurons were identified by the presence of serotonin-like immunoreactivity, tryptophan hydroxylase-like immunoreactivity, or high affinity uptake of the auto-fluorescent serotonin analogue, 5,7-dihydroxytryptamine. When stimulated with a single intracellular current pulse, approximately 3% of the neurons possessing serotonergic markers evoked slow inhibitory postsynaptic potentials (ipsp's) in themselves or in other neurons; another 50% of these neurons evoked fast excitatory postsynaptic potentials (epsp's), 17% evoked biphasic fast epsp/slow ipsp responses, and 30% failed to evoke an electrogenic postsynaptic effect. Fast epsp's were observed in microcultures containing solitary, cytochemically-identified serotonergic neurons. Glutamate receptor antagonists such as kynurenate, APV and CNQX blocked the fast epsp's, while $5HT_{1A}$ receptor antagonists such as propranolol and spiperone blocked the slow ipsp's. These data indicate that single pontomesencephalic raphe neurons possess both serotonergic and glutamatergic properties under these conditions, and raise the possibility that some raphe neurons release both serotonin and glutamate in vivo. Supported by an American Psychological Association Minority Fellowship, a Harvard Ryan Fellowship, the Freudenberger Fund, and NS 02253-32.

639.15

THE EFFECT OF TRYPTOPHAN DEPLETION ON THE ACOUSTIC STARTLE RESPONSE IN DEPRESSED SUBJECTS H. L. Miller*, C. A. Morgan III, P. L. Delgado, L. Karper, M. Davis, D. S. Charney. West Haven VAMC and Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06508.

Acoustic startle is a reflex with a well-characterized neural pathway that is useful as a model system to investigate effects of drugs on sensorimotor reactivity. Preclinical data indicate that the startle reflex is modulated by serotonin. Brain serotonin can be reduced by depleting plasma TRP by dietary manipulations. TRP depletion increases the startle reflex in rats. As part of a research program assessing the role of serotonin in depression, startle might provided a sensitive measure to ascertain whether TRP depletion was having central effects. **METHOD**: 14 depressed patients participated in two tests, one week apart. Each test involved 24 hrs. of a low TRP diet followed by a 15 amino acid drink. During one test, TRP was added to the diet and drink (control); during the other test TRP was not added (depletion). Eyeblink response to 36 tones of 5 different intensities was measured 5 hrs. after subjects received the depleting drink or the control drink. **RESULTS**: Plasma TRP was depleted by 70 to 90% 5 hrs. after the amino acid drink. The amplitude of the startle response was significantly increased after TRP depletion compared to control test ($p < .04$). **IMPLICATIONS**: These findings suggest that plasma TRP depletion has central effects, and, consistent with the preclinical literature, that the startle reflex is modulated by serotonin in humans.

639.16

DIFFERENTIAL EFFECTS OF RAPHE GRAFTS IN THE HIPPOCAMPUS, AMYGDALA OR THE HYPOTHALAMUS. G. Richter-Levin* and M. Segal. Dept. Neurobiol., The Weizmann Inst., Rehovot 76100, Israel.

Serotonin depletion in rats affected spatial memory ability, body weight and thermoregulation. In an attempt to localize these functions, we studied the behavioral and physiological effects of raphe graft-induced differential restoration of the serotonergic innervation of the hippocampus, amygdala and the hypothalamus, in serotonin depleted rats. Control (n=8), serotonin depleted (5,7-DHT, 200 ug, icv) (DHT, n=8), and DHT rats with raphe grafts in the hippocampus (HG, n=7), amygdala (AG, n=8), and hypothalamus (HTG, n=9), were tested in the Morris water-maze (+ atropine, 40 mg/kg, ip). All lesioned groups, with the exception of the HG rats, performed significantly worse than controls. There was a significant reduction in body weight in all the lesioned groups including the HG rats. Exposure of the rats to 3 min ice cold water, led to a significantly greater reduction in body temperature in all lesioned groups, except for the HTG rats which were not different from controls. These results indicate that the effects of the serotonergic lesion on spatial memory are not due to its effects on body weight or thermoregulation. The graft can thus be used as a tool for studying local serotonergic functions in the brain.

639.17

SEROTONIN1A AGONIST REDUCED CONDITIONED FREEZING BEHAVIOR. T.Inoue*, T.Koyama and I.Yamashita. Dept. of Psychiat. and Neurol., Hokkaido Univ. Sch. of Med., Sapporo 060, Japan.

We have found that conditioned fear stress (CFS) increased serotonin (5-HT) metabolism in the medial prefrontal cortex with an induction of freezing behavior. These results could support the 5-HT hypothesis of anxiety. In the present study, the effects of various serotonergic agents and diazepam on shock-induced freezing behavior were examined using time-sampling procedure. Various doses of diazepam (0.1-5mg/kg), ipsapirone (0.1-10mg/kg), ICI169,369 (5-20mg/kg), DOI (0.1-1mg/kg) or mCPP (0.1-10mg/kg) were administered subcutaneously to rats 24 hours after the last session of repeated footshock for 5 days. Rats were again placed in the shock chamber without shocks 20 min after treatment and observed. Diazepam (1mg/kg), ipsapirone (0.5-10mg/kg), DOI (0.1-1mg/kg) and mCPP (0.5-10mg/kg) significantly reduced freezing behavior. ICI169,369 failed to change freezing behavior. In conclusion, these results suggest an anxiolytic potential of 5-HT1A agonist and a possible role of 5-HT1C and 5-HT2 agonist in the treatment of anxiety.

640.1

DIFFERENTIAL EFFECT OF α_1-ADRENERGIC RECEPTOR ACTIVATION ON ADENOSINE A_2 AND β_2-ADRENERGIC RECEPTOR DESENSITIZATION. H.L. Wiener*, G.P. Thalody, J.M. Murray, R. Osman, J. Goldfarb, and S. Maayani. Dept. Pharm. Sci., Coll. Pharm., St. John's Univ., Jamaica, NY 11439, and Depts. Anesthesiol., Physiol. and Biophys., and Pharmacol., Mt. Sinai Sch. Med., CUNY, New York, NY 10029.

The contractile response mediated by α_1-adrenergic receptors and the relaxation responses mediated by either adenosine A_2 or β_2-adrenergic receptors in the isolated adventitia- and endothelium-denuded rabbit thoracic aorta were selected as a model to study functional antagonism between simultaneously activated membrane-bound receptors. The present study focused on the effect of α_1-adrenergic receptor activation on the desensitization of the response mediated by adenosine A_2 or β_2-adrenergic receptors. Neither adenosine nor isoproterenol altered basal tissue tension in naive rings. Prior incubation with adenosine or isoproterenol (10 min) had minimal effect on the concentration-response curve to phenylephrine suggesting that both receptors have fully desensitized in the absence of the α_1-adrenergic stimulus. Adenosine and isoproterenol rapidly relaxed phenylephrine precontracted rings in a concentration-dependent and saturable manner. The relaxation response to adenosine was monotonic and stable for over 30 min. In contrast the relaxation response to isoproterenol was biphasic, consisting of a rapid relaxation and partial regaining of tissue tension. In rings preincubated with low concentrations of phenylephrine and relaxed with adenosine, concentration-response curves to higher concentrations of phenylephrine were shifted dextrally. Taken together, these results suggest that while the adenosine A_2 receptor and the β_2-adrenergic receptor desensitize in the presence of their respective agonists, their desensitization is differentially attenuated by prior α_1-adrenergic receptor activation: the desensitization of the adenosine A_2 receptor is prevented while that of the β_2-adrenergic receptor is not. USPHS GM34852.

640.2

SYMPATHETIC INNERVATION OF THE BULLFROG AORTA BY 2 CO-TRANSMITTING SYNAPSES IN SERIES. JP Horn*, WD Stofer and R Thorne Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

We seek to understand the integrative significance of slow synaptic potentials in sympathetic ganglia. Our strategy has been to develop an isolated preparation containing ganglia and an end-organ. Here we describe a preparation made from a bilateral dissection of paravertebral ganglia 7-10 and the abdominal aorta. It has 2 co-transmitting synapses in series and is suitable for observing the transformation between controlled patterns of preganglionic activity and vascular contractions.

Anatomically, the lumbar ganglia innervate the aorta. In addition to the rami between each ganglion and its corresponding spinal nerve, smaller accessory nerves connect each lumbar ganglion to the aorta. From dissection it is clear that some axons project to the kidneys and gonads. However, tracing with DiI reveals that other axons in the accessory nerves enter the wall of the aorta where they form a fine plexus. When stained as a wholemount for neuropeptide Y, the aorta contains a dense plexus of immunoreactive axons and varicosities. This anatomy suggests that C-type sympathetic neurons innervate the aorta.

Nerve-evoked contractions can be recorded for many hours after coupling a 1 cm length of the aorta to a tension transducer. Stimulation of the preganglionic C, but not the B, pathway causes contraction. The aorta is bilaterally innervated. Nerve cuts show that ganglia 9 & 10 provide at least 90% of the innervation to the caudal aorta. Blockers of nicotinic and α-adrenergic receptors antagonize the evoked aortic contractions. Evidence for synaptic gain is presented in the companion paper. Supported by NIH grants NS21065, NS01427 and HD07343.

640.3

HOMOSYNAPTIC AND HETEROSYNAPTIC GAIN MODULATION AT CO-TRANSMITTING SYNAPSES IN THE BULLFROG SYMPATHETIC VASOMOTOR C SYSTEM. R Thorne* and JP Horn Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

Based on their known actions in the bullfrog, luteinizing hormone releasing hormone (LHRH) and neuropeptide Y (NPY) are capable of endowing the vasomotor sympathetic system with use-dependent synaptic gain. To test this possibility, we studied the relation between patterns of preganglionic stimuli and arterial contractions in an isolated preparation of sympathetic ganglia and the aorta (see companion paper).

Short stimulus trains (<50) evoke contractions whose amplitudes saturate in a frequency-dependent manner at 1-5 Hz. Such responses are blocked by curare and phentolamine. However, small contractions return with longer stimulus trains. This suggests that co-transmitters can overide the blockade of primary transmitters. When trains are lengthened in the absence of drugs, contractions do not saturate but continue to grow. In 1 case, the growth in contractions was logrithmic between 2 and 1000 stimuli. These results suggest that co-transmitters produce homosynaptic gain modulation in this circuit.

Because the aorta is bilaterally innervated, one can test for interactions between separate pools of ganglionic C neurons. Using one side to evoke a test contraction and the other for conditioning, we found that long conditioning trains can potentiate the amplitude of subsequent test responses. This demonstrates that interactions occur between postganglionic synapses and is most simply described in terms of enhanced heterosynaptic gain.

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640.4

CHARACTERIZATION OF POLYAMINE UPTAKE AND RELEASE IN CHICK RETINA CELLS IN CULTURE. A.L.M.Ventura* and R.B.S.Pedro. Dept. Neurobiologia, Univ. Fed. Fluminense, Niterói, Brasil.

Putrescine is an abundant polyamine that may serve as an alternative precursor for GABA synthesis in the CNS. In this work we show that retina cells in culture are able to incorporate and release (^{3}H)-putrescine. Two types of cul tures were used: cultures containing predominantly neurons and mixed, containing neurons and glia. Both types were able to incorporate radioactivity in a manner dependent on temperature and pH of the medium but independent on the presence of extracellular Na^+ions. Nipecotic acid, a potent inhibitor of GABA uptake, did not interfere with the uptake of (^{3}H)-putrescine, suggesting that the me chanisms of polyamine uptake is distinct of the mechanisms of GABA uptake. In mixed cultures, KCl 50 mM was able to induce the release of radioactivity in a calcium dependent manner. In cultures containing predominantly neurons, both KCl 50 mM and Veratridine 100uM released radioactivity, the effects also being Ca^{++} dependent. Glutamate, a com pound that releases GABA in the retina, did not induce the release of radioactivity in neuronal cultures, suggesting that the radioactivity released did not represent GABA. Our results suggest that polyamines, although participa ting in the synthesis of GABA, may have a neuromodulatory role in the retina.

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640.5

GLUTAMATE MODULATED RELEASE OF 3H-GABA FROM RAT OLFACTORY BULB. E.H. Jaffé* and I. García, Laboratorio Neuroquímica, Instituto Venezolano de Investigaciones Científicas, Apartado 21827, Caracas 1020-A, Venezuela.

We studied the possible neurotransmitter involved in the modulation of GABA release from granule cells of the olfactory bulb. The GABAergic granule cell has been well characterized. However the neurotransmitter of the mitral cell, which establishes reciprocal synapsis with the granule cell controversial. A strong candidate is glutamate. In previous work (Jaffé, Vaello 1989, J. Neurochem. 52, 1766-1774) we observed that glutamate had only a week response on the release of GABA and was only seen after a previous K depolarization. This effect of Glutamate was indirect since it was inhibited by TTX. We further characterized the effect using continous superfusion of olfactory bulb slices prelabled with 3H-GABA. Nipecotic acid a GABA uptake inhibitor enhanced the GABA releasing effect of Glutamate, Kainate and AMPA. The effect of NMDA was only seen after a previous depolarization of the tissue with K or Kainate. The effect of Kainate and AMPA was inhibited by CNQX but not by AP7. TTX inhibited Glu and Kainate effect. The exitatory amino - acid, cysteinsulphinic acid was able to induce GABA release. At the level of the olfactory bulb several glutamate receptors are able to induce GABA release through a polysynaptic mechanism.

640.6

THE EFFECT OF CHRONIC HALOPERIDOL ADMINISTRATION ON GABA-IMMUNOREACTIVE AXON TERMINALS IN RAT MEDIAL PREFRONTAL CORTEX. E. Adamec*, S.L. Vincent, I. Sorensen and F.M. Benes. Department of Anesthesia, Massachusetts General Hospital, Boston, MA; Department of Psychiatry and Program in Neuroscience, Harvard Medical School; Mailman Research Center, McLean Hospital, Belmont, MA.

Several reports provide evidence that chronic haloperidol treatment induces ultrastructural changes in synapses of substantia nigra, corpus striatum and medial prefrontal cortex (mPFC). Recent studies suggesting that there is a loss of GABAergic cells in anterior cingulate cortex of schizophrenic subjects have prompted interest in the question of whether dopamine blocking agents can influence this transmitter system. This study provides a quantitative light microscopic analysis of GABA-immunostained axosomatic terminals in mPFC of rats treated with haloperidol decanoate (0.5 mg/kg/day i.m., McNeal Labs). GABAergic terminals were visualized with an immunoperoxidase method for localizing anti-GABA antibodies developed in rabbits (Incstar) using the avidin-biotin procedure. Computer-assisted image processing was used to determine the total number of pixels occupied by the reaction product in GABA-positive axosomatic terminals on individual neuronal cell bodies in various layers of mPFC. Drug-treated animals showed a significant increase in the number of GABA-positive pixels in layers 2, 3, 5 and 6 (93%, 63%, 31% 43%, respectively). These data suggest that chronic haloperidol administration induces a significant increase in the amount of GABA in rat mPFC. The fact that neurons in layer 2 showed the largest increase in GABA-immunoreactive terminals parallels the losses of interneurons and the up-regulation of GABA-A receptor binding observed in the same lamina of anterior cingulate cortex in schizophrenic patients. Supported by MH 00423, MH 42261 and the Scottish Rite Foundation.

640.7

EFFECTS OF TYPICAL AND ATYPICAL ANTIPSYCHOTIC DRUGS ON EXTRACELLULAR GABA LEVELS IN THE PREFRONTAL CORTEX. D. Cameron*, A. J. Bourdelais, and A. Y. Deutch. Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06508, and VA Medical Center, West Haven, CT 06516.

Dopamine (DA) afferents to the prefrontal cortex (PFC) inhibit cortical pyramidal neurons through both direct and indirect means. Direct symmetric synapses between DA terminals and pyramidal neurons are present. DA indirectly inhibits pyramidal cell activity by enhancing GABA release from interneurons. We have used *in vivo* microdialysis to characterize the effects of typical and atypical antipsychotic drugs (APDs) on extracellular GABA levels in the PFC. Animals were implanted with chronic indwelling guide cannulae, and one week later a dialysis probe placed in the PFC. The freely-moving rats were then perfused with dialysis buffer until a stable baseline was obtained, and then either the typical APD haloperidol or the atypical APD clozapine was injected subcutaneously. Haloperidol resulted in a marked decrease in extracellular GABA levels as compared to vehicle control. In contrast, CLZ did not significantly reduce extracellular GABA levels in the PFC compared to its vehicle. Previous reports have indicated that clozapine results in a greater enhancement of DA release in the PFC than does haloperidol; coupled with the lower affinity of clozapine for the D_2 receptor, clozapine may not block postsynaptic activity of DA as much as haloperidol. The present data suggest that the atypical APD clozapine may act on negative symptoms by not enhancing GABA-induced inhibition over cortical pyramidal neurons. Supported by MH-45124 and VA National Center for Schizophrenia Research to the West Haven, CT VA Medical Center.

640.8

EFFECTS OF TYPICAL AND ATYPICAL ANTIPSYCHOTIC DRUGS ON GLUTAMIC ACID DECARBOXYLASE GENE EXPRESSION. P.Z. Gallipoli* and A. Y. Deutch. Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06508 and VA Medical Center, West Haven, CT 06516.

Dopamine (DA) appears to regulate the activity of GABAergic neurons in both the striatum (CP) and in the prefrontal cortex (PFC). We have therefore examined the regulation of glutamic acid decarboxylase (GAD) gene expression in the PFC following chronic (21 day) administration of antipsychotic drugs (APDs); these include the typical APDs haloperidol and raclopride, the atypical APD clozapine, and the putative atypical APD remoxipride. Preliminary data suggest that haloperidol and raclopride increased expression of both GAD_{65} and GAD_{67}; remoxipride only marginally increased GAD_{67} expression, but appeared to more robustly increase GAD_{65} expression. The effects of haloperidol and raclopride on GAD mRNAs appeared to be more pronounced on GAD_{67} mRNA. These data suggest that APDs increase GAD gene expression in the striatum, but that there may be differences between two typical APDs (haloperidol and raclopride) and remoxipride, a putative atypical APD.

Supported by MH-45124, the VA National Centers for Schizophrenia Research and Post Traumatic Stress Disorders at the West Haven VA Medical Center, and the National Parkinson Foundation at Yale University.

640.9

Partial Characterization of Excitatory Amino Acid-Evoked Striatal GABA Release in vivo: Comparison of Kainate and NMDA. T.N. Ferraro*, D.P. Carrozza, G.T. Golden, P.F. Reyes and T.A. Hare. Thomas Jefferson University, Philadelphia, PA and VAMC, Coatesville, PA.

Excitatory amino acid (EAA) receptors in the mammalian CNS are involved in a number of aspects of neuronal function. We have investigated the effects of intrastriatal (i.s.) administration of kainate (KA) and NMDA on GABA release using intracerebral microdialysis in freely-moving rats. An in vivo standardization procedure involving a brief (10 min, i.s.) perfusion with a Ringer's solution containing 100mM potassium (Brain Res. 543:69, 1991) was utilized to quantitate GABA release for a dose of KA or NMDA given subsequently. Doses of KA and NMDA were manipulated by varying the time (up to 10 min) that the drugs (12.5mM in normal Ringer's) were perfused through the microdialysis probe. Probes were of concentric design with tip parameters of 4 mm x 0.2 mm; perfusion flow rate was 2.75 ul/min with 15 min sampling intervals. Microdialyzate GABA levels were measured using an ion-exchange HPLC/fluorometric method. Results showed that KA and NMDA mediate dose-dependent striatal GABA release of a similar magnitude. KA-evoked GABA release was not affected by pretreatment with cadmium (1.0mM, up to 45 min) but was partially inhibited by pretreatment with kynurenic acid (1.0mM, 10 min). NMDA-evoked GABA release was partially inhibited by pretreatment with cadmium, dextromethorphan (1.0mM, 5 or 15 min) and "low" doses (0.04mM, 5 min) of aminophosphonovalerate (APV). Pretreatment with "high" doses (1.0mM, 5 min) of APV potentiated NMDA-evoked GABA release. Results suggest that KA and NMDA mediate striatal GABA release via distinct mechanisms and/or from distinct pools. Observed differences may be related to the striatal distribution of receptors which respond to these two EAAs. The ability of the higher dose of APV to potentiate NMDA-evoked GABA release may be explained by a mechanism involving relief of an NMDA receptor-regulated interneuronal negative feedback circuit. Supported in part by the Veterans Administration.

640.10

SEROTONIN-DOPAMINE INTERACTIONS IN THE REGULATION OF NEOSTRIATAL GENE EXPRESSION J. G. Capodilupo* & P. D. Walker, Department of Anatomy & Cell Biology, Wayne State University School of Medicine, Detroit, MI 48201.

Serotonin (5-HT) manipulation alters tachykinin biosynthesis in the neostriatum (Walker et al., Brain Res., 546 [1991] 33-39). However, the influence of 5-HT on neostriatal gene expression may ultimately be controlled via dopamine pathways (Walker et al., Brain Res., 557 [1991] 31-36). To further investigate possible 5-HT/dopamine co-regulation, we tested the ability of quinpirole, a dopamine D2 agonist, to regulate neostriatal gene expression during pharmacological inhibition of 5-HT synthesis.

Daily injections of *p*-chlorophenylalanine, *p*CPA, (100mg/kg/day i.p. for 7 days) blocked acute (single 1 mg/kg i.p.) but not chronic (7 daily 1 mg/kg i.p.) quinpirole-induced increases in neostriatal preprotachykinin (PPT) mRNA detected within hours of the last daily injection. Alone, *p*CPA did not influence PPT mRNA at this time. However, in another series of animals, *p*CPA significantly lowered PPT mRNA levels 1 week after the end of 7 daily injections. Such delayed effects were observed in additional *p*CPA experiments where increases in the length of *p*CPA treatment shifted the mRNA response to later periods. Therefore, later examination following combined quinpirole/*p*CPA administration may uncover the ability of *p*CPA to block the chronic effects of quinpirole.

These results provide further evidence for co-dependence between 5-HT and dopamine in the regulation of neostriatal gene expression. However, since 5-HT appears to influence glutamic acid decarboxylase (GAD) mRNA levels in a manner opposite to that of dopamine, further analysis is required to tease apart independent influences of these monoamines on basal ganglia neurotransmission. (Supported by 1991 WSU Research Grant to P.D.W.)

640.11

SIGMA RECEPTOR LIGANDS REGULATE N-METHYL-D-ASPARTATE (NMDA)-STIMULATED [^{3}H]DOPAMINE ([^{3}H]DA) RELEASE FROM RAT STRIATAL TISSUE. **G.M. Gonzalez* and L.L. Werling, Dept. Pharmacology, The George Washington University Medical Ctr., Washington, D.C. 20037.**

We have previously reported inhibition of NMDA-stimulated [^{3}H]DA release in rat striatal slices by sigma receptor agonists (+)pentazocine and (+)SKF10,047. We have investigated several potential sigma antagonists in this system.

Striata were dissected, chopped, and washed in Mg^{++}-free modified Krebs-HEPES buffer, then incubated with 15 nM [^{3}H]DA for 30 min. Slices were loaded into a superfusion apparatus and superfused with buffer to establish a low, stable baseline release. Tissue was stimulated to release [^{3}H]DA by a 2 min exposure to 25 µM NMDA. Inflow was returned to non-stimulating buffer, with or without potential inhibitor of release, for 10 min. Tissue was stimulated a second time (S2) for 2 min in the presence or absence of a potential inhibitor.

Both (+) and (-) isomers of SKF10,047 and pentazocine inhibited NMDA-stimulated [^{3}H]DA release from rat striatal slices in a naloxone-insensitive manner. In contrast, the sigma antagonist haloperidol and PRE084, a novel, selective sigma ligand, reversed the inhibition by (+)-pentazocine. BMY14802, an experimental antipsychotic drug, and NPC16377, another novel sigma ligand, not only reversed (+)pentazocine-induced inhibition, but potentiated NMDA-stimulated [^{3}H]DA release as well. Sigma ligands DTG, 3-(+)-PPP, and ifenprodil did not antagonize (+)pentazocine-induced inhibition. In addition, (+) pentazocine (10 µM) also inhibited NMDA-stimulated [^{3}H]DA release from rat striatal synaptosomes, suggesting that sigma receptors are located on dopaminergic nerve terminals. These data suggest a potential involvement of sigma receptors in the regulation of DA release from striatum. (Supported by a grant from NIDA to LLW and by NIGMS predoctoral fellowship to GMG.)

640.12

INTRAMEMBRANE INTERACTIONS BETWEEN CHOLECYSTOKININ (CCK) AND DOPAMINE (DA) RECEPTOR SUBTYPES IN THE RAT STRIATUM. X-M. Li, P.B. Hedlund, G. von Euler, F. Benfenati*, L. F. Agnati and K. Fuxe. Dept. of Histology & Neurobiology, Karolinska Institute, Stockholm, Sweden.

The interactions between CCK and DA receptors have been analyzed in the rat striatal membrane preparations. CCK-8 (0.1 and 1 nM) reduced the affinity of striatal D_2 agonist ^{3}H-N-propylnorapo-morphine (^{3}H-NPA) binding sites by about 25% without altering the B_{max} values (n=8). A kinetic analysis (n=8) indicated that this action was related to a reduction of the association rate constant for the radioligand. In contrast, 0.1, 1 (peak effect) and 10 nM of CCK-8 decreased the K_H and K_L values of DA for the D_2 antagonist ^{3}H-raclopride (^{3}H-RAC) binding sites by 40-57% (n=22). This action was completely antagonized by the D_1 antagonist SCH23390 (200 nM), which by itself significantly increased the K_H and K_L values of DA for ^{3}H-RAC binding sites (n=6) without directly affecting the D_2 receptors (IC_{50}=3516±279 nM). CCK-8 (1 nM) did not affect the K_H and K_L values of DA for ^{3}H-SCH23390 binding sites (n=7). All the effects of CCK-8 on D_2 receptors were blocked by the CCK_B antagonist PD134308 (100 nM). The results suggest the possibility that CCK-8 via CCK_B receptors produces its neuroleptic activity by reducing the affinity and possibly the transduction of D_2 receptors. However, when D_1 and D_2 receptors are coactivated CCK-8 may instead increase D_2 receptor activity leading to a synergistic interaction with D_2 receptors.

640.13

ACTH INPUT TO MIDBRAIN DOPAMINERGIC NEURONS: LIGHT AND ELECTRON MICROSCOPIC EXAMINATION. C.-L. Liang, G.P. Kozlowski, S.A. Joseph and D.C. German*. Depts. of Psychiatry and Physiology, UT Southwestern Med. Cntr., Dallas, TX 75235, and The Neuroendocrine Unit, Univ. of Rochester Med. Sch., Rochester, N.Y. 14642.

Stress activates the mesocorticolimbic dopaminergic (DA) neurons (Thierry et al., Nature **263**: 242, 1976). The present study sought to determine whether the stress-related peptide, adrenocorticotropin (ACTH), makes synaptic contacts with mesocorticolimbic DA neurons. Single- and double-labeling immunocytochemical staining procedures were used to examine the relationship between ACTH-containing nerve terminals, and DA neurons in the rat midbrain. ACTH nerve terminals were found extensively in regions occupied by the mesocorticolimbic DA neurons, such as in the interfascicular n., paranigral n. and central linear n. (CLi). In the CLi, ACTH axon terminals made both symmetric and asymmetric synaptic contacts with DA dendrites, and putative axo-axonic contacts with unlabeled axon terminals which, in turn, made contacts with DA dendrites. The ACTH-DA synapses may play a role in stress-induced changes in mesocorticolimbic DA neuronal activity. Supported by grant DA-05314.

640.14

EFFECT OF 6-HYDROXYDOPAMINE (6-OHDA) LESIONS IN NEONATE RATS ON mRNA LEVELS ENCODING THE ENZYME GLUTAMATE-DECARBOXYLASE (GAD) AND THE DOPAMINE D2 RECEPTOR. J-J Soghomonian*. Centre de Rech. en Neurobiologie, Univ. Laval, Québec, (Qc) Canada G1K 7P4.

6-OHDA lesions of dopamine neurons in adult rats increase mRNA levels encoding the dopamine D2 receptor and GAD enzyme in projection neurons of the striatum. When dopamine neurons are lesioned in neonates, adults exhibit specific behavioral and neurochemical features not observed after adult lesions. In this study, we determined if 6-OHDA injections (intraventricular; 150µg) in neonate Sprague-Dawley rats induce changes in mRNA levels encoding the enzyme GAD (Mrs 67,000) and the dopamine D2 receptor at adulthood. Brain sections were processed for in situ hybridization histochemistry with 35S-labeled probes. Labeling was visualized by X-ray film radioautography and measured by computerized densitometry. In agreement with previous studies, we found increased and decreased levels of mRNAs encoding the peptides enkephalin and substance P, respectively, in sections of the striatum. Adjacent sections of 6-OHDA-treated rats exhibited an increased labeling when processed with the dopamine D2 receptor probe (+114% as compared to controls) or with the GAD(Mrs 67,000) probe (+119% and +135% as compared to controls for stereotaxic levels IA 10 and 10.6, respectively). Both D2 and GAD mRNA increases appeared homogeneously distributed in the dorsal-ventral and lateral-medial portions of the striatum. The results indicate that injection of 6-OHDA in neonate rodents can modify the level of expression of GAD and dopamine D2 receptor mRNAs in a direction similar to that observed after adult lesions. (supported by FRSQ)

BEHAVIORAL PHARMACOLOGY: DOPAMINE, SEROTONIN, NE

641.1

UNCONDITIONED BEHAVIORAL EFFECTS OF SELECTIVE DOPAMINE D_1 AND D_2 AGONISTS IN SQUIRREL MONKEYS. I. Bergman*, P. Hesterberg, and S. Rosenzweig-Lipson. Harvard Medical School/NERPRC, Southborough, MA 01772.

Recent evidence suggests that the behavioral effects of high- and limited-efficacy D_1 agonists may differ in primates. In the present study, the unconditioned behavioral effects of the high-efficacy D_1 agonists SKF 81297 and SKF 82958, the limited-efficacy D_1 agonists R-SKF 38393 and SKF 75670, and the D_2 agonists (+)-PHNO and quinpirole were compared by videotaping unconditioned behavior of squirrel monkeys in their home cages following the administration of drug or vehicle. Videotapes were scored by at least 2 observers for different behaviors using a continuous observation procedure. The high-efficacy D_1 agonists SKF 81297 (0.1 - 3.0 mg/kg) and SKF 82958 (0.03 - 1.0 mg/kg) produced dose-dependent increases in the frequency of visual scanning and had no effect on either huddling or scratching. The limited-efficacy D_1 agonists R-SKF 38393 (0.1 - 1.0 mg/kg) and SKF 75670 (0.1 - 1.0 mg/kg) produced dose-dependent increases in the duration of huddling and had no effect on visual scanning or scratching. The D_2 agonists (+)-PHNO (0.0003 - 0.01 mg/kg) and quinpirole (0.003 - 0.3 mg/kg) produced dose-dependent increases in the frequency of scratching and had no effect on visual scanning or huddling. The present results indicate that the unconditioned behavioral effects of high- and limited-efficacy D_1 agonists differ in primates and that the unconditioned behavioral effects of D_2 agonists differ from those of D_1 agonists. Supported by USPHS Grants DA03774, DA00499, MH07658, and RR00168.

641.2

Intra-striatal injections of kainic acid induces contralateral rotation in rats. I.D. Smith* and R.J. Beninger, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

The role of striatal kainate receptors in the control of locomotor activity was investigated with intra-caudate injections of kainic acid (KA). Kainate was injected in three concentrations (5 µM (0.53 ng), 50 µM (5.3 ng), 250 µM (26.6 ng) dissolved in 0.5 µl saline) into the dorsal striatum of 15 rats. A significant increase in contralateral turning was observed after 250 µM ($p<.05$) and 50 µM KA ($p<.05$) whereas the 5 µM dose had no effect. In addition, 250 µM KA resulted in an increase in the number of rotations exhibited during the observation period ($p<.05$). Behavioral evidence of seizure activity was not observed at any dose level.

Excitatory corticostriatal projections have been shown to depolarize striatal output neurons via a non-NMDA glutamate receptor subtype. Thus a KA receptor-mediated increase in striatal cell firing may underlie the activation of motor systems, resulting in rotation. Striatal glutamate receptor activation also stimulates the release of neurotransmitters such as dopamine (DA). Since rats with imbalances in striatal DA receptor stimulation tend to rotate away from the side of higher DA, it is possible that the contralateral turning resulted also from a KA-induced increase in extracellular DA levels. Additional experiments using systemic amphetamine injections will examine the involvement of an interaction between glutamate and dopamine receptor stimulation in the observed behavior. (Supported by NSERC)

641.3

Blockade of S_2 receptors in rats enhances D1-mediated repetitive jaw movements (RJM) induced by SKF 38393. H.Rosengarten*, J.W. Schweitzer and A.J.Friedhoff
Millhauser Laboratories NYU School of Medicine
Department of Psychiatry , New York ,NY 10016

We have previously demonstrated that the D_1 dopamine system mediates behavior we have named (RJM) repetitive jaw movements in rats.This behavior can be induced by the D1 agonist SKF 38393 and inhibited by the D_2 agonist,LY171555, or enhanced by blockade or inactivation of D_2 dopamine receptors(Rosengarten et al. 1983, 1986,1988). In the present study we explored the effect of inactivation or bockade of S_2 serotonin receptors with cyproheptadine on this behavior. We found that inactivation or blockade of S_2 receptors greatly augmented SKF 38393 - inducible RJM.The present findings demonstrate that the S_2 system inhibits D_1 mediated RJM. These finding would provide a mechanism underlying the clinical use of serot nin-enhancing drugs such as fluoxetine in the treatment of tardive dyskinesia.

641.4

THE EFFECTS OF DOPAMINE AGONISTS AND ANTAGONISTS IN MICE PRETREATED WITH RESERPINE. M.J. Piesla, E.A. Muth*, and K.L. Marquis, Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543.

Typical stereotypy (sniffing-licking-biting) and climbing in normal rodents can be induced only by stimulation of both D-1 and D-2 receptors (Braun and Chase, 1986; Moore and Axton, 1988). However, either D-1 or D-2 agonists can reverse reserpine-induced akinesia, as measured by an increase in locomotor activity, in mice (Rubinstein et al., 1988). Therefore, the effect of dopamine agonists and antagonists on stereotypy (S) and climbing (C) in mice with supersensitive receptors (produced by 20-24 hour pretreatment with 5.0 mg/kg sc reserpine) was determined. The mixed D-1/D-2 agonist, apomorphine, dose-dependently increased S and C in supersensitive mice (S-ED50=0.05 and C-ED50=0.09 mg/kg sc). Quinpirole, the full D-2 agonist, significantly increased S and C (S-ED50=0.2 and C-ED50=0.3 mg/kg ip) in mice. SKF 38393, the full D-1 agonist, had a slight effect on C, but produced no S. However, mice treated with a fixed dose of SKF 38393 (10.0 mg/kg ip) and varying doses of quinpirole displayed S and C similar to that produced by apomorphine (S-ED50=0.09 and C-ED50=0.05 mg/kg ip). In antagonism studies, the selective D-1 antagonist, SCH 23390, failed to antagonize apomorphine-induced (1.0 mg/kg sc) S and C. Conversely, the selective D-2 antagonist, raclopride, reduced C, but not significantly, and dose-dependently blocked S (ED50=1.2 mg/kg ip). Partial dopamine agonists were also evaluated in this paradigm. For example, (+)3PPP induced these behaviors (S-ED50=2.5 and C-ED50=1.9 mg/kg sc) to a greater extent than (-)3PPP (S-ED50=9.0 and C-ED50=10.0 mg/kg sc). The very weak D-2 agonist, OPC 4392, failed to have any effect on these behaviors. Also, the putative D-2 agonist, Lu 22-106-C, produced both behaviors (S-ED50=0.7 and C-ED50=2.4 mg/kg ip) while the enantiomer Lu 22-105-C, a putative D-2 weak partial agonist/antagonist, had no effect. Thus, these data demonstrate the prevalent role for D-2 agonism in producing S and C through supersensitive receptors in mice. In addition, the level of intrinsic activity can be inferred from this model.

641.5

LOCOMOTOR ACTIVITY IN CONTROL AND CHRONIC CAFFEINE-TREATED MICE: INTERACTIONS OF ADENOSINE ANALOGS, CHOLINERGIC AND DOPAMIN-ERGIC AGENTS AND XANTHINES. O. Nikodijevic, K. A. Jacobson, and J. W. Daly,* NIDDK/NIH, Bethesda MD 20892.

Chronic caffeine ingestion (CCI) by NIH Swiss male mice results in a prolonged reduction in locomotor activity and alterations in response to caffeine, other xanthines, adenosine analogs, and nicotinic and muscarinic agents. Caffeine (IP) and an A_2-selective xanthine (3,7-dimethyl-1-propargylxanthine) remain stimulatory, and the typical bell-shaped locomotor dose-response curve to caffeine is left-shifted after CCI. Mice became more sensitive to depressant effects of A_1 and A_2 agonists. Depressant effects of xanthines that are potent PDE inhibitors are either blunted or enhanced by CCI. Depressant effects of nicotine are abolished. Responses to muscarinic agents are either little affected (oxotremorine, agonist) or right-shifted (scopolamine, antagonist). Depressant effects of the mixed A_1/A_2 agonist NECA, either alone or in combination with caffeine or other xanthines, are altered after CCI. Depressant effects of NECA in the presence of scopolamine or amphetamine, are similar in controls and after CCI. The depressant effects of a low dose of NECA in the presence of cocaine are reduced after CCI. The results suggest complex effects of CCI on adenosine-, dopaminergic-, and cholinergic-mediated behaviors.

641.6

EFFECTS OF DOPAMINE D1 AND D2 RECEPTOR INACTIVATION ON LOCOMOTOR ACTIVITY AND SNIFFING IN 11- AND 17-DAY-OLD RATS. M. Mestlin and S.A. McDougall*. Dept. of Psychology, CSUSB, San Bernardino, CA 92376.

Behavioral effects of dopamine (DA) receptors were assessed in 11- and 17-day-old rat pups using the irreversible DA antagonist, EEDQ. In Experiment 1, the locomotor activity and sniffing of 11- and 17-day-olds was assessed after treatment with the nonselective DA receptor agonist, R-propylnorapomorphine (NPA; 0.00, 0.01, 0.1, 1.0, and 5.0 mg/kg). Behaviors were assessed during a 20-min testing session which began 5-min after NPA treatment. Testing occurred across four days using a 25 X 25 X 18 cm grey heated chamber. Sniffing was recorded every 20-sec using a time-sampling technique. In Experiment 2, 10- and 16-day-old rat pups received a single dose of EEDQ (7.5 mg/kg) or vehicle after DA receptors were left either unprotected or protected using a combination of sulpiride (100.0 mg/kg) and SCH 23390 (1.0 mg/kg). NPA (0.00, 0.01, or 5.0) was then administered to rat pups 24, 48, and 96 hours after EEDQ treatment. Results from Experiment 1 suggest that NPA did not increase activity in 11-day-old pups; whereas, 17-day-olds showed an increase in activity, with 0.01 mg/kg NPA producing the greatest effect. NPA produced a dose-dependent increase in sniffing in both aged pups. In Experiment 2, EEDQ did not affect the locomotor activity or sniffing of either 11- or 17-day-old pups. Results indicate that the response of preweanling and adult rats to EEDQ is fundamentally different and may reflect a drug-induced change in receptor affinity.

641.7

THE NUCLEUS ACCUMBENS AND CAUDATE AS NEURAL MEDIATORS OF AMPHETAMINE-INDUCED LOCOMOTOR STEREOTYPY. D.E. Krug*and K. Mueller. Dept. of Psychology, Texas Christian University, Fort Worth, TX 76129.

Amphetamine-induced behaviors in rats include focused stereotypy, hyper-locomotion, and locomotor stereotypy (patterned locomotion). The mechanisms controlling hyper-locomotion (nucleus accumbens [NAC]) and focused stereotypy (anterior caudate [CAUD]) are well understood, but how locomotor stereotypy is produced is not well understood. Two experiments examined the CAUD and NAC for the production of locomotor stereotypy as measured by the gammahat procedure. In experiment 1, fluphenazine [FLU] (0.0, 1.25, or 2.50 µg) was infused into either the NAC or CAUD prior to systemic amphetamine (2 mg/Kg). In the NAC, both doses of FLU reduced hyper-locomotion and locomotor stereotypy. In the CAUD, neither dose affected hyper-locomotion, but the lower FLU dose was more effective than the higher dose at reducing locomotor stereotypy. In experiment 2, 100 µg amphetamine was infused into either the NAC or CAUD. In the NAC, amphetamine produced hyper-locomotion but did not produce locomotor stereotypy. Amphetamine in the CAUD produced focused stereotypy without producing locomotor stereotypy. These results suggest the involvement of both the NAC and CAUD in the production of amphetamine-induced locomotor stereotypy.

641.8

APPARENT MEDIATION OF THE STIMULUS PROPERTIES OF A LOW DOSE OF QUINPIROLE BY DOPAMINERGIC AUTORECEPTORS. D.V. Widzowski* and D.A. Cory-Slechta. Environ. Health Sci. Ctr., Univ. Rochester Med. School, Rochester, NY 14642.

Drug discrimination (DD) studies have established that the stimulus properties of quinpirole (QUIN) are mediated by dopaminergic (DA) D2-type receptors, although the involvement of autoreceptors vs postsynaptic D2 receptors in such effects remains to be established. This study examined the contention that the stimulus properties of QUIN were mediated via autoreceptors, engendering a decline in DA release and consequent decrease in postsynaptic stimulation. Thus pharmacological treatments which engender a decline in postsynaptic DA availability should result in QUIN-appropriate responding, while those that activate postsynaptic DA receptors either directly or indirectly should result in saline-appropriate responding. To test this hypothesis, pharmacological treatments which either ultimately produce or decrease postsynaptic DA receptor stimulation were examined in rats trained to discriminate QUIN (0.05 mg/kg) from saline. At the training dose, QUIN maintained levels of responding on the drug lever of about 90%, and a dose-related decline in QUIN responding was observed in the presence of doses of QUIN below the training dose. Two other D2 agonists which have been reported to primarily stimulate autoreceptors at low doses (apomorphine (APO), 0.04-0.167 mg/kg, and n-propylnorapomorphine,0.04-0.12 mg/kg) produced a dose-related increase in QUIN lever responding to levels of 70 and 90%, respectively. The DA-synthesis blocker and DA depleter alpha-methyl-p-tyrosine (AMPT, 25-75 mg/kg) substituted for QUIN when given alone and potentiated QUIN stimulus properties when coadministered (75 mg/kg) with QUIN, shifting the QUIN dose-effect curve to the left. Treatments known to stimulate postsynaptic DA receptors, including the D1 agonist SKF38393 (6-12 mg/kg) and d-amphetamine (1-6 mg/kg), resulted in saline responding when given alone and antagonized QUIN stimulus properties when coadministered with QUIN. In addition, a very high dose of APO (0.5 mg/kg), i.e., one known to stimulate postsynaptic D1 and D2 receptors, resulted in saline responding. Taken together, these findings are consistent with the contention that the stimulus properties of 0.05 mg/kg QUIN are primarily mediated by D2-type presynaptic autoreceptors, and that these stimulus properties involve a decline in DA release and consequent decrease in postsynaptic stimulation. ES05017, ES05093.

641.9

CHOLINERGIC, GLUTAMATERGIC AND OPIOID INDUCTION OF DOPAMINE (DA) RELEASE: IMPACT ON THE STIMULUS PROPERTIES OF D1 AND D2 DA AGONISTS. R.A. Fox, S.C. Johnson and D.A. Cory-Slechta. Environ. Health Sci. Ctr. and Interdepartmental Neuroscience Program, Univ. Rochester Med. School, Rochester, NY 14642.

It has become increasingly clear in recent years that neurotransmitter systems do not operate in isolation, but are interactive, regulating aspects of each other's function. Non-competitive NMDA receptor antagonists, mu opiate agonists and muscarinic cholinergic agonists have all been reported to increase DA release, as measured by in vivo microdialysis. To determine whether this DA release has functional or behavioral properties, the ability of these compounds to substitute for DA agonists in a drug discrimination (DD) paradigm was examined. Rats were trained to discriminate either the D1 agonist SKF38393 (6.0 mg/kg) or the D2-type agonist quinpirole (0.5 mg/kg) from saline using a standard two-lever operant food-reinforced DD paradigm. Following acqusition of the discriminations, the ability of the non-competitive NMDA antagonist MK-801 (0.05-0.3 mg/kg), the mu opiate agonists morphine (3.0-10.0 mg/kg) and fentanyl (0.025-0.3 mg/kg), and the muscarinic cholinergic agonist arecholine (1.75-3.5 mg/kg) to substitute for the DA agonist training drugs was examined and compared to non-DA releasing compounds such as pentobarbital (5.0-10.0 mg/kg) and the kappa opiate agonist U50488 (2.0-4.0 mg/kg). The peak level of drug lever responding across the range of doses was averaged across individual rats, since dose-effect curves were not always lineraly dose-related. Under those conditions, both quinpirole and SKF38393 resulted in peak levels of drug lever responding of about 90%. Mu opioid agonists evoked peak levels of drug lever responding averaging 70-80% in D2-trained rats, but only 45-55% in D1-trained rats. MK-801 and arecoline evoked peak levels of drug lever responding of about 50-60% in both the D1- and D2-trained groups. In contrast, compounds not inducing DA release (pentobarbital) or known to decrease DA release (U50488) provoked levels of drug lever responding of 34% and 15% in D2-trained and 42% and 23% in D1-trained rats, respectively. Taken together, these data suggest that some behavioral properties of compounds that act directly on glutamatergic, opioid or cholinergic neurotransmitter systems and lead to DA release, may be at least partially mediated by DA systems. In particular, behavioral effects of mu opioid agonists may include DA D2 mediation. ES05017, ES05093.

641.10

SENSITIZATION AND TOLERANCE TO HALOPERIDOL'S EFFECTS ON OPERANT BEHAVIOR. S.C. Fowler* and J.-R. Liou. Depts. of Psychol. and Pharm., Univ. of Miss., University, MS 38677

Each of 5 groups of male rats received a different daily dose of haloperidol (0, 20, 40, 80, or 120 ug/kg) 45 min before a water-rewarded operant session for 21 days. All haloperidol groups exhibited across session decrements in response rate, with rates in the two lower dose groups reaching a minimum about day 10. By day 21, rate for the two lower dose groups only substantially recovered. Operant response duration also displayed progressive slowing across the first 10 days of dosing, but unlike the case for rate, little tolerance to the maximal duration effect was observed in any haloperidol group. At the higher doses, muzzle entry into the reward well persisted in the absence of operant responding. Thus whether tolerance follows sensitization depends on dose, length of treatment, and type of behavioral measurement. Neither haloperidol accumulation in brain nor dopamine receptor supersensitivity fully account for these effects. Supported by MH43429.

641.11

DOPAMINE D1 AND D2 MEDIATION OF THE DISCRIMINATIVE STIMULUS PROPERTIES OF TRIADIMEFON. A.N. Perkins* and D.A. Eckerman. Dept. Of Psychology, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Triadimefon (TDF), a triazole fungicide has been found to increase motor activity and to induce stereotypy, behavioral effects that are characteristic of the psychomotor stimulants. Triadimefon was also found to function as a discriminative stimulus, and its stimulus properties were found to be qualitatively similar to those produced by the psychomotor stimulant, methylphenidate.

A variety of evidence suggests that dopamine (DA) may be important in mediating the behavioral effects of TDF. The purpose of the present study was to address the role of DA in the discriminative stimulus effects of TDF. Rats (N=6) were trained to discriminate an i.p. injection of 40 mg/kg TDF from vehicle under an FR-20 schedule of milk reinforcement. Haloperidol, a D2 antagonist, in combination with TDF was found to antagonize the TDF stimulus in the absence of marked rate-decreasing effects. The D2 agonist quinpirole substituted for the TDF stimulus, whereas the D1 agonist dihydrexidine at dosages of up to 4 mg/kg did not substitute for the TDF stimulus. These preliminary data suggest a role for the involvement of the DA receptor in the discriminative stimulus properties of TDF.

641.12

SENSITIZATION OF HALOPERIDOL-INDUCED HYPOPHAGIA: ROLE OF INTERDOSE INTERVAL AND BEHAVIORAL EXPERIENCE. D. L. Wolgin*, J. Moore and L. Dalzell. Dept. of Psychology, Florida Atlantic Univ., Boca Raton, FL 33431.

Two experiments were conducted to elucidate the variables that control the development of sensitization to the hypophagic effects of haloperidol (HAL). In the first experiment, groups of rats were given chronic injections of either HAL (0.62 mg/kg) or saline at interdose intervals (IDIs) of 1, 2, 7, or 14 d. Following each injection, sweetened milk was presented for 30 min. HAL given at IDIs of 1 or 2 d produced neither tolerance nor sensitization to the initial hypophagic effect of the drug, whereas at IDIs of 7 or 14 d, sensitization developed. In the second experiment, groups of rats were given injections of HAL (0.31 mg/kg) at 7-day intervals either before (Before Group) or after (After Group) access to sweetened milk. Control groups were given injections of saline prior to milk access. After sensitization developed in the Before Group, all groups received a single injection of HAL (0.15 mg/kg) before access to milk. On this final test, only the Before Group showed sensitization of hypophagia. The After Group ingested as much as the control groups. These results demonstrate that sensitization of HAL-induced hypophagia develops at IDIs greater than 2d and is contingent on access to milk while in the drugged state.

(Supported in part by grant DA 04592 from NIDA)

641.13

REINFORCING EFFECTS OF THE D1 DOPAMINE AGONIST SKF 81297 IN RHESUS MONKEYS. M. R. Weed, K. E. Vanover and W. L. Woolverton.* Drug Abuse Research Center, The University of Chicago, Chicago, IL. 60637

The partial D1 agonist SKF 38393 has previously been found not to function as a positive reinforcer in rhesus monkeys (Woolverton et al., JPET 230: 678, 1984). The present experiment was designed to evaluate the reinforcing effect of the full D1 agonist SKF 81297 under similar conditions. Two rhesus monkeys were prepared with chronic intravenous catheters and lever pressing was maintained by cocaine (0.03 mg/kg/inj, FR10, 1 hr/day) under baseline conditions. When responding was stable (+/- 10% of 3 day mean), saline was substituted for cocaine. Responding declined to low levels (<10 inj/hr) within 4-6 sessions. Doses of SKF 81297 (SKF; 0.003-0.3 mg/kg/inj) were then made available for at least 4-6 sessions or until responding was stable. Baseline conditions were reinstated between SKF doses. Responding was maintained above saline levels at least at two doses in both monkeys. Injections of the D1 antagonist SCH 39166 (SCH; 0.003-0.03 mg/kg, i.m., 30 min pre-session) were administered before alternate sessions over the last several sessions of availability of the lowest SKF dose self-administered (0.01 mg/kg/inj). SCH decreased responding maintained by SKF in a dose-related manner in both monkeys. At the highest SCH doses, rate and pattern of responding were similar to that seen when saline was available for self-administration. Thus, the D1 agonist SKF functioned as a positive reinforcer and its reinforcing effect appeared to be blocked by the D1 antagonist SCH. These results further implicate D1 receptors in the reinforcing effects of drugs which increase dopamine neurotransmission. (Supported by NIDA grants DA-00250, DA-00161 and NIGMS grant GM-07151).

641.14

THE HIGHLY SELECTIVE α2-ADRENOCEPTOR ANTAGONIST RX811059 PRODUCES A CENTRALLY MEDIATED DISCRIMINABLE CUE IN RAT DRUG DISCRIMINATION STUDIES. S. Jordan, †H. C. Jackson, †D. J. Nutt, †R.B. Holman* and S. L. Handley. Pharm. Sci. Inst., Aston Univ., Birmingham. B4 7EA . UK. †Reckitt & Colman Psychoph. Unit, Bristol. BS8 1TD. UK.

Idazoxan is the most selective α2-adrenoceptor antagonist to date which produces an interoceptive discriminative stimulus or 'cue' in rat drug discrimination (DD) studies (Sanger et al, 1989, Psychopharm. 99, 117-121). However, this drug also binds with high affinity to the non-adrenoceptor idazoxan binding site (NAIBS, Michel & Insel,1989, Trends Pharmacol. Sci. 10, 342-344). The 2-ethoxy analogue of idazoxan, RX811059 is a highly selective α2-adrenoceptor antagonist with minimal NAIBS affinity (Mallard et al, 1991, Br. J. Pharmacol. 102, 221P).

The purpose of this study was to examine the ability of RX811059 to produce a discriminable cue in rats. A group of male Lister-Hooded rats (n = 6) learned to discriminate RX811059 (2.5 mg/kg, IP) from saline in a fixed ratio (FR = 10) DD schedule. A series of α2-adrenoceptor antagonists were tested for their ability to generalise to (i.e. mimic) the RX811059-induced cue.

RX811059 itself dose-dependently mimicked the RX811059-induced cue (i.e. >80% total responses were RX811059 associated), as did the α2-adrenoceptor antagonists idazoxan, fluparoxan and 1-(2-pyrimidinyl)-piperazine (1-PP), using doses up to 2.5, 10, 3 and 3 mg/kg respectively. However, the peripherally acting α2-adrenoceptor antagonist L659,066 (Clineschmidt et al, 1988, J. Pharmacol. Exp. Ther. 245, 32-40) failed to generalise to the RX811059-induced cue (i.e. <10% total responses were RX811059 associated) as did clonidine, an α2-adrenoceptor agonist, using doses up to 8 and 0.04 mg/kg respectively.

Thus the highly selctive α2-adrenoceptor antagonist, RX811059 produces a discriminable cue in rats which appears to be central in origin.

641.15

THE INVOLVEMENT OF β-ADRENERGIC MECHANISMS IN STRESS-RELATED BEHAVIORAL CHANGES.

A. Laurel Gorman and Adrian J. Dunn*. Dept of Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130.

Substantial evidence suggests that noradrenergic systems are involved in stress-like behavioral responses. Previous studies showed that α_1-adrenergic agonists induced defensive withdrawal in rats (Yang *et al.*, 1990) and decreased mean stimulus-contact times in mice tested in the multicompartment chamber (MCC: Berridge & Dunn, 1989). Because the α_1-agonist-induced behaviors were attenuated by pretreatment with the CRF antagonist, alpha-helical CRF_{9-41}, these responses may have resulted from α_1-adrenergic stimulation of CRF release. However, β-antagonists exhibit anxiolytic properties in defensive withdrawal, suggesting that noradrenergic systems play additional roles in anxiety-like responses. L-Propranolol (2.5-5 mg/kg ip) decreased defensive withdrawal in naive rats and reversed that induced by restraint or icv CRF (Yang *et al.*, 1990). We now show that propranolol also reversed restraint-induced changes in the behavior of mice in the MCC and the elevated plus-maze.

To investigate further the role of central noradrenergic systems in defensive withdrawal behavior, the effects of β-adrenergic stimulation were assessed using the β-agonist isoproterenol. Isoproterenol (1-10 μg icv) produced a dose-dependent increase in defensive withdrawal, significant at the 10 μg dose. L-Propranolol (0.1-3 mg/kg ip) reversed isoproterenol-induced defensive withdrawal, suggesting that the response to isoproterenol resulted from the activation of β-adrenergic receptors. These results support earlier data indicating the involvement of central β-adrenergic receptors in stress-related behavioral responses (Yang & Dunn, 1990).

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641.16

INTERACTION OF GEPIRONE AND HOMOCYSTEIC ACID ON ULTRASONIC VOCALIZATIONS AND OTHER FEAR-RELATED BEHAVIORS IN ADULT RATS. D.J.Knapp, D.Benjamin, Y.Ahmad, J.Stern,* & L.A. Pohorecky. Center of Alcohol Studies and Department of Psychology, Rutgers University, Piscataway, NJ 08855-0969.

Previous experiments in this laboratory have found that the administration of 1-3 air puffs to rats elicits robust 22 kHz ultrasonic vocalizations (USVs) which continue, without further stimulation, for up to 20 minutes. In the present experiments, the ability of pharmacological agents to modify ultrasonic vocalizations (USVs) and other fear or anxiety related behaviors was studied in male Long-Evans rats. Previous investigators reported that the 5-HT1A agonist, 8-OH-DPAT, could reverse fear-inducing effects of the excitatory amino acid, D,L-homocysteic acid (DLH), administered to the dorsal periaqueductal gray (DPAG). In our investigations, the peripheral administration of the 5HT1A agonist, gepirone, potently reduced air-puff induced USVs. Intra-DPAG DLH (5 nmol/250 nL) induced a range of fear-like behaviors including USVs. Lower doses (1 nmol) or administrations of DLH onto the DPAG induced a behavioral response characterized primarily by immobility and USVs which were gepirone reversible (30 nmol/250 nL), while more dorsal administrations induced contralateral rotations or no response. Higher doses (3-5 nmol) or administrations more ventral or ventrolateral resulted in violent defensive reactions or running fits which were apparently incompatible with USVs and irreversible with gepirone (30 nmol/250 nL). Furthermore, administration of air puffs to these rats did not elicit USVs during or after this robust behavioral response to DLH. These data in part corroborate previous findings of anti-aversive 5-HT1A effects on excitatory amino acid stimulation in the central gray, and expand this interaction to include USVs. Rat USVs may serve as a useful model of human panic anxiety or fear.

641.17

OPERANT RESPONSE SUPPRESSION (RS) INDUCED WITH SYSTEMIC ADMINISTRATION OF 5-HYDROXYTRYPTOPHAN (5-HTP) IS CENTRALLY MEDIATED. E.A. Engleman*, J.M. Murphy, F.C. Zhou, M.H. Aprison, and J.N. Hingtgen. Depts Psychiat; Biochem; Anat; Psychol; Prog Med Neurobiol; Inst Psychiat Res; Indiana U Sch Med; Purdue Sch Sci; Indianapolis, IN 46202-4887

Systemic administration of 5-HTP produces behavioral disruptions in animals performing food reinforced operant tasks. Previous studies have demonstrated that these effects are likely mediated through serotonin 5-HT_2 and/or 5-HT_{1C} receptors. In the current study, intracerebroventricular (ICV) administration of LY53857, a potent and selective 5-HT_2 and 5-HT_{1C} receptor antagonist, was used to test the hypothesis that 5-HTP induced RS is centrally mediated. Male Wistar rats trained to lever press for milk reinforcement were stereotaxically implanted unilaterally with chronic 22 ga guide cannulae just above a lateral ventricle. DL-5-HTP (25mg/kg,IP) given 15 min. into a VI 1' operant session produced a baseline period of RS. ICV injections (1.0, 3.75, or 7.5ug in 5ul) of LY53857 immediately prior to systemic 5-HTP (25mg/kg) sessions, dose dependently reduced the period of RS. These results provide additional evidence that 5-HTP induced RS is centrally mediated through 5-HT_2 and/or 5-HT_{1C} receptors. (Indiana Dept. Mental Health, IUPUI Res. Invest. Fund, AA08553, Assn. Adv. of Mental Health Res and Edu. Inc.)

641.18

5-HT_{1A} RECEPTOR AGONIST AND ANTAGONIST EFFECTS ON SUPPRESSION OF RESPONDING IN A MODEL OF DEPRESSION. T.A. Lovell, E.A. Engleman, M.H. Aprison, J.N. Hingtgen* and J.M. Murphy. Dept. Psychology, Purdue Sch. Sci.; Prog. Med. Neurobiol., Inst. Psychiat. Res., Dept. Psychiatry, Indiana U. Sch. Med., IUPUI, Indianapolis, IN 46202-4887.

Previous studies have indicated the involvement of 5-HT_2 and/or 5-HT_{1C} receptors in 5-HTP-induced operant response suppression. This study tested whether 5-HT_{1A} receptors are also involved in the response suppression effects of 5-HT agents. Male Wistar rats (n=8), maintained at 80-85% of free-feeding weight, were trained to bar press for sweetened milk on a VI-60" reinforcement schedule. The 180-min sessions were given every other day, 3 days/week. After responding stabilized, rats received IP injections of saline or drug 15 min into at least one of 3 weekly sessions. Suppression of responding averaged 34±6 min after IP injection of L-5-HTP and 45±7 min after 8-OH-DPAT (1 mg/kg). Propranolol (5 mg/kg), which has 5-HT_{1A} antagonist properties, did not significantly block the 5-HTP-induced suppression, but it did significantly ($p<0.05$) attenuate the 8-OH-DPAT-induced response suppression by approximately 75%. 5-HT_{1A} receptors may partially mediate suppression seen after injection of some 5-HT agents in this pharmacological model of depression. (Supported by Indiana Dept. Mental Health & AA08553)

RECEPTOR MODULATION, UP AND DOWN REGULATION IV

642.1

THE EFFECT OF CONTINUOUS AND INTERMITTENT LEVODOPA ADMINISTRATION ON STRIATAL DOPAMINE METABOLISM: A MICRODIALYSIS STUDY. J.L. Juncos*, M.S. Hooks, J.B. Justice, Jr., Departments of Neurology and Chemistry, Emory University, Atlanta, GA 30322.

Behavioral and biochemical indices of dopaminergic function are affected differentially by continuous and periodic levodopa administration. To elucidate the mechanisms of this differential effect, male SD rats were treated for 7 days with levodopa methylester (LDME, 100 mg/kg/d), a soluble form of levodopa, either continuously (ALZA® minipump) or intermittently (i.p., b.i.d.). Control rats received saline. Following a 24 hour washout, all groups were challenged with i.p. injections of either a dopamine receptor blocker (haloperidol, 1 mg/kg), or a dopamine transporter blocker (GBR-12909, 20 mg/kg). Outcome measures included locomotor activity, catalepsy scores, and dopamine concentration in the caudate-putamen using in-vivo microdialysis and HPLC.

During the 7 days of treatment there were no differences in locomotor activity; after the 24 hour washout there were no differences in baseline dopamine levels. Following haloperidol challenge there was a time-dependent threefold increase catalepsy scores ($p<0.05$) in all LDME-treated rats compared to the saline-treated rats. Haloperidol-induced elevations in dopamine levels were 55% higher in the continuously-treated group compared to the other two groups ($p>0.01$) which did not differ from each other. This difference consisted of plateau of dopamine levels evident in the 60-180 minute interval post challenge. GBR-12909-induced elevations in dopamine levels were 2-4 times higher than those induced by haloperidol. The saline- and continuous LDME-treated rats exhibited plateau responses comparable to the above, but in contrast, the saline group reached levels that were 55% higher than those in the continuous group ($p<0.01$). During the plateau the intermittent LDME-treated rats exhibited a biphasic response with high levels at 45-90 minutes and lower levels at 90-180 minutes ($p<0.0025$). The results suggest that continuous and periodic levodopa administration have a differential and probably reciprocal effect on extracellular dopamine modulation by the dopamine autoreceptor and the dopamine transporter.

642.2

AUTO-RECEPTOR (autoR) SUPERSENSITIVITY (SS) IS ASSOCIATED WITH PERMANENT, BUT NOT TRANSIENT, DA RECEPTOR (DAr) SUPERSENSITIVITY. JZ Fields-, GE Drucker, R Song & JH Gordon. Res Svce 151, VA Hosp, Hines IL 60141.

Female rats on chronic neuroleptics show a transient increase in postsynaptic D2 DAr density (TRANS rats). In contrast, in ovariectomized (OVX) rats on neuroleptics, the D2 upregulation is permanent (PERM rats). We hypothesized that an upregulation of presynaptic autoR occurs in PERM but not TRANS rats. This autoR SS should lead to lower DA levels in the synaptic cleft. This would then attenuate the normal ability of DA to downregulate D2 DAr and restore homeostasis. Testing PERM and TRANS rats, we evaluated nigrostriatal D2 autoR by 3 independent methods: i) apomorphine (APO) inhibition of in vivo DA synthesis in gamma-butyrolactone pretreated rats; ii) hypolocomotion induced by low dose APO; iii) in vitro APO inhibition of efflux of [3H]DA from superfused striatal slices. Our data show a significant increase in APO potency at autoR in PERM rats. We interpret these data as an autoR SS associated with decreased cleft DA levels. Our proposed mechanism for the permanence of the DAr upregulation in PERM rats thus involves autoR SS and decreased cleft DA levels which potentiate the D2 DAr SS; this leads to a vicious cycle that maintains upregulation of both pre- and post-synaptic DAr in PERM rats. The lack of an autoR SS in TRANS rats suggests that they retain the ability to compensate by increasing DA release. The PERM rat may be useful for modelling permanent DAr changes such as occur in tardive dyskinesia and schizophrenia. (Supported by VA Med Res & RoINS26449)

642.3

MODULATORY EFFECT OF SEROTONIN ON HALOPERIDOL-INDUCED UP-REGULATION OF DOPAMINE D-2 RECEPTORS. S.Matsubara*, R.Matsubara, T.Koyama, A.Ishikane and I.Yamashita. Dept. of Psychiatry Hokkaido Univ. School of Med., Sapporo, Hokkaido 060, Japan

Chronic treatment of rats with haloperidol (HAL,0.25-1mg/kg,3wks) increased the number of D-2 receptors in the striatum, while no increase was obseved by that with risperidone (1mg/kg), an atypical antipsychotic drug which has high affinity at serotonin(5-HT)2 receptor sites with lower affinity at D-2 sites. Chronic treatment with MK-212, a nonselective 5-HT agonist (2.5mg/kg), or with citalopram, a 5-HT reuptake inhibitor (10mg/Kg), both of which had no effect on the number of D-2 receptor sites by themselves, potentiated the up-regulation of D-2 receptor sites when coadministrated with HLA (0.5mg/kg). Coadministration of ritanserin, a 5-HT2/5-HT1c antagonist (1mg/kg), on the other hand, had no influence on the HAL-induced increase of D-2 receptor sites. These results suggest that serotonergic activity may have a complex modulatory influence on the up-regulation of D-2 receptor sites by HAL. Study on the effect of another manipulation of serotonergic activites is ongoing.

642.4

DENERVATION OF MOUSE STRIATUM DECREASES D_1 RECEPTOR mRNA AND THE RATE OF SYNTHESIS OF D_1 RECEPTORS AND INCREASES D_2 RECEPTOR mRNA AND THE RATE OF SYNTHESIS OF D_2 RECEPTORS. Z.H. Qin, J.F. Chen and B. Weiss*. Div. of Neuropsychopharmacology, Dept. of Pharmacology, Medical College of PA, Philadelphia, PA 19129.

To study the effects of dopaminergic input to the corpus striatum on the expression of D_1 and D_2 dopamine receptors, mice were unilaterally lesioned with 6-hydroxydopamine and then injected with a single dose of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) which irreversibly inactivates both D_1 and D_2 receptor sites. D_1 and D_2 receptors and D_1 and D_2 receptor mRNAs were analyzed at various times after EEDQ treatment. D_1 and D_2 receptors were analyzed in situ by receptor autoradiography, using [^{3}H]-SCH23390 and [^{3}H]-raclopride for D_1 and D_2 receptors, respectively. D_1 and D_2 receptor mRNAs were analyzed by in situ hybridization histochemistry, using radiolabeled oligodeoxynucleotide probes. The results showed that 4 hr after EEDQ treatment, more than 90% of the D_1 and D_2 receptors were inactivated. The rate of recovery of D_1 receptors following inactivation by EEDQ was lower in the lesioned striatum than in the unlesioned striatum while the rate of recovery of D_2 receptors was higher in the lesioned striatum than in the unlesioned striatum. Denervated striata showed small but significant decreases in D_1 receptor mRNA and increases in D_2 receptor mRNA. In both cases, the largest percentage changes were seen in the lateral striatum. These studies suggest that dopaminergic denervation results in a decreased synthesis of D_1 receptors and an increased synthesis of D_2 receptors and that these changes are due to alterations in the levels of the respective mRNAs for the D_1 and D_2 receptors. (Supported by MH42148).

642.5

MODULATION OF DOPAMINE D1 RECEPTOR mRNA IN THE STRIATUM AND NUCLEUS ACCUMBENS OF RAT DURING THE ESTROUS CYCLE BY IN SITU HYBRIDIZATION. M. Morissette*, P. Falardeau and T. Di Paolo. Mol. Endocinol., CHUL Res. Centre, G1V 4G2 and Sch. Pharm., Laval Univ., G1K 7P4, Québec, CANADA.

Dopamine (DA) D1 receptor density was previously shown to increase after chronic estradiol treatment and to fluctuate during the estrous cycle. In order to investigate the mechanism of the hormonal modulation of D1 DA receptors, the present study investigated brain D1 receptor mRNA changes during the estrous cycle. The level of DA D1 mRNA in the rat striatum and nucleus accumbens was evaluated by *in situ* hybridization during the estrous cycle and compared to ovariectomized (OVX) rats. During the estrous cycle, rats in the morning of estrus, diestrus I, diestrus II, and proestrus (PAM) were studied as well as rats in the afternoon of proestrus. A groups of rats was OVX and killed 14 days after their surgery. A fragment from the rat striatal D1 receptor cDNA corresponding to the carboxy terminal tail of the receptor +2300bp of the 3' untranslated area was subcloned into pBluescript. ^{35}S-labelled antisense- or sense-strand RNA probes were prepared by *in vitro* transcription and hybridized with 4% paraformaldehyde-fixed adjacent coronal rat brain sections (10 µm). In OVX rats, a high level of DA D1 receptor mRNA was observed in striatum, nucleus accumbens and olfactory tubercle whereas no signal was detectable in the substantia nigra. During the estrous cycle, the levels of D1 mRNA in the striatum and in the nucleus accumbens were decreased in PAM as compared to rats in the other stages of the cycle and to OVX rats, the levels of D1 mRNA being similar in the latters. Fluctuation of striatal D1 DA receptors showing a peak in diestrus and of striatal D1 receptor mRNA during the estrous cycle probably reflected a different mechanism of action of steroid hormones on these dopaminergic components. Supported by a MRC of Canada grant to T.D.P..

642.6

DOPAMINE RECEPTORS AND BEHAVIOR: MODIFICATION BY DOPAMINERGIC LESIONS AND INTRASTRIATAL FETAL CELL GRAFTS. **L. Pundt*, L. Fisher†, F. Gage†, N. Narang and J.K. Wamsley**. Dept. of Pharm. Sci., NDSU and Neuropsych. Res. Ins., Fargo, ND 58103; †Dept. of Neurosci., UCSD.

Studies were undertaken to investigate alterations in dopamine receptors (D_1 and D_2), D_2 message, and dopamine uptake sites in the striatum of 6-hydroxydopamine (6-OHDA) lesioned rats after intrastriatal fetal cell transplantation. Attempts were made to determine the relationship between these alterations and behavior (amphetamine-induced rotations). It was observed that D_2 receptor binding on the ipsilateral (grafted) side was most highly correlated with the degree of behavioral recovery observed. Recovery was also found to be significantly related to the alterations of dopamine uptake sites on the ipsilateral side, but not on the contralateral side. There were no significant correlations between behavior and D_1 receptors or D_2 message. This study suggests that alterations in D_2 receptor binding and dopamine uptake sites on the ipsilateral side of a lesioned and subsequently grafted brain have the most influence on behavioral recovery.

642.7

BILATERAL INTERDEPENDENCE OF DA SYSTEMS IN THE BASAL GANGLIA. **N. Narang*, L. Pundt, M.E. Hunt, M.E. Alburges and J.K. Wamsley. Neuropsychiatric Res. Institute, 700 1st Ave. S., Fargo, ND 58103.**

Changes in dopamine (DA) receptors in basal ganglia result from many movement disorders. Injection of ibotenic acid (IA) in the caudate-putamen (CPU) of the rat has been proposed as an animal model for Huntington's disease. We examined the effect of unilateral IA lesions on D_2 receptor binding and message on both sides of the CPU. One group of male LE rats was stereotaxically injected with saline and the second group with IA (20 µg/2 µl) in the CPU. After 2 weeks, in situ hybridization and D_2 receptor binding were performed using [^{35}S]ATP radiolabeled DA D_2 receptor oligonucleotide probe and [^{3}H]raclopride. A significant increase in D_2 receptor mRNA (113%) with no change in D_2 receptor binding, was found in the CPU on the contralateral side when compared to saline injected animals. These results suggest that compensatory changes may be occurring on the unlesioned side of the brain which involve regulation of D_2 receptor transcription. This observation of bilateral interdependence in the DA system may be of importance in understanding movement disorders.

642.8

DOPAMINERGIC CHARACTERISTICS IN NEUROBLASTOMA CELL LINES FOLLOWING DIFFERENTIATION WITH RETINOIC ACID. D. R. NEW, H. A. GELBARD*, and M. F. D. NOTTER, Department of Neurobiology and Anatomy and Department of Pediatric Neurology, University of Rochester Medical Center, Rochester, N.Y. 14642.

The neuroblastoma originates from a solid tumor of the primitive cells of the neural crest, specifically from embryonic autonomic neuroblasts. Neuroblastoma cell lines are a commonly used model to study neuronal development and the influence of growth modulators on neural differentiation. The neuroblastoma cell lines, SK-N-AS and SK-N-SH, have been found to have functional D-2 dopamine subtype receptors. A functional D-1 dopamine subtype receptor that tightly couples to adenylate cyclase has been identified in the SK-N-MC cell line that is similar to striatal D-1 receptors. Studies on intracellular dopamine content in the LA-N-1 cell line have found that dopamine can be increased, while norepinephrine content decreases following differentiation with the morphogen retinoic acid.

Our studies utilized the neuroblastoma cell lines SK-N-MC and LA-N-1 to characterize the D-1 and D-2 dopamine subtype receptor expression following treatment of these cell lines with retinoic acid. Our preliminary results revealed that both cell lines can be morphologically differentiated with retinoic acid consistent with observations reported by others. While D-1 and D-2 receptor subtypes cannot be enhanced with retinoic acid in the SK-N-MC cell line. The LA-N-1 cell line has been found to contain a D-2 dopamine subtype receptor that can be upregulated with retinoic acid, with the appearance of two binding sites with two different affinities. Studies on the D-1 subtype receptor are in progress at this time. These results suggest that the LA-N-1 cell line can be upregulated with retinoic acid to express two different classes of the D-2 dopamine subtype receptor.

642.9

INDUCTION OF EXPRESSION OF ENDOGENOUS D2 DOPAMINE RECEPTOR IN GH4C1 CELL. S. Allard, M. Labbé and P.Falardeau*, Génétique Moléculaire, CHUL, and School of Pharmacy, Laval Univ., Québec, Canada, G1V 4G2

GH4C1 cells are subclone of the GH3 cells (a prolactin-secreting cell line) which do not respond to dopamine agonists. In contrast with GH3 cells (Missale et al, J.Biol. Chem., 266: 23392, 1991), native GH4C1 do not show any D2 receptors when assessed by PCR for mRNA levels or by [3H]spiperone binding. After transfection of pRSVNeo, a plasmid which procure neomycin resistance, all selected colonies expressing neomycin resistance gene showed the presence of both dopamine D2 receptor mRNA and [3H]spiperone binding. cDNA of this D2 receptor was amplified and sequenced. No difference was observed in the coding region of this receptor when compared with the cloned rat D2 dopamine receptor. In the transfected cells, level of binding for D2 receptor range between 150-500 fmol/mg of protein. Moreover, selective D2 agonist such as quinpirole can inhibit forskolin- as well as VIP-stimulated cAMP formation by up to 90% in a dose related fashion. Cells grown in presence of 0.25mg/ml Geneticin (G418 sulfate) for 3 weeks lead to a two fold increase in the amount of [3H]spiperone binding. The precise mechanisms associated with the induction of D2 dopamine receptor in these cells are still not well understand. However this model can be used to study gene regulation of D2 dopamine receptor. (Supported by MRC grant)

642.10

REPRESSION OR ACTIVATION OF REGULATORY (RI-RII) SUBUNITS OF cAMP DEPENDENT KINASE mRNA LEVELS FOLLOWING CHRONIC BLOCKADE OF D1 OR D2 DOPAMINE (DA) RECEPTORS IN RAT STRIATUM

G. Schettini*, C. Ventra, A. Porcellino ^, M. Grimaldi and E. Avvedimento ^. Dip. di Farmacologia, and ^ Dip. di Biol e Patol. Mol. e Cell, II Facolta'di Medicina, Universita'di Napoli, Italy.

We studied the modulation of RI and RII regulatory subunits of cAMP-dependent kinase mRNA expression in the development of DA receptors supersensitivity. We found both genes expressed in the striatum. The blockade of D1 receptor by SCH 23390 for 1, 3, 7, 14, and 21 days, likely lowering striatal cAMP levels, reduced RII mRNA content, measured 1 h after the last injection. This effect occurred after 7 days, lasted till the 21st day, and was no more detectable 72 h after the last injection. No apparent changes of RI mRNA content were observed. The blockade of D2 receptors by haloperidol, likely increasing striatal cAMP content, enhanced both RI and RII mRNA levels. Such changes occurred very rapidly. We suggest that high cAMP levels specifically increase RI and RII striatal mRNA, while low cAMP concentrations suppress RII mRNA.

642.11

REGULATION OF THE ALPHA-2C ADRENERGIC AND 5-HT_{1B} SEROTONERGIC RECEPTORS BY DEXAMETHASONE IN AN OPOSSUM KIDNEY (OK) CELL LINE. H.S. Blaxall,* R.C. Pleus, D.R. Cerutis, N.A. Hass and D.B. Bylund, Dept. of Pharmacology, Univ. of Nebraska Med. Ctr., Omaha, NE 68198-6260.

Previous pharmacological studies from our laboratory have characterized the alpha-2 adrenergic receptor expressed by the OK cell line as the alpha-2C subtype and the serotonergic receptor expressed as the 5-HT_{1B} subtype. Both of these receptors are down-regulated by treatment with their respective agonists, norepinephrine and serotonin.

To further investigate the regulation of these receptors OK cells were treated with dexamethasone. Cells were grown in serum free, steroid free media (Ultroser SF, Sepracor, Columbia, MD) in the presence and absence of 100 nM dexamethasone. After 72 hours, control and treated OK cells were harvested and membranes were prepared for saturation binding assays. The B_{max} values were determined for the alpha-2C adrenergic receptor using [^{3}H]rauwolscine and for the 5-HT_{1B} serotonergic receptor using [^{125}I]iodocyanopindolol. The alpha-2C receptor number was decreased by ~ 30% and the 5-HT_{1B} receptor number was increased by ~ 55% with dexamethasone treatment. Thus, it appears that in OK cells dexamethasone causes a down-regulation of alpha-2C receptors and an up-regulation of 5-HT_{1B} receptors. (Supported by NIH grants GM40784 and MH47354).

642.12

REGULATION OF A2 ADENOSINE RECEPTOR mRNA BY PHORBOL ESTERS IN THE PC12 CELL LINE. JS Fink* and R A Peterfreund, Depts. of Anesthesia and Neurology, Massachusetts General Hospital, Boston, MA 02114

A rat cDNA which encodes a high affinity A2 adenosine receptor (A2R) was recently cloned and expressed in our laboratory (Mol Br Res, 1992, *in press*). PC12 cells, a clonal rat cell line known to express functional high affinity A2Rs (J. Neurochem. **37**, 1431: 1981) were found to produce high levels of mRNA which specifically hybridized to a rat A2R cDNA probe. Activation of the protein kinase C (PKC) second messenger system is known to regulate mRNA levels for other G protein-linked receptors. We asked if activation of the PKC second messenger system regulates A2R mRNA levels in PC12 cells.

Treatment with tetradecanoyl phorbol acetate (TPA), which activates PKC, reduced the levels of A2R specific mRNA by 50-75% in two different subclones of PC12 cells. The effect was dose dependent; inhibition was observed for doses greater than 10 nM. A reduction in A2R mRNA levels was first detected after 2 hours and was maximal by 5-6 hours. Structural analogs of TPA known to have reduced efficacy to activate PKC were less effective in reducing A2R mRNA levels. Cells treated with TPA and allowed to recover overnight exhibited an increase in A2R mRNA levels. The A2R is believed to exert its intracellular effects by a signal transduction pathway involving activation of adenylate cyclase and production of cAMP, but not through activation of PKC. Our results are consistent with heterologous regulation of transcription or stability of A2R mRNA by PKC activation.

642.13

THE G PROTEIN βγ SUBUNIT ACTIVATES AGONIST-DEPENDENT PHOSPHORYLATION OF β ADRENERGIC AND MUSCARINIC RECEPTORS by β ADRENERGIC RECEPTOR KINASE 1 AND MUSCARINIC RECEPTOR KINASE. Kimihiko Kameyama, Kazuko Haga, and Tatsuya Haga*. Dept. Biochem., Inst. Brain Res., Fac. Med., Univ. Tokyo, Hongo, Tokyo 113, Japan

We have previously shown that the βγ subunit of G proteins stimulates the agonist- or light-dependent phosphorylation of muscarinic receptors (mAChRs) and rhodopsin by a protein kinase (mAChR kinase) partially purified from porcine cerebrum (J.BiolChem.,267,2222 (1992)).The light dependent phosphorylation of rhodopsin by rhodopsin kinase, however, is not stimulated by the βγ subunit, and it is not known if the agonist-dependent phosphorylation of β adrenergic receptor (βAR) by βAR kinase is stimulated by the βγ subunit. We report here that the βγ subunit also stimulates the agonist-dependent phosphorylation of bovine lung βARs by the mAChR kinase and the agonist-dependent phosphorylation of mAChRs (m2 subtype) by recombinant βAR kinase 1 extracted from cos-7 cells. These results suggest that the βAR kinase 1 is the same as or very similar to the mAChR kinase but is distinguished from the rhodopsin kinase with respect to activation by the βγ subunit. We hypothesize that the βγ subunit activates the βAR kinase thereby facilitating the desensitization of phosphorylated receptors. The cDNA for βAR kinase 1 was kindly provided by Dr. R.J. Lefkowitz.

642.14

DIFFERENTIAL EFFECTS OF PHORBOL ESTER ON THE FUNCTIONAL COUPLING OF THE RAT MUSCARINIC M_1 AND M_2 RECEPTORS. S.W. Ma, W.R. Roeske, H.I. Yamamura and J. Lai*. Departments of Pharmacology and Internal Medicine, The University of Arizona Health Sciences Center, Tucson, AZ 85724.

Carbachol (CCh) mediated a stimulation of [^{3}H]IP_1 accumulation in transfected B82 cells expressing the M_1 receptors with an EC_{50} value of 22.6 μM and a maximal stimulation of 22 fold above basal level. Pretreatment of these cells for 60 min at 37°C with phorbol 12-myristate, 13-acetate (PMA) resulted in a rapid desensitization of the M_1 receptors with an IC_{50} value of 9.1 nM and a maximal inhibition of 84% of the effect of 100 μM CCh. PMA (0.1 μM) pretreatment had no effect on the multiple affinities of CCh for the M_1 receptors in intact cells or membrane preparations (K_H = 2.85 μM; K_L = 389 μM), contrasted with a single low affinity of 124 μM for CCh in the presence of 100 μM GTP-γ-S. Treated cells showed a reduction in total M_1 receptor density (67.4 ± 6.6 % of [^{3}H](-)QNB binding in control cells) as well as the number of receptors on the cell surface (76.7 ± 3.3 % of [^{3}H](-)MQNB binding in control cells). The affinity of the antagonists was not affected ($p>0.05$). The M_2 receptors expressed in B82 cells were coupled to an inhibition of cAMP formation and a small stimulation of [^{3}H]IP_1 accumulation. Neither of these functions nor the density of the M_2 receptors was altered by 0.1 μM PMA pretreatment. These data demonstrate clearly a differential sensitivity of the M_1 and M_2 receptors to PMA in their functional coupling. The rapid desensitization of the M_1 receptors may be due to a PMA-induced uncoupling of phospholipase C from the G-proteins. Supported by AHA and NIMH.

642.15

G PROTEIN LEVELS AND EXPERIMENTAL ASTHMA J.Y. Lee#+*, Y. Uchida+, T. Sakamoto+, A. Nomura+ and F. Hirata#+. #Dept. of EHS, The Johns Hopkins Univ., Baltimore, MD 21205, and +Depts. of Pharm. Sci., and Pharmacol., and Inst. of Chem. Tox., Wayne State Univ., Detroit, MI 48202

Asthma is characterized by the hyperresponsiveness to constrictors and hyporesponsiveness to relaxants. Since most, if not all, receptors for constrictors and relaxants are members of "G protein coupled receptor" family, we hypothesized that an altered expression of G proteins is responsible for such receptor dysfunctions. Mild (acute) and severe (chronic) asthma were produced in previously sensitized guinea pigs by a single or multiple challenges with aerosolized immunogen, ovalbumin. Levels of Gq_α and Gi_α but not of Gs_α and G_β increased as a function of time when measured at 0, 12 and 24 hr postimmunochallenge. Guinea pigs with severe asthma had larger increases in Gq_α and Gi_α than animals with mild asthma. The in vitro assay of isolated tracheas showed that the hyperreactivity to cholinergic agents takes place only in the animals with severe asthma immediately after the challenge, while the hyporeactivity to isoproterenol progressed in both of the animals with mild and severe asthma. Our results suggest that an altered expression of Gq and Gi proteins in airway smooth muscle is partly, if not totally, responsible for receptor dysfunctions observed in asthma.

642.16

SEROTONIN 1_A AND 1_B RECEPTOR BINDING IS INCREASED IN THE RAT HYPOTHALAMUS FOLLOWING ACUTE DENERVATION. M. Frankfurt*, S.D. Mendelson, C.R. McKittrick and B.S. McEwen. Neuroendocrinology Lab., The Rockefeller University, New York, NY 10021.

Alterations in serotonin (5-HT) receptor binding were assessed in male rats by quantitative autoradiography following intrahypothalamic injection of the serotonin neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT). [^{3}H]8-OH-DPAT binding was used to label 5-$HT1_A$ receptors and [^{125}I]-iodocyanopindolol binding in the presence of isoproterenol (to mask ß-adrenergic receptors) was used to label 5-$HT1_B$ receptors. Seven days after 5,7-DHT, when 5-HT levels are lowest, [^{3}H]8-OH-DPAT binding was increased in the ventromedial and dorsomedial hypothalamic nuclei (VMN, DMN). In the same animals, [^{125}I]-iodocyanopindolol binding was increased in the VMN, but unchanged in the DMN. No changes in 5-$HT1_A$ or 5-$HT1_B$ binding were observed in the lateral hypothalamic area, the dentate gyrus, or the CA1 region of the hippocampus, in spite of a large decrease in the binding of [^{3}H]paroxetine, which labels the presynaptic 5-HT transporter site. These results demonstrate differential regulation of 5-HT receptors in response to denervation and also suggest that some of the 5-$HT1_B$ receptors in the VMN are located post-synaptically. Studies in females are in progress to determine possible sex differences in the response of 5-HT receptors to 5,7-DHT. Supported by NS07080.

642.17

ANTISENSE OLIGODEOXYNUCLEOTIDE INHIBITION OF NEUROPEPTIDE Y (NPY) Y1-RECEPTOR EXPRESSION. F. Yee*, M. Heilig and C. Wahlestedt. Div. Neurobiol., Dept. Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021 and Dept. Neuropharmacol., Scripps Res. Inst., La Jolla, CA 92037.

The Y1-receptor mediates behavioral and vascular actions of NPY. Since specific Y1-receptor antagonists are not available, we have employed the antisense oligonucleotide approach to suppress Y1-receptor protein synthesis *in vitro* and *in vivo* (Heilig *et al.*, this meeting). Based on the recent cloning of human and rat NPY Y1-receptor (Larhammar et al., *J. Biol. Chem.*, in press), antisense and sense (control) oligodeoxynucleotides (D-oligos) corresponding to different regions of the receptor were synthesized. These D-oligos were then added to media of cultured cells, *e.g.* rat primary cortical neurons and human neuroblastoma cells (SK-N-MC), which we had previously found to express the Y1-receptor by RT-PCR and/or Northern analyses (*ibid*). In both these cell types, antisense D-oligos directed to regions immediately downstream of the initiation codon were found to reduce, by up to 90%, high affinity binding sites labeled by ^{125}I-peptide YY (PYY). D-oligo concentrations as low as 0.1-1 μM (maintained over 3-5 days) were sufficient for reducing ^{125}I-PYY binding sites in cortical neurons under serum-free conditions. In contrast, SK-N-MC cells, which were trypsinized prior to addition of D-oligos and grown in the presence of heat inactivated sera, required 10 μM concentrations for similar suppression. Comparable data (70% inhibition of full NPY response) were obtained when assessing the ability of NPY to reduce forskolin stimulated cAMP accumulation in SK-N-MC cells treated with the antisense D-oligo. No biochemical (protein content and LDH release) or morphological abnormalities were observed to be induced by the D-oligos at the above concentrations. The described antisense approach may thus be useful in attempts to specifically affect Y1-receptor synthesis and function, resulting in the reduction of NPY efficacy.

642.18

***IN VIVO* DOWNREGULATION OF NEUROPEPTIDE Y (NPY) Y1-RECEPTORS BY I.C.V. ANTISENSE OLIGODEOXYNUCLEOTIDE ADMINISTRATION IS ASSOCIATED WITH SIGNS OF ANXIETY IN RATS.** M. Heilig, E. Merlo Pich, G.F. Koob, F. Yee and C. Wahlestedt*. Dept. of Neuropharmacol., Scripps Res. Inst., La Jolla, CA 92037 and Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

Central NPY transmission has been suggested to be altered in depressive illness, in which anxiety is a common symptom. In rats, centrally administered NPY produces anticonflict/antianxiety effects by activating NPY Y1-receptors. The importance of endogenous NPY for anxiety remains unclear due to the absence of specific Y1-receptor antagonists. We therefore designed an 18-mer antisense oligodeoxynucleotide (D-oligo) corresponding to the amino-terminus of the rat Y1-receptor (Larhammar et al., *J. Biol. Chem.*, in press); this D-oligo was tested *in vitro* (Yee et al., this meeting). In this study, the antisense D-oligo was injected twice daily for two days into the CSF of awake, freely moving rats. Control animals received the matching sense D-oligo or saline. On the third day, antisense D-oligo treated rats showed marked signs of anxiety in an established animal model of anxiety, the elevated plus-maze. Sense D-oligo treated animals did not differ from saline controls. No effects on feeding or locomotor activity were observed. In cerebral cortex of antisense D-oligo treated rats, there was a time dependent reduction of the density (B_{max}) of binding sites labeled by either ^{3}H-NPY or ^{125}I-PYY. Since the cortex contains both Y1- and Y2-type NPY receptors, we used a Y1/Y2 masking approach to determine that the downregulation involved Y1-receptors only. Using the described antisense approach, it was thus possible to selectively inhibit cortical synthesis of the Y1-receptor protein. Since this inhibition was accompanied by marked signs of anxiety, we suggest that the anxiolytic effects of exogenous NPY may represent a physiological function of this endogenous transmitter.

642.19

DEVELOPMENTAL EXPRESSION OF CANNABINOID RECEPTOR mRNA C.R. McLaughlin*, W.L. Dewey and M.E. Abood. Dept. of Pharm., Med. Coll. of Virginia, Virginia Commonwealth University Richmond, VA 23298.

The cloning of a putative cannabinoid receptor affords the opportunity to examine its developmental expression and distribution. Other receptor systems, notably those for the opioids, have been shown to have distinct developmental time frames. For the initial study, Sprague-Dawley rats from the following age groups were employed: postnatal days 2, 5 and adults. The brains were grossly dissected into cerebellum and forebrain, and total RNA was extracted by a modified acid-extraction method. Expression of cannabinoid receptor mRNA was analyzed by two methods: Northern blot analysis and polymerase chain reaction (PCR). The probe used in the Northern blot analysis was a full length cDNA corresponding to the rat cannabinoid receptor. The probe was cloned in our lab based on published sequence information. Oligonucleotide primers based on bp 1-21 and bp 824-843 on the opposite strand were chosen for use in the PCR. Preliminary results indicate that by postnatal day 2, the cannabinoid receptor mRNA can be detected in the brain. In addition, spinal cords from 13 day old rats were analyzed. Cannabinoid receptor message was present, but at low levels, consistent with published receptor autoradiographic data from adult animals.

This research was supported by DA05274 and DA07027.

642.20

HEPARIN INHIBITS HIGH-AFFINITY BINDING OF [^{125}I]MELATONIN IN CHICK BRAIN. C.Harjivan, L.P. Niles, A. Nanji and E.S. Werstiuk*. Department of Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

It is now known that high-affinity binding sites for melatonin exist in diverse regions of the chick brain. Moreover, these sites are present on receptors which are coupled to inhibition of adenylate cyclase activity via a pertussis toxin-sensitive G protein. Recently, heparin was found to decrease the affinity of the α_2-adrenoceptor and to block adrenaline-induced inhibition of adenylate cyclase in human platelet membranes. Therefore, we have examined the effects of heparin on the G protein-coupled melatonin receptor in chick brain.

Heparin caused a dose-dependent inhibition of [^{125}I]MEL binding in chick brain synaptosomal membranes. In the presence of 100 units/ml heparin, binding was inhibited by about 50-75%. Typical biphasic agonist inhibition curves, which exhibit high- and low-affinity binding, were shifted to the right with loss of the high-affinity state in the presence of heparin. As previously observed for sodium, lithium inhibited high-affinity binding of [^{125}I]melatonin in chick brain membranes. A combination of maximally effective concentrations of sodium and lithium did not produce additional inhibition of binding. However, a combination of heparin and lithium resulted in an additive suppression of binding, indicating that these agents act on different sites on the receptor - G protein complex. The ability of heparin to abolish the high-affinity state of the melatonin receptor in chick brain, supports earlier evidence that this receptor is coupled to a G_i protein.

Supported by the OMHF, Canada.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION: STRESS

643.1

FORCED AMBULATION IN AN EXTREME POSTURE INCREASES TONIC CORTICOSTERONE BUT NOT ACTH LEVELS IN RATS. J. Hulse-Neufeld*, L. Breen, and R. Hauger. VA Medical Center (151), San Diego, CA 92161.

An animal model of low back dysfunction has been described (1991 meeting #120.4). Now tonic serum levels of corticosterone (B) and ACTH after six weeks of forced ambulation are reported so as to assess the degree of stress between groups of rats. Three groups of rats were compared: rats forced to ambulate on a flat surface (normal posture), rats forced to ambulate in rotating cylinders (extreme posture), and rats not forced to ambulate. After six weeks, rats forced to ambulate in cylinders and rats not forced to ambulate had higher tonic levels of B than rats forced to ambulate on a flat surface. The relationships of tonic levels of ACTH to B were different between the rats forced to ambulate in cylinders and on a flat surface. It is suggested from the results that forced ambulation in a normal posture lowers tonic levels of B, and forced ambulation in an extreme posture is more stressful than in a normal posture. Since the adaptation to exercise stress was associated with higher B levels when exercise was performed in an extreme posture, the voluntary assumption of an extreme posture in the low back is stressful in exercising (forced ambulation) rats.

643.2

GLUCOCORTICOID REGULATION OF THE ADRENOMEDULLARY CATECHOLAMINERGIC SYSTEM FOLLOWING A MILD ACUTE STRESSOR. P. Boksa*, K. Betito, S. Bhatnagar, J.B. Mitchell, J. Diorio & M.J.Meaney. McGill University, Depts. of Psychiatry, Pharmacology, and Neurology & Neurosurgery, Douglas Hospital Research Center, Montreal, Quebec H4H 1R3, Canada.

Adrenal medullary enzymes are regulated both by splanchnic innervation and by glucocorticoids (GCs). Phenylethanolamine N-methyltransferase (PNMT), the final enzyme in the catecholamine biosynthetic pathway, is predominantly regulated by GCs. Changes in the activity of PNMT in rats have been observed following a variety of chronic stressors, as well as with more acute stressors, such as an intermittent 2h swim stress or a 2.5h immobilization stress. We have previously shown that bovine adrenomedullary cells respond to a pulse of GCs as short as 15 min by increasing PNMT 2d later (Betito et al, 1992, J. Neurochem 58, 1853). In the present study, we looked at the adrenomedullary response of male rats to a more moderate acute stressor, 20 min restraint stress, where the increase in plasma corticosterone (CORT) is relatively short (1h). A significant increase in PNMT activity was observed only at 18h (17%) and 24h (14%) following restraint, but not before. Adrenaline and noradrenaline content of the adrenal increased significantly at 18h with no increase in tyrosine hydroxylase (TH). Splanchnic nerve transection did not alter basal PNMT and TH activities (as previously shown) and did not prevent an increase in PNMT activity following restraint. Inhibition of CORT synthesis by metyrapone (100 mg/kg, injections 24h and 2h prior to stress) prevented a stress-induced increase in PNMT activity at 24h. Suppression of CORT release by dexamethasone (DEX; 100 μg/kg injection 3h prior to stress) did not affect basal levels of PNMT, however the stress-induced increase in PNMT activity was prevented at 24h. These results demonstrate that a relatively short moderate stressor, which elevates GCs for only 1h, can elevate the activity of PNMT following a period of 18-24h. The regulation of this activity appears to be independent of neural input and dependent on the release of CORT during the period of stress.

643.3

REGULATION OF ACTH CO-SECRETAGOGUES DURING THE ESTROUS CYCLE IN THE RAT. V.Viau* & M.J.Meaney. Douglas Hospital Research Ctr., Depts. of Psychiatry and Neurology/Neurosurgery, McGill Univ., Montreal H4H 1R3, Canada

We have previously shown that the HPA axis in the female rat is most sensitive to stress during the proestrous phase of the estrous cycle (Viau and Meaney, Endocrinology, 1991). This suggests that high, physiological levels of estrogen secreted during proestrous could regulate ACTH co-secretagogues. We examined median eminence CRH and AVP content during the estrous cycle and in ovariectomized-estrogen replaced females. CRH and AVP content was higher in tissue obtained under basal conditions from females during proestrous and estrous compared to diestrous: CRH=11.1 $\pm$ 2.3, 7.2 $\pm$ 1.3, and 5.3 $\pm$ 0.4 pg/μg protein, respectively; AVP= 47.5 $\pm$ 10.0, 49.8 $\pm$ 14.1, and 30.9 $\pm$ 7.7 pg/μg, respectively. No differences were observed in median eminence content for either CRH or AVP between OVX and OVX-E2 replaced females, suggesting a concerted role for both estrogen and progesterone in the regulation of these peptides. AVP content, but not CRH, was higher in tissue obtained from OVX-E2 replaced animals following 20 min of restraint stress.

Plasma ACTH levels 5 min following icv administration of the α-1 adrenoreceptor agonist phenylephrine (50 μg) were significantly higher in rats during proestrous compared to diestrous; 1060 $\pm$ 130 vs 646 $\pm$ 175 pg/ml, respectively. Consistent with these data, AVP has been previously shown to mediate α-1 adrenergic stimulation of ACTH secretion (Al-Damluji et al, Endocrinology, 1990). Taken together, these results suggest that estrogen enhances the HPA response to stress during proestrous by enhancing the synthesis/release of CRH and, possibly to a greater extent AVP via an α-1 adrenergic receptor mediated mechanism.

643.4

EFFECTS OF ACUTE RESTRAINT STRESS ON CORTICOSTERONE, ACTH, CRH, AND VASOPRESSIN DIFFER ACROSS SEX AND STRAIN. S.L. Cummings,*[1] A.C. Griffin,[2] and C.C. Whitacre[2]. University of California Davis, CA 95616 and The Ohio State University, Columbus, OH 43210.

Experimental autoimmune encephalomyelitis (EAE), a model for the human demyelinating disease multiple sclerosis, is frequently studied in the highly susceptible Lewis (LEW) strain of rat. We and others have reported that restraint stress delays the onset and decreases the severity of EAE, particularly in the LEW female. The hypothalamic-pituitary-adrenal axis (HPA) has been hypothesized to play a role in the innate as well as stress-induced resistance to various autoimmune diseases, including rheumatoid arthritis and EAE. Therefore, we have examined corticosterone (CORT), adrenocorticotropic hormone (ACTH), corticotropin releasing hormone (CRH) and vasopressin (VP) levels in response to restraint in both sexes of 4 rat strains with varying susceptibility to inflammatory disease (LEW, Lewis Resistant, Brown Norway, and Fischer (F344). Plasma CORT and ACTH levels were determined by radioimmunoassay, and expression of CRH and VP mRNA in the hypothalamic paraventricular nucleus (PVN) was examined using in situ hybridization histochemistry. CRH and VP levels in the median eminence (ME) were visualized immunohistochemically. Levels of CRH and VP mRNA within the PVN were similar across strains in non-stressed animals, though steady state peptide levels within the ME of the highly resistant F344's consistently were higher than in the susceptible LEW. In response to 30 min of restraint stress, CORT and ACTH levels increased in both sexes of all strains, but to a greater degree in F344's than LEW's. CORT increased to higher levels in females than males, regardless of strain. After 30 min of stress, small increases in CRH and VP mRNA already were evident in the PVN of F344's, but not LEW animals. Taken together, these data suggest that susceptibility of EAE and other inflammatory diseases may be related to differential genomic regulation within the HPA axis.USPHS:MH44660

643.5

PITUITARY-ADRENOCORTICAL AND ADRENOMEDULLARY RESPONSES TO A NOVEL STRESS IN CHRONICALLY COLD STRESSED RATS. K. Betito*, S. Bhatnagar, J.B.Mitchell, P. Boksa., & M.J. Meaney. McGill University, Depts. of Pharmacology, Neurology & Neurosurgery, and Psychiatry, Douglas Hospital Research Center, Montreal, Canada H4H 1R3.

Chronically stressed rats exhibit an adaptation of hypothalamic-pituitary adrenocortical and adrenomedullary systems when reexposed to the chronic stressor. Basal levels of adrenomedullary enzymes (phenylethanolamine N-methyltransferase, PNMT; tyrosine hydroxylase, TH), catecholamines (epinephrine, E; norepinephrine, NE), and pituitary-adrenocortical hormones may or may not be elevated depending on the chronic stress. However, these systems hyperrespond when challenged with a novel stressor. In the present study, we have exposed male rats to 3 week intermittent cold stress (4°C, 4h/d; CHR rats) and present a comprehensive endocrine profile. Adrenomedullary measures following chronic cold stress show an increase in adrenal NE and E content, and in basal TH but not in PNMT activities (as previously shown). Similar levels of free and total levels of plasma corticosterone (CORT) were seen in control and CHR rats, whereas an increase in ACTH was observed in CHR rats, suggesting loss of adrenocortical sensitivity in these animals. Exposure to a novel stressor (20 min restraint) tended to increase TH activity and adrenal catecholamines in both CHR and control rats, whereas little increase in PNMT activity was observed. However, CHR rats exposed to restraint had higher levels of plasma CORT compared to control rats exposed to restraint, suggesting a hyperresponsiveness of the CHR rats to the novel stressor. ACTH levels following restraint were similar in both groups, suggesting increased adrenal sensitivity in CHR rats. Therefore, adrenal medullary and adrenal cortical systems may not respond uniformly to chronic stress or to exposure to a novel stressor, but may be responding to particular physiological/metabolic requirements that are specific to this stressor. Alterations in the central regulation of both these systems following chronic cold stress and exposure to heterotypic stress are currently being studied.

643.6

HYPOTHALAMIC-PITUITARY-ADRENAL DYSREGULATION ASSOCIATED WITH A HIGH FAT DIET. B.M. Tannenbaum*, D.N Brindley, M.F. Dallman, M.J. Meaney. Douglas Hosp. Res. Ctr., McGill Univ., Montreal, Canada H4H 1R3; Dept. of Biochemistry, Univ of Alberta, Edmonton, Canada, T6G 2C2; Dept of Physiology, UCSF., San Francisco, CA, 94143

Current models of stress-induced pathology often lack the most serious factors which have been identified in human pathology. High-fat intake is an excellent case in point. Increased fat intake leads to insulin resistance; subsequently this results in a high cost metabolic strategy which includes increased secretion of glucocorticoids and free fatty acids as well as altered hepatic production rates and clearance of high-and low-density lipoproteins. Alone, this regimen presents a health risk; however, because of the hormonal adaptations to diet an additional risk lies in altered responses to acute, superimposed stress. We have begun a series of longitudinal, epidemiological studies in rats to explore the consequences of acute stress imposed on a common maladaptive nutritional state seen in man. Plasma corticosterone levels were examined before, during and following a 20 min restraint stress after 1 week exposure to either a high fat (23%) or control (3%) diet. Basal plasma corticosterone levels were slightly elevated in high-fat rats. Sixty min after the end of restraint, high-fat rats still had significantly elevated corticosterone levels (22±7 vs. 6±2 ug/dl in control). In a parallel set of rats, adrenalectomized one day earlier, glucocorticoid receptor binding measured in the hypothalamus was significantly reduced in rats on high-fat diets compared with controls; no differences in binding were observed in hippocampus, frontal cortex or pituitary. A decrease in brain corticosteroid receptors has been frequently associated with decreased inhibition of the hypothalamic-pituitary-adrenal (HPA) axis and chronically increased glucocorticoid secretion. Thus, these studies show that even short term (1 week) exposure to a high fat diet can augment the HPA axis response to acute stress. The augmented HPA axis response to acute stress should serve to worsen the insulin sensitivity imposed by the diet, and repeated acute stressors may result in provoking the capacity for metabolic adjustments to its limits.

643.7

MODEST INCREASE IN NADIR CORTICOSTERONE (B) SECRETION IN OBESE (fa/fa) ZUCKER RATS ALTERS FEEDBACK SENSITIVITY ON BASAL ACTH RELEASE. C-D. Walker, J.S. Stern, L.S. Myers*, M.F. Dallman. Dept of Physiology, UCSF; Dept of Nutrition UCD, Dept of Psychology, CSU Stanislaus, CA 94143.

Genetically obese Zucker (fa/fa) rats exhibit a number of metabolic and endocrine disorders, most of which can be reversed by adrenalectomy (ADX). We studied basal adrenocortical activity in intact lean (Fa/Fa) and obese (fa/fa) rats and the ability of B to affect ACTH and insulin secretion and fat deposition in both phenotypes. The effects of ADX with various B pellets replacements (15-120%B) over 5 days were measured on basal nadir (AM) and stress-induced ACTH secretion. Intact obese rats showed a modest increase in basal B secretion in the AM, despite no changes in ACTH secretion or ACTH responses to stress. The chronic increase in B in obese rats was evidenced by decreased thymus and increased adrenal weights and PNMT activity. The ability of B to suppress basal ACTH was diminished in obese (IC50=4.17+0.7) vs lean (IC50=3.16+0.5) rats. After stress, similar suppression by B was observed in both phenotypes. Hyperinsulinemic obese rats did not show greater increases in insulin secretion with increasing B than lean rats. Fat deposition slightly increased with B in obese, but not in lean rats. Like many models of chronic stress, small increases in nadir B secretion in obese rats are associated with a decline in feedback sensitivity on basal ACTH secretion and subtle changes in the central regulation of ACTH secretion occur to maintain adequate responses to stressful stimuli. Supported by Swiss NRF (CDW), DK28172 (MFD), DK18899 (JSS).

643.8

ADRENALECTOMY-INDUCED FOS-LIKE IMMUNOREACTIVITY (FLI) IN THE PVN OF STRESS-HYPORESPONSIVE RATS **N. Wintrip, D. M. Nance and M. Wilkinson* Depts. of Obst. and Gynecol., Physiol. and Biophys., and Anatomy, Dalhousie U., Halifax, N.S., B3H 4H7, and Dept. of Pathology, U. of Manitoba, Winnipeg, Manitoba, R3E 0W3, Canada**

Adrenalectomy (ADX) is reported to induce the appearance of FLI in the rat PVN (Jacobson et al, Endocr., 128:1709). However, we have observed FLI in the PVN of adult Sprague-Dawley rats 2h and 4h following ADX, unilateral ADX, sham ADX, OVX, sham OVX, and a number of other stressful challenges (antibody: Cambridge Res. Biochem.).

We have sought to dissociate the induction of FLI which results from generalized stress from that specifically due to ADX. A stress-hyporesponsive period is reported to occur in neonatal rat pups (approx. day 4 to day 15) (Endocr., 110:1676). At postnatal day 11, FLI was observed in the PVN 4h after ADX or unilateral ADX but not following sham ADX or other stress. However, at postnatal day 18, sham ADX or other stress did elicit FLI in the PVN. Lightman and Young (Endocr., 124:2358) have also reported an association between lactation and a stress-hyporesponsive period. We have found an increase in FLI in the PVN of lactating rats at 4h following ADX but not following anaesthetic stress. This phenomenon appears to be restricted to the immediate perinatal period.

In conclusion, during early postnatal life, the c-fos response to stressors other than ADX appears to be attenuated in both mothers and pups. **Supported by Can. MRC (M.W. and D.M.N.)**

643.9

PATTERN OF IMMEDIATE EARLY GENE ACTIVATION IN RAT BRAIN FOLLOWING ACUTE STRESS. [1]W.E. Cullinan*, [2] J.P. Herman, and [1]S.J. Watson [1]University of Michigan, Mental Health Research Institute, Ann Arbor, MI, 48109-0720, and [2]University of Kentucky Medical Center, Dept. Anatomy and Neurobiology, Lexington, KY, 40536-0084.

In an effort to produce a functional map of the neural circuitry related to activation of the hypothalamic-pituitary-adrenal axis, we examined the pattern of induction of the immediate early gene c-fos in rat brain at 0, 30, 60 and 120 min. following acute immobilization stress. A radiolabelled cRNA probe was used to detect c-fos mRNA using *in situ* hybridization histochemistry. Results indicated regionally specific patterns of c-fos expression. Prior to stress c-fos was undetectable in most brain areas, and was markedly induced at 30 min. post-stress in the cingulate, infralimbic and orbitofrontal cortices, the piriform cortex, and in several neocortical areas including frontal and parietal regions. Also prominently labelled at 30 min. were the lateral septal nucleus, portions of the lateral hypothalamus, the hypothalamic paraventricular nucleus, the mammillary hypothalamic region, the medial and cortical amygdaloid nuclei, as well as a number of thalamic and brainstem regions. In the majority of these regions message levels were reduced at 60 min., and generally undetectable at 120 min. Exceptions included the piriform and parietal cortices, which exhibited a slower rate of decline, remaining detectable at 120 min post-stress. We are currently examining the induction c-fos in these regions at earlier and intermediate time points, and have begun characterization of c-fos and other immediate early genes (c-jun, zif/268) with respect to chemically defined neuronal populations.

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643.10

INVOLVEMENT OF THE VENTRAL HIPPOCAMPUS IN REGULATION OF THE HYPOTHALAMO-PITUITARY-ADRENOCORTICAL AXIS. J.P. Herman*, W.E. Cullinan, M.J. Morano and S.J. Watson, Univ. Kentucky Med. Ctr., Lexington, KY 40536 and Univ. Michigan, Ann Arbor, MI 48109.

The present studies were designed to address the hypothesis that ventral hippocampal structures play a role in modulation of hypothalamo-pituitary-adrenocortical (HPA) function. Male Sprague-Dawley rats received stereotaxic injections of ibotenic acid or saline (SAL) into the ventral subiculum (VSUB)/ventral hippocampus (VHIPP). An additional group remained unhandled (CON). Seven days after surgery rats were subjected to acute restraint stress and blood collected 0, 30 and 120 m after stress induction. Animals were sacrificed 4 d later and brains harvested for in situ hybridization analysis of paraventricular nucleus (PVN) corticotropin releasing hormone (CRH) mRNA levels. There were no differences among the groups in basal CORT secretion. The VSUB group did not differ from SAL or CON rats at either the 30 m or 120 m post-stress time points. However, animals with damage confined to the VHIPP (including portions of CA1, CA3 and the dentate gyrus, but *excluding* the subiculum) showed significantly increased CORT levels at 30 and 120 m post-stress. The decay in plasma CORT from 30-120 m showed a similar time-course in all four groups, suggesting that VHIPP lesions alter amplitude but not duration of HPA stress response. CRH mRNA levels were tonically increased (30-80%) in the medial parvocellular PVN in both the VSUB and VHIPP groups relative to SAL and CON rats, indicating tonic up-regulation of these important hypophysiotrophic neurons. The data suggest the existence of distinct hippocampal pathways involved in tonic regulation of the HPA axis and in modulation of the magnitude of the stress response.

643.11

HABITUATION OF THE CORTICOSTERONE STRESS RESPONSE TO REPEATED RESTRAINT STRESS IN SPRAGUE-DAWLEY, FISCHER, AND LEWIS RATS. F. S. Dhabhar*, R. L. Spencer, and B. S. McEwen, Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY 10021.

We have examined the corticosterone (CORT) response to repeated restraint stress in three related strains of rats: Sprague-Dawley, Fischer 344, and Lewis. These strains are known to differ in their CORT response to acute stress. Here we describe how they also differ in their response to repeated stress. Rats were subjected to 1 h restraint stress daily for 10 days. Blood (tail clip) was sampled on days 1, 5, and 10. Four samples: basal (0 min), stress (30 & 60 min), and recovery (120 min) were collected for RIA determination of plasma CORT. Fischers had significantly higher stress CORT levels than Sprague and Lewis rats on all test days. Sprague and Lewis rats showed both within session and across session habituation. Fischers showed only across session habituation, and the magnitude of this habituation was much smaller than that shown by the other strains. On day 5, the decreases in peak CORT levels (compared to peak at day 1) were: Fischer (10 %), Sprague (13 %) and Lewis (42 %). On day 10, the decreases in peak CORT were: Fischer (13 %), Sprague (27 %), and Lewis (41 %). Stressed animals from all three strains showed a smaller percent increase in body weight compared to controls. Stressed Fischers showed adrenal hypertrophy whereas Spragues and Lewis did not. Repeated stress had no effect either on thymus and spleen weights, or on plasma corticosterone binding globulin levels. Our results suggest that these strains differ considerably in their reactivity to repeated stress. Comparisons between these strains provide an attractive model for studying the mechanisms underlying the processing of, and adaptation to, stressful information. (MH 41256)

643.12

AN INTEGRATIVE ROLE OF NGF IN CNS MECHANISMS OF STRESS. L. Angelucci*, G. Cigliana, P.J. Foreman[1], L.A.A. Muscolo, R. Nicolai, J.R. Perez-Polo[1], A. Porcu, S. Scaccianoce, G. Taglialatela[1]. Institute of Pharmacology II, La Sapienza Univ. of Rome, Italy; [1]Human Biochemistry Dept., Texas University at Galveston.

The increase in plasma corticosterone (B) by exogenous NGF (Otten et al., 1979) is abolished in hypothalamus (HY)-blocked rats but not by the CRH antagonist alpha-Helical CRH (9-41) indicating that the main releaser of ACTH is a non-CRH factor. In vitro NGF while inactive on pituitary and adrenal, stimulates the release from and the synthesis in the HY of CRFs. Cold stress reduces ^{125} I-NGF binding and increases the levels of NGF and NGF-mRNA in the hippocampus (HI) as well of p75 ^{NGFR} in the basal forebrain; these actions are not mimicked by exogenously produced equivalent increases in plasma B, nor abolished by adrenalectomy. Considering that NGF concentration in blood can be increased by aggression (Aloe et al., 1986) and that NGF can be exogenously increased in HI, a structure involved by its glucocorticoid receptor in the regulation of the HPAA, we conclude that NGF has a role in the mechanisms of stress activation, possibly integrating HI and HY activities.

643.13

HIPPOCAMPAL REGULATION OF THE PITUITARY-ADRENAL AXIS IN MONKEYS. H. Uno,* A. Sakai, S. Shelton and S. Eisele, Regional Primate Res. Ctr. and Psychiat. Res. Inst., Univ. of Wisconsin, Madison, WI 53706

Our previous studies revealed that prenatal administration of dexamethasone to pregnant rhesus monkeys at 132 and 133 gestation days caused degeneration of the CA3 pyramidal neurons and retarded growth of the dentate granular neurons in the hippocampuses of neonates. Three juveniles treated with prenatal dexamethasone (DEX) showed normal physical development compared to two vehicle-treated, sex- and age-matched animals. MRI of the hippocampal gyrus examined at 9 months of age showed no striking difference between the DEX and the vehicle group. However, the serum concentration of cortisol showed higher base line and post-stress levels in the DEX-treated animals. For the stress study, the infants were isolated from their mothers and kept in a restraining cage for 30 min. The average levels of cortisol were: DEX group: AM=34, PM=25, Post-isolation=52, 1 hr after =48, 2 hr=28; Vehicle group: AM=19, PM=14, Post-isolation=39, 1 hr=20, 2 hr=20 μg/dl). ACTH in the DEX group became elevated to 138 after isolation from 19 pg/ml. Developmental deficiency of the hippocampal neurons caused hypercortisolemia in both base line and post-stress conditions. These results suggest that the hippocampus appears to be a suprahypothalamic site for regulation of the pituitary-adrenal axis. (Supported by NIH RR00167.)

643.14

ALTERED CORTISOL RESPONSE TO STRESS AFTER FOUR MONTHS' PRACTICE OF THE TRANSCENDENTAL MEDITATION PROGRAM. C.R.K. MacLean, K.G. Walton*, S.R. Wenneberg, D.K. Levitsky, J.V. Mandarino, R. Waziri[1] and R.H. Schneider, Depts. of Physiology and Chemistry, Maharishi International University, Fairfield, IA 52556 and [1]University of Iowa, Iowa City, IA 52242.

Recent studies on the wild baboon suggest that low basal cortisol levels and high cortisol response to stressors is a more stable, more adaptive profile than its opposite. Previous research on the Transcendental Meditation (TM) technique has reported decreased basal cortisol levels both acutely with the practice and longitudinally. Using a random-assignment, pre-post design, the present research examined before and after the effects of four month's of TM or stress education class (SEC) on basal cortisol levels and the dynamic response of plasma cortisol to laboratory stressors. Twenty-nine healthy Caucasian males (ages 18-32) were randomly assigned to TM or SEC. Plasma for cortisol was sampled using a continuous withdrawal pump (Dakmed) during a one-hour laboratory stress session (which included three 5 minute tasks) . Samples were assayed for cortisol by RIA (DPC) and statistically analyzed by t-test and ANCOVA.

The decrease in basal cortisol from pretest to posttest was significant for the TM group when compared to the SEC group ($t(2,15) = 2.21$, $p = 0.043$). In addition, the TM group exhibited higher cumulative magnitude of the cortisol values relative to baseline ($F(2,26) = 4.814$ $p = 0.038$) and increased range of cortisol secretion during the session ($F(2,27) = 4.825$, $p = 0.037$). These results suggest that practice of the TM technique is associated with lower baseline cortisol and increased cortisol response to stressors. Such alterations are proposed to result from changes in neural modulation of the hypothalamic-pituitary-adrenal axis by TM practice. Supported by NIH Research Grant # 1 R15HL 40495 01A1, and NIH Grant # RR59, Clinical Research Centers Branch.

643.15

ADRENOCORTICAL FUNCTION IN PTSD

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Patients with Post-traumatic Stress Disorder (PTSD) have been found to have lower 24-hour urinary free cortisol levels than do controls. To test whether PTSD patients have less cortisol available for release in response to maximal stimulation by ACTH, we administered the ACTH analog Cosyntropin to healthy, medication-free male patients with PTSD and controls. On the study day, i.v. catheters were inserted and 45 minutes later 3 basal blood samples for cortisol were drawn 10 minutes apart. Cosyntropin 0.25 mg. was given i.v., and then cortisol levels were drawn at 15, 30, 60, 90, 120, 150, 180 and 210 minutes. T-tests revealed no differences in RIA-determined basal plasma cortisol (7.8+1.9 vs. 10.5+2.7 mg/dl), or area under the cortisol response curve (4373+366 vs 3265+1197 mgxmin/dl) for PTSD (N=6) or control subjects (N=4) (mean+SD). With the small N tested thus far, there is no evidence for a smaller cortisol response to maximal stimulation with ACTH in PTSD.

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS: DEVELOPMENT AND PLASTICITY

644.1

DEVELOPMENT OF METABOLIC ACTIVITY PATTERNS IN THE VENTROBASAL THALAMUS AND SOMATOSENSORY CORTEX OF THE NORMAL AND BASAL-FOREBRAIN-LESIONED MOUSE. S. Noctor[1], D. Eslin[2], C. Hohmann[3], S. Juliano[1,2]*. Depts. of [2]Anatomy and [1]Neuroscience, USUHS, Bethesda., MD, & [3]Kennedy Krieger Research Inst., Baltimore, MD.

Although a wealth of knowledge exists regarding the morphologic development of the barrel cortex and thalamocortical relations, very little is known about the development of functional activity in these regions. In order to further understand mechanisms that participate in development of the barrel field, mice (ages 1 wk to 6 mos) that were either normal or unilaterally basal-forebrain-lesioned (BFL) at birth underwent a 2-deoxyglucose (2DG) experiment. Following the 2DG injection, each mouse was either returned to its cage with whiskers intact, or received bilateral stimulation to a single whisker. At one wk of age, for both normal and BFL mice, although barrels were fully formed as indicated by cytochrome oxidase staining, above background stimulus-evoked 2DG activity was difficult to visualize in the barrel field. At this age the label was distinctly different from the pattern evoked in the adult and occurred in layer 4, the supragranular layers, and layer 6, with a distinct activity gap in layer 5. Activity specifically elicited by stimulation of single vibrissae was smaller in dimension than comparable activity evoked in the adult. At 2 wks of age, the whisker-stimulated activity was greater than that observed in the 1 wk animals, but was not close to the adult pattern until 4 wks of age. In contrast to weak activity observed in the barrel field, the ventrobasal thalamus demonstrated a remarkably high level of 2DG uptake, which was significantly stronger in the 1-2 wk old animals compared to the 4 wk old animals. At early developmental ages 2DG uptake in the somatosensory cortex ipsilateral to the BFL did not significantly differ from the contralateral or normal hemispheres. Supported by RO7064.

644.2

DEVELOPMENT OF NON-NMDA GLUTAMATE RECEPTORS IN RAT BARREL FIELD CORTEX. M.E. Blue*, M.Fotuhi, T.M. Dawson, S.H. Snyder and M.V. Johnston. Kennedy Krieger Research Institute and Depts. of Neuroscience and Neurology, The Johns Hopkins Univ. Sch. of Med. Baltimore, MD 21205.

The ontogeny of ionotropic (AMPA) and metabotropic (mGluR) glutamate receptors in barrel field cortex was studied in rats between postnatal ages P4 and P17 and in adults. To examine mGluR sites, flattened sections of cortex were labeled using ^{3}H-glutamate with AMPA and NMDA displacers. Autoradiographically labeled mGluR sites form a vibrissa-related map at P4, with higher densities of receptors in barrel centers than in barrel septa or surrounding tissue. Densities of mGluR sites in barrels reach their highest levels on P10 and then decline. Over time, the difference in density between barrel centers and surrounding tissue becomes less striking. At P10, the difference in density between barrels and surrounding cortex is 42% (2.94 versus 2.34 pmol/μg protein), in the adult, it is only 13% (1.69 versus 1.50 pmol/μg protein). The ontogeny of mGluR sites also was examined using an affinity purified antibody to the mGluR1 receptor which recognizes both α and β isoforms. Staining of mGluR receptors is enhanced in the immature brain, with greater concentrations of mGluR1 immunoreactivity in barrel centers than in septa and surrounding cortex. Within barrel centers, numerous varicose processes (presumably axonal) are stained. Relatively few neurons within the barrels are stained compared to surrounding cortex; these immunoreactive cells are predominantly located in barrel walls and are often contacted by immunoreactive boutons. In the adult, the relatively low density of staining in barrels contrasts with that in surrounding cortex; many fewer neurons and processes are stained. Ionotropic sites, labeled with ^{3}H-AMPA, show a contrasting developmental pattern of expression to that of mGluR sites. At all ages examined, barrel centers contain fewer AMPA sites than barrel septa or surrounding cortex. The density of AMPA sites increases to its highest value at P17 (1.95 pmol/mg protein) and then declines slightly to the adult value (1.76 pmol/μg protein). These results show a differential ontogeny of AMPA and mGluR receptors. Based on distribution and timecourse, the mGluR sites appear more likely to influence the process of barrel formation. Supported by NIH grants NS28208 and NS29167.

644.3

DEVELOPMENT OF CORTICO-CORTICAL CONNECTIONS IN THE PRIMARY SOMATOSENSORY CORTEX OF KITTENS. R. Sonty, R.A. Code*, S.L. Juliano. Dept. of Anatomy, Cell Biology, and Neuroscience, USUHS, Bethesda, MD 20814.

Stimulus-evoked metabolic activity in the primary somatosensory cortex (SI) of kittens is initially more diffuse and less distinctly patch-like than in adults, but becomes patchy and nearly adult-like by 5 weeks of age. To examine a possible anatomical substrate underlying these metabolic changes, we studied the development of SI cortico-cortical connections using DiI. Crystals of DiI were implanted in post-mortem, fixed SI of kittens yielding typical injection sites of about 1250 μm. Tissue was examined using confocal microscopy, fluorescence microscopy, and Nissl staining. At 3 days of age, there was a single, dense, continuous band of label in layer V, 750 μm deep to the cortical surface, spreading for long distances from the injection site. The label extended into the subplate where labelled cells were seen. At 6 days of age, the label was present as two distinct bands. A superficial, discontinuous, 400 μm thick band located in the cortical plate and a deep, continuous, 350 μm thick band located in layer V. A few labelled cells were seen in the subplate. At 9 days age, the label was present in two bands as given above, but fewer labelled cells were found in the subplate. At all ages, many cells with large nuclei, of varied morphology (including pyramidal and fusiform), and with complex dendritic branching were seen. The transformation of the diffuse, horizontally connected SI cells into discretely connected, patch-like clusters may serve as a basis for the observed change in the pattern of evoked metabolic activity. In turn, both phenomena may be activity-dependent. Supported by NIH Grant NS24014.

644.4

THE LAMINAR DISTRIBUTION OF CHOLINE ACETYLTRANSFERASE IMMUNOREACTIVITY DURING POSTNATAL DEVELOPMENT IN THE PRIMARY SOMATOSENSORY CORTEX OF THE CAT. C.S. Heck[1] and P.A.McKinley[2]. [1]Department of Physical Therapy, The University of Western Ontario, London, Ontario N6G 1H1, and [2]School of Physical and Occupational Therapy, McGill, Montreal, Quebec H3G 1Y5.

Acetylcholine has been implicated as playing an important physiological role in the modulation of several brain functions. Since choline acetyltransferase (ChAT) is presently regarded as the most definitive and reliable marker for the localization of cholinergic neurons, we decided to investigate the changes that occur in laminar distribution of ChAT during the postnatal development of the primary somatosensory cortex (SI) in cats, and furthermore, to compare its distribution with that of acetylcholinesterase (AChE) during the same time period. The immunohistochemical demonstration of ChAT in the layers of SI cortex was carried out in cats at various ages between 4 and 144 days of postnatal age. Results indicated that immunolocalization over the developmental period studied could be characterized by 3 distinct phases: 1) restriction of stained cell processes to the interface of layers V and VI at 4 days, 2) by 14 days, immunoreactivity was detected in layers I, V and VI, and 3) from 28 days onwards, positively labelled structures were localized in layers I, interface between IV and V, and interface between V and VI. Furthermore, the co-localization of ChAT and AChE shows a high correlation during the developmental period studied. The time course of development of cholinergic innervation appears to relate well to that of overall maturation of cortical structure and function.

644.5

CHANGES IN CORPUS CALLOSUM INPUTS TO BARREL FIELD DURING POSTNATAL DEVELOPMENT IN ALBINO RATS REVEALED BY DiI. L. Zhang*[1], A.J. Elberger[1] and N.G.F. Cooper[2]. [1]Dept. of Anatomy and Neurobiology, University of Tennessee, Memphis TN 38163; [2]Dept. of Anatomical Sciences and Neurobiology, University of Louisville, Louisville KY 40292.

In adult albino and pigmented rat SI cortex, the corpus callosum (CC) inputs to the barrel field in layer IV were shown to fill the septae outlining the barrels (Koralek et al., J. Comp. Neurol. 1990). The developing CC projections to rat SI cortex have not been examined in tangential sections. Studies of the development of thalamocortical (TC) inputs to the barrel field of albino and pigmented rats have shown that TC inputs fill the hollow core of the barrel from postnatal day (PND) 2 (day of birth=PND 1) to adult (e.g., Zhang and Cooper, Ann. NY Acad. Sci. 1991). In the present study we have examined the development of CC inputs to the barrel field by placing crystals of the carbocyanine dye, DiI, in the mid-sagittal CC in aldehyde-fixed tissue from PND 1 to adult in albino rats. Tangential sections 100 μm thick were examined with epifluorescence using a standard rhodamine filter set.

The CC inputs do not form a detectable barrel pattern until after PND 5. By PND 9 the CC input takes the form of filled hollows throughout the barrel field. At PND 14 and 17 the CC inputs are in a state of transition, with filled hollows in the anterolateral 2/3 and filled septi in the posteromedial 1/3 of the barrel field. At PND 24, 27 and adult the CC barrel field input is restricted to filled septae. Thus, the CC input to the barrel field in albino rats changes substantially during development; results from pigmented rats will be compared. Supported by NIH grants EY08466 (AJE) and EY02708 (NGFC).

644.6

α_1 ADRENOCEPTOR DISTRIBUTION IN THE BARREL CORTEX AND THE EFFECTS OF VIBRISSECTOMY AND NOREPINEPHRINE DEPLETION. A. Dunn-Meynell* and B.E.Levin, Neurol. Svc., VA Med. Ctr., Tremont Ave., E. Orange, NJ 07018

α_1 adrenoceptors (α_1AR) modulate neuronal firing and therefore may be involved in the metabolic plasticity seen in adult rat barrel cortex following vibrissectomy. α_1AR distribution in the adult rat barrel cortex was assessed autoradiographically using 1 nM ^{3}H prazosin with nonspecific binding defined in the presence of 100 μM phentolamine. In coronal sections, a discontinuous band of high prazosin binding was seen in layer IV of the somatosensory cortex. In tangentially sectioned cortices, the pattern of barrels was defined by the contrast between higher levels of binding in barrel septae (267±10 fmol/mg protein) than in the barrel centers (229±11 fmol/mg protein). Chronic (2 mo) total vibrissectomy with sparing of the C3 whisker produced no apparent change in α_1-AR density or distribution. However, 2 months after cortical norepinephrine depletion by unilateral locus coeruleus lesioning with 6-hydroxydopamine, specific binding was increased 16.4% over the unlesioned side, P=.057). Results therefore suggest an upregulation of α_1AR in layer IV of the barrel cortex but show little effect of spared C3 vibrissectomy on α_1AR regulation in the adult. Supported by the Research Service of the Department of Veterans Affairs.

644.7

THE EFFECT OF CORTICAL ACETYLCHOLINE DEPLETION ON SENSORY PROCESSING IN RATS. S.E. Jacobs[1]* and S.L. Juliano[1,2], Dept. Anat.[1] and Dept. Neurosci.[2], USUHS, Bethesda, MD

A number of studies have proposed a role for acetylcholine (ACh) in learning and memory, yet almost no research has explored its impact on sensory discriminative behavior. We studied the role of ACh on a rat's ability to perform a task of tactile sensory perception. Rats were trained in a T-maze to discriminate between a deflection of the vibrissae and a sham deflection. When a pre-determined level of performance was achieved, rats received either a sham lesion (SL) or an excitotoxic lesion of the basal forebrain (BFL). Behavioral testing continued until pre-lesion criteria were again met. All SL rats were immediately able to perform at pre-lesion levels. All BFL rats experienced difficulty with the pre-learned task. In addition, there was a strong correlation between the number of sessions it took the BFL rats to re-achieve criteria and the degree of cortical ACh depletion. A terminal 2-deoxyglucose (2-DG) experiment conducted on all rats allowed us to compare functional activity evoked by whisker stimulation in the somatosensory cortex with the behavioral performance. While all BFL rats returned to pre-lesion criteria, their 2-DG maps showed continued reduction of somatosensory cortical activity. This pattern of 2-DG activity is similar to that found in earlier studies from our lab where the animals sustained relatively short-term ACh depletion. Supported by RO7064.

644.8

PARALLEL DISTRIBUTED PROCESSING IN SOMATOSENSORY CORTEX: EFFECTS OF SII INACTIVATION ON SI RESPONSES IN CAT. A.B. Turman*†, J.W. Morley and M.J. Rowe. School of Physiology and Pharmacology, Univ. of NSW, and †Dept. of Biol. Sci., Faculty of Health Sciences, Univ. of Sydney, NSW, Australia.

The first and second cortical somatosensory areas (SI and SII) in primates appear to be organized in a serial scheme in which tactile information is conveyed from the thalamus to SI and thence to SII. However, in the cat, the SII responses are largely independent of SI although some facilitation appears to be exerted by SI on a small proportion (20 %) of SII neurons. In the present study we have used a very rapid, reversible procedure based on cooling to inactivate the forelimb SII area in order to extend previous investigations (Burton and Robinson, Somatosen. Res. 4, 1987) on whether responses in the SI area of the anesthetized cat are independent of the SII area. The distal forelimb areas in SI and SII were initially mapped by recording surface evoked potentials and a circular metal cooling block (6 mm diameter) equipped with a Peltier thermal cooling device was positioned over the distal forelimb focus of SII. Inactivation of SII by cooling, usually to 8-13°C, left SI evoked potentials intact in half the experiments or reduced in amplitude in the other half. The effects of SII inactivation on the responsiveness of single SI neurons activated by tactile stimulation of the glabrous or hairy skin of the forelimb were investigated before, during and after SII inactivation. The response level (impulses/s) remained unchanged in half the SI neurons studied, while there was a reduction in response in the other half. These results suggest that in the cat, the responsiveness of SI neurons to tactile stimulation depends to some extent on the functional integrity of SII. (Supported by NH&MRC and ARC).

644.9

EVIDENCE FOR RAPID CHANGES IN THE RECEPTIVE FIELD ORGANIZATION OF SI CORTEX IN SQUIRREL MONKEY: AN INTRACELLULAR RECORDING STUDY COMBINED WITH REVERSIBLE DEAFFERENTATION. R.S. Waters*, C.X. Li. Dept. of Anatomy and Neurobiology, UT, Memphis, Col. of Medicine, Memphis, TN 38163.

The ability of the cortex to rapidly reorganize following peripheral nerve injury suggests the unmasking of previously undetected inputs. We examined intracellularly recorded evoked responses in SI cortex following reversible deafferentation of the ulnar nerve and then re-examined SI cortex for changes in receptive field organization. We report that removal of peripheral nerve input from a single nerve alters the responsiveness of SI neurons and that the effect is reversible.

Adult squirrel monkeys were anesthetized with Ketamine (35mg/kg), the head and left arm were stabilized in custom made holders, and the ulnar nerve was exposed and placed on a pair of cuff-stimulating/recording electrodes; the nerve was also placed upon a modified cooling device. Following forepaw preparation, the contralateral SI cortex was exposed, and an acrylic recording chamber was fashioned on the surrounding bone. Carbon fiber electrodes were used to record evoked responses elicited by mechanical and/or hand held stimulators, and the forepaw region of SI cortex was identified and mapped. An intracellular electrode was then inserted into the forepaw representation in SI and the receptive field of the recorded neuron was identified in terms of suprathreshold and subthreshold components. The mechanical stimulator was then fixed to stimulate either component on the skin surface, the ulnar nerve was reversibly deafferented, and the receptive field was reexamined. Using these techniques the following result is noteworthy:

1. Following deafferentation, subthreshold responses may be altered and/or elevated to suprathreshold firing levels. These results bear directly on the unmasking process.

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644.10

RADIAL NERVE INPUT TO THE MEDIAN NERVE REPRESENTATION IN MONKEY AREA 3B. *C.E. Schroeder *, S. Seto, M. Steinschneider, J.C. Arezzo and P.E. Garraghty. Depts. Neurosci. & Neurol., Albert Einstein Coll. Med., and Dept. Psychology, Indiana Univ.*

The pattern of reorganization in Area 3b of adult primates after median nerve section suggests that somatic afferents carried by the radial nerve have preferential access to median nerve territory. A likely mechanism underlying preferential access is pre-existing, but silent, radial nerve inputs. We tested this by comparing effects of electrical stimulation (100μsec, 4-6mA, 2/sec) of median versus radial nerve, on responses in the median nerve representation of Area 3b. Laminar current source density and multiunit activity profiles were recorded with linear array multicontact electrodes spanning the laminae of Area 3b. Isolation of peripheral nerve stimulation was confirmed by simultaneous recording of EMG from appropriate distal muscles. Repeated sampling of one site was obtained with an implanted electrode in a squirrel monkey anesthetized during recording. Subsequent sampling was conducted during acute penetrations in an awake (conditioned) macaque. In both monkeys, compared to colocated median nerve responses, radial nerve responses typically had longer latencies (by 11-16 msec), lower amplitude, longer duration, more variability and different laminar distribution. Since median and radial nerve primary cortical responses differ by only 1.8 ms: 1) the latency difference is not due to differences in median and radial nerve conduction times to cortex; 2) the "delayed" radial nerve response is not due to a trivial cause such as volume conduction. Rather, it may reflect indirect or extralemniscal input. (MH06723 and DC00657).

644.11

THE EXTENT OF CORTICAL REORGANIZATION AFTER NERVE INJURY IS LIMITED BY THE CONTENT OF THE DEPRIVATION. P.E. Garraghty*[1,2], D.P. Hanes[2], S.L. Florence[2] and J.H. Kaas[2]. [1]Dept. of Psychology, Indiana Univ., Bloomington, IN 47405 and [2]Dept. of Psychology, Vanderbilt Univ., Nashville, TN 37240.

When multiple digits are amputated in adult monkeys [JCN, 224 (1984) 591], cortical reorganization is incomplete. Yet when a much larger area of cortex is deprived by transection of the median and ulnar nerves, deafferenting the entire volar surface of the hand [PNAS, 88 (1991) 6976], apparently complete reorganization ensues, with the dorsum of the hand and digits expanding their representations. Is it possible that incomplete reorganization follows multiple digit amputation because both the dominant and preferred latent set of inputs have been removed (i.e., the glabrous and hairy surface of given digits). We report here on squirrel monkeys in which the ulnar and radial nerves were transected and ligated. This manipulation completely eliminates both glabrous and hairy surface inputs from D5, ulnar D4, and the ulnar hand. Yet the total amount of cortex deprived is less than with median and ulnar nerve cut. We find in these animals, as in monkeys with multiple digit amputations, that cortical reorganization in areas 3b and 1 is incomplete. Large sectors of deprived cortex are unresponsive to cutaneous stimulation, though noncutaneous stimulation can frequently drive responses. Thus, the specific pattern of peripheral deafferentation and not the absolute amount of deprived cortex limits the extent of reorganization that is possible. (Supported by N.I.H. NS16446 and HD15052.)

644.12

THE ORGANIZATION OF SOMATOSENSORY AREA 3A IN THE NEOCORTEX OF THE FLYING FOX (*PTEROPUS POLIOCEPHALUS*). S. Finnigan, L. Krubitzer*, J. C. Clarey, and M. Calford. VTHRC, Department of Physiology and Pharmacology. University of Queensland, Australia 4072.

Although there is some evidence for the existence of a deep representation rostral to 3b in a number of mammals, there are no descriptions of the overall organization of this deep rostral field, area 3a, in any mammal. Because neurons in 3a respond to joint manipulation and hard taps to the body surface, it is believed to be involved in proprioception and motor control. Thus, how this field is organized and interconnected with cutaneous representations and motor cortex is of great interest. The flying fox was chosen to study the organization of area 3a for several reasons. First, as an archontan, the flying fox shares a close phylogenetic relationship with primates, and information gained on the somatosensory cortex of the flying fox may be applicable to all archontans, including primates. Second, much is already known about the organization and connections of somatosensory cortex in the flying fox. Finally, the flying fox has a smooth neocortex and most of 3a is on the dorsolateral surface. In many primates, 3a is inaccessible because it is located on the fundus of the central sulcus. Using microelectrode recording procedures, receptive fields were obtained for single neurons and neuron clusters at a number of closely spaced sites across the cortex, and detailed maps of area 3a were obtained. In these same animals, the myeloarchitecture of 3a was matched to recording sites and architectonic borders were added to physiological maps. Most neurons in 3a responded to light pressure and joint manipulation, although some neurons responded to cutaneous stimulation as well. Receptive field sizes for neurons in 3a varied from very small (located just on distal digit 1), to very large (located on the trunk and portions of the forelimb). Mediolaterally, the topographic organization of 3a mirrored that of 3b, although 3a appeared to be less topographically precise than 3b. In cortex that has been stained for myelin, 3a stains very lightly relative to 3b caudally and motor cortex rostrally.

644.13

INTERHEMISPHERIC CONNECTIONS OF SOMATOSENSORY CORTEX IN THE FLYING FOX (*Pteropus poliocephalus*). L. Krubitzer, R. Tweedale, J. C. Clarey, and M. Calford*. VTHRC, Department of Physiology. and Pharmacology, University of Queensland, Australia 4072.

The precise role of the corpus callosum in connecting the two cerebral hemispheres is unknown. However, we do know that cutting the corpus callosum in humans results in unique impairments in coordinating opposite sides of the body. One theory is that the corpus callosum fuses midline representations in the somatosensory and visual systems so that an integrated sense of the body and visual world is formed in the neocortex. In the present investigation we examined the connections of area 3b, 1/2, SII and PV with the opposite hemisphere in the flying fox. Using microelectrode recording procedures, the area of interest was mapped in detail and anatomical tracers were restricted to that area. Cortex was flattened and cut parallel to the cortical surface so that areal patterns of connections could be observed. Alternate sections were processed for anatomical tracers or stained for myelin, and a comprehensive reconstruction based on electrophysiological mapping, cortical connections, and myeloarchitecture was obtained. Injections in 3b resulted in very sparse connections with 3b in the opposite hemisphere regardless of the body part injected. Most connections were with the lightly myelinated area 1/2 interspersed within 3b. Connections of area 1/2 were moderate to dense with area 1/2 in the opposite hemisphere. Injections in SII resulted in dense label in the contralateral SII, and sparse label in VS and area 1/2 in representations similar to those injected. Finally, injections in PV resulted in moderate to dense transported tracer in PV, SII and VS in the opposite hemisphere, in representations similar to those injected. Our results indicate that midline body part representations are not preferentially interconnected. Another interesting finding was that anterior parietal areas , 3b and 1/2, were only sparsely to moderately interconnected between hemispheres, while lateral parietal fields such as SII and PV were more densely interconnected.

644.14

PATHWAYS OF INTERHEMISPHERIC TRANSFER OF DYNAMIC PLASTICITY REVEALED BY FOCAL COOLING OF SOMATOSENSORY CORTEX. J. C. Clarey*, R. Tweedale, and M. Calford, Vision, Touch, and Hearing Research Centre, Department of Physiology and Pharmacology, University of Queensland, Australia 4072.

A small denervation in the periphery results in an immediate expansion of the neuronal receptive field representing the affected body area in primary somatosensory cortex (area 3b) in *both* hemispheres (Calford & Tweedale, Science 249: 805, 1990). This expansion is explicable in terms of a rapid unmasking of existing but normally inhibited inputs, and is termed dynamic plasticity here. Since each half of the body is represented in the contralateral hemisphere only, it is likely that these plastic changes are transferred to the ipsilateral hemisphere via the corpus callosum. However, in the animal under study (flying foxes), the callosal connections of area 3b are sparse, while those of an adjacent and interconnected somatotopically-organised field (area 1/2) are relatively dense (see Krubitzer et al., this meeting). To uncover the pathway(s) responsible for the interhemispheric transfer of dynamic plasticity, focal cortical cooling experiments were performed in adult, ketamine-anaesthetised flying foxes (*Pteropus scapulatus*). Cooling the forelimb digit 1 (D1) representation of area 3b, until neural activity beneath the cooling probe decreased, resulted in an expansion (of approximately 2-4 times in area) of a D1 receptive field (RF) recorded from an extracellular microelectrode in area 3b of the opposite hemisphere (n=8). The RF rapidly contracted to its original dimensions when the cortex was rewarmed to normal temperatures. Expansion and contraction of D1 RFs in area 3b of *both* hemispheres was also obtained when the D1 representation of area 1/2 in one hemisphere was cooled and rewarmed (n=2). In two other animals, this effect was observed only in the area 3b RF ipsilateral to the cooling probe. These results suggest that blocking the callosal pathway(s) produces a disinhibition that allows unmasking of large RFs and is probably mediated by the interhemispheric connections of area 1/2 via its intracortical connections with area 3b.

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS: PSYCHOPHYSICS AND NETWORKS

645.1

BODY SITE DIFFERENCES IN SENSITIVITY TO HEAT PAIN AND COOLNESS. J.D. Greenspan* and D.J. Taylor, Depts. of Neurosurgery and Physiology, SUNY Health Science Center, Syracuse, NY 13210

Thresholds for 1) slight heat pain, 2) moderate heat pain, and 3) coolness were determined for 32 subjects at four body sites, bilaterally: thenar eminence, dorso-lateral forearm, lateral calf, and plantar surface of the foot. A multiple staircase procedure was used to assess all three thresholds within the same session.

Heat pain thresholds (both slight and moderate) were significantly higher on glabrous skin sites than on hairy skin sites (ANOVA $F=37.64$, $p<0.001$). In contrast, cool thresholds were lowest on the hand, intermediate on the forearm, and highest on the leg and foot (ANOVA $F=19.94$, $p<0.001$). Heat pain thresholds were slightly higher on the dominant side of the body (mean difference for slight pain = 0.3°C; ANOVA $F=5.72$, $p<0.05$). Cool thresholds were also slightly higher on the dominant side of the body, but not at a statistically significant level (mean difference = 0.4°C; ANOVA $F=1.76$, $p>0.05$). There was a significant trend for heat pain thresholds to increase with repeated testing. Over a 2-3 week period, slight heat pain thresholds increased an average of 0.8°C. In contrast, cool thresholds decreased an average of 0.8°C. over the same time period.

There are highly significant differences in heat pain and cool sensitivity at various body sites. However, dissimilar factors appear to be responsible for the regional variation of these two sensory systems.

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645.2

INDIVIDUAL MAGNITUDE ESTIMATES CORRELATE WITH NEURAL INTENSITY CHARACTERISTICS. S.J. Bolanowski, G.A. Gescheider* and R.T. Verrillo, Institute for Sensory Research, Syracuse University, Syracuse, NY 13244.

Four distinct channels of information can combine to signal the mechanical aspects of touch (Bolanowski, et al, 1988), the particular combination of channels mediating a given tactile sensation being dependent upon stimulus conditions. For example, a 250 Hz vibratory stimulus of large size (2.9 cm^2) and of low and moderate intensities is signaled only by the Pacinian (P) channel, while stimuli at higher intensities are signaled by both the P and the non-Pacinian II (NPII) channels. Additionally, the P channel includes Pacinian corpuscle (PC) fibers that display entrainment plateaus in their intensity characteristics as obtained with sinusoidal stimuli, while the SA II fibers of the NP II channel do not. Lastly, a psychophysical correlate of neural intensity characteristics are the magnitude estimation (ME) functions which for results averaged across observers approximate power functions. We performed absolute ME experiments on observers [n=5; stimulus frequency, 250 Hz and noise (175-350 Hz); location, thenar eminence; contactor size, 2.9 cm^2 and 0.008 cm^2] to determine if breaks in the individual functions occur since: a) crossing from one information channel (e.g., P) to another (e.g., NP II) should result in a break in the overall function; b) PCs entrain while SA IIs do not and c) averaging data across observers may obscure ideosyncratic relationships. The individual functions obtained were scalloped in shape, as predicted, with breaks correlated with the P/NPII crossover and PC entrainment levels. The breaks correlating with the P channel were eliminated with the noise stimuli, also as predicted and consistent with the fact that noise precludes PC entrainment. Supported by NIH grants DC00380 and DC00098.

645.3

DOES PERFORMANCE OF TACTUAL TEXTURE DISCRIMINATION DEPEND ON SCANNING VELOCITY?

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The ability of humans to tactually recognize or discriminate between different textures improves when they are allowed to move their fingers across these surfaces. Several research groups have claimed that the velocity of movement is irrelevant to the subject's performance. Yet, this claim was not tested for naive subjects that were free to utilize their own scanning strategies. Previously, we suggested a model (the "PLL model") predicting that when the task becomes difficult, requiring subjects to reach optimal performance, scanning velocity (V) will depend on the spatial frequency (SF) of the texture (Ahissar and Vaadia (1990) *Proc. Natl. Acad. Sci. USA* **87** (22): 8935-8939). The model further predicts that scanning velocity will change in a way that will keep the resulting temporal frequency (f = V * SF) as close as possible to a preferred value. Three such preferred values were suggested, as expected from the tri-modal distribution (0-15 Hz, 15-50 Hz and 80-250 Hz) of the oscillating frequencies of cortical local oscillators found in the second somatosensory cortex of the monkey. This distribution corresponds also to the distribution of the best frequencies for activating mechanoreceptors in the finger tips of humans and monkeys.

Naive human subjects were tested for tactile recognition and discrimination tasks. The subjects were allowed to choose their own scanning strategies. The locations of the scanning fingers of both hands were sampled in 20 ms time resolution and 0.1 mm spatial resolution by an infra-red, ultra-sonic location detector. The analysis of scanning strategies revealed that when the task became difficult subjects adjusted their finger velocities according to the spatial frequencies, as predicted by the PLL model.

645.4

ROBOTIC TACTILE STIMULATOR FOR NEUROBEHAVIOR EXPERIMENTS WITH PRIMATES. H. Burton* and R.J.Sinclair, Dept. Anatomy & Neurobiology, Washington University Sch. Med., St. Louis, MO 63110

We developed a tactile stimulator for passive stimulus presentation paradigms using mostly "off-the shelf" products. The device provides controlled mechanical stimulation across an extensive range of stimulus patterns with a degree of flexibility previously available only with visual or auditory stimuli. The stimulator automatically positions and moves one of 11 different Nyloprint etched surface strips (20x100mm) linearly against a fingertip with controlled force and velocity. Force resolution is 1 gm and ranges to 1 Kg. Velocity resolution is <5mm/sec and ranges from 10-180mm/sec. Hydraulic pistons damp vibrations during strokes whose maximum length is 280mm. Commercial software integrates stimulator controls with simultaneous recordings from single neuron electrodes. Simple programs present surface strips in any sequence. Different sets of stimulus patterns interchange within seconds, permitting uninterrupted study with varied tactile stimuli. Stimulation is possible with any object by attaching it to the movable, magnetized tablet. Initial observations from neurons in primary somatosensory cortex of *M. mulatta* show ascending, monotonic response functions to differences in groove width of gratings rubbed along the fingertip at different velocities. (Supported by McDonnell Ctr. for Studies of Higher Brain Funct. & NIDCD 00096)

645.5

CONTEXT DEPENDENT MODULATION IN MONKEY SECOND SOMATOSENSORY AREA (SII) DURING ACTIVE TOUCH OF TEXTURED SURFACES. R.J. Sinclair* and H. Burton. Dept. Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Two *M. mulatta* stroked their fingertips over pairs of horizontal gratings and discriminated differences in their groove widths. Hand position and downward applied force were measured. We recorded from 127 single units and compared response periods with similar average force (1) before, (2) during, and (3) after strokes. The hand was stationary in 1 & 3. Task dependent modulation was seen in 6 cells with suppressed activity levels during contact with gratings. Firing rates decreased approximately 150ms prior to hand movement, but increased after movement ended. Comparable modulation was seen in 3 other cells with vigorous responses during strokes over gratings. Firing rates were higher during the hold time before movement began compared to the period following stroke termination. Thus, in both cell types responses depended on behavioral context of the task; either pre or post movement. This resembles sensory "gating" during locomotion or ballistic reaching. Activity changes in condition 1 could reflect anticipating hand motion. Facilitation or inhibition may relate to task relevance or attention. Changed responsivity after strokes (condition 3) may result from altered task requirements. Recordings from VPL and SI neurons in the same animals performing this task showed no similar response modulation. (Supported by NIDCD 00096)

645.6

THE EFFECT OF ATTENTION ON SPATIAL FORM PROCESSING IN THE FIRST AND SECOND SOMATOSENSORY CORTICES. S.S. Hsiao*, D.M. O'Shaughnessy, and K.O. Johnson, Phillip Bard Laboratories of Neurophysiology, Dept. of Neuroscience, The Johns Hopkins University Sch. of Medicine, Baltimore, MD. 21205.

The effect of selective attention on the responses of neurons in the primary (SI) and secondary (SII) somatosensory cortex were studied in a Macaque monkey trained to perform a tactile letter discrimination and a visual light detection task. Embossed letters of the alphabet were scanned continuously across each neuron's receptive field while the animal's focus of attention was switched between the tactile and visual tasks. In the tactile task the animal was rewarded for responding when the letter scanning across its finger matched the letter on the video screen. In the visual task the animal was rewarded for responding when one of 3 illuminated squares dimmed slightly. Because the same tactile stimulus patterns were presented continuously during both tasks differences in the neural responses can be attributed to differences in the animal's focus of attention.

A total of 104 neurons in SI cortex and 158 neurons in SII cortex were studied for attentional effects. The results show that attentional focus plays a major role in somatosensory form processing. Approximately 10% of neurons in SI cortex (3/55 in area 3b and 8/49 in area 1) and 40% of neurons in SII cortex (65/158) were affected. The effects were expressed not simply as an overall change in neuronal excitability but as specific modifications in the patterns of neuronal responses. Two main kinds of attentional effects were observed. The first was a modification of the discharge pattern that occurred before or during the stimuli. These effects were particularly prominent for the target letters. The second was a change in the evoked neural activity between relevant and irrelevant letters. For example, letters following target letter often evoked reduced responses perhaps because the animal had learned that target letters never occurred twice in a row in our design. The first effect may reflect enhanced signal processing for critical stimuli. The second may reflect a variation in signal processing related to the animal's expectation. Supported by NIH R01 NS18787.

645.7

TACTILE RECOGNITION THROUGH INTERMEDIATE SURFACES.

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The effects of placing an intermediate surface between the fingertip and a tactile stimulus were studied in combined neurophysiological and psychophysical experiments. This investigation had two purposes. The first was to develop the use of intermediates as a method of immobilizing the skin during somatosensory experiments. The second was to study how intermediates, such as gloves, alter somatosensory perception, and how the alterations affect the responses of slowly-adapting (SA) and rapidly-adapting (RA) afferents.

Psychophysical letter recognition experiments were performed on 33 human subjects using various intermediate surfaces, such as paper, different types of tape and surgical gloves. Subjects were asked to identify randomly presented embossed letters with a bare finger, which is the control condition, or with an intermediate between the letter and the finger. Overall, subjects' performance was 60% correct with the bare finger, 58-65% for a range of surgical gloves, and 35-44% for other intermediates, i.e tapes and paper. Mechanical tests performed on the skin with and without intermediates provide a potential explanation for these results. Surgical gloves increased the stiffness of the finger by a factor of 2 or less, permitting the skin to conform to the embossed surface, whereas the other materials increased the stiffness by 2.5-10 times, preventing the skin from conforming to the letters.

Neurophysiological recordings were made from the peripheral nerve of macaque monkeys to study the effects of surgical glove rubber on the spatial and temporal responses of the afferent fibers. The surgical glove rubber produced a significant decrease in RA mean firing rate but had relatively little effect on the response properties of SA fibers. Since the glove intermediate did not diminish perceptual performance, the differential effects on SA and RA responses support the notion that the SA afferents are critical for spatial form perception and the RA afferents encode other aspects of tactile perception.

645.8

A QUANTITATIVE STUDY OF THE CODING OF DIRECTION AND MOTION IN THE SOMATIC SENSORY CORTEX OF AWAKE MONKEYS. S. Ruiz, P. Crespo and R. Romo*. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México. 04510 México D.F.

A first step in the study of the representation of tactile signals in the somatosensory cortex, is to see whether in the neuronal responses there is information regarding the physical properties of the stimuli. We studied the responses of single neurons of areas 3b and 1 in the primary somatic cortex of awake monkeys, to a single 1 mm probe scanned across their receptive field in eight different directions, variable speeds, and constant traverse distance and force. The mean discharge rate of 129 of 178 neurons changed as a function of the speed. No differences were encountered between areas and submodalities, suggesting that neurons in both areas 3b and 1 can encode motion across the skin of the hand. An analysis of variance (ANOVA, F test, $p < 0.05$) detected 129 neurons that varied significantly with the direction of the stimulus. A linear regression model was used to test whether the mean discharge rate varied orderly with the direction of movement. Sixty seven neurons showed good fits to the model, ($p < 0.05$, $r > 0.7$). These observations suggest that single neurons in areas 3b and 1 possess radially tuned functions and that there is a relationship between the directional properties and the speed of the stimulus. It is hypothesized that the direction of the stimulus is encoded across a neuronal population distributed in primary somatic cortex in the form of a neuronal population vector. The research of R. Romo was supported in part by an International Research Scholar Award from the Howard Hughes Medical Institute, DGPAPA-UNAM (Proyecto IN206491) and CONACyT (Proyectos D111-903556 and F0006). R. Romo is a fellow of the Guggenheim Foundation.

645.9

ACTIVITY OF PARIETAL CORTICAL AREA 7B NEURONES DURING ACTIVE AND PASSIVE TOUCH. F. Tremblay*, S.A. Ageranioti-Bélanger, I. Zompa, C.E. Chapman. Centre de recherche en sciences neurologiques, Université de Montréal, Québec, Canada, H3C 3J7.

While it is known that lateral area 7 (7b) in the monkey contains cells with complex somatic and somatomotor response properties, the function of such cells, particularly those related to active hand movements, is at present unclear. In the present study, we were interested in determining if such cells play any role in a task requiring active scanning of textured surfaces. Recordings were performed in 3 monkeys (*macaca mulatta*) trained to discriminate textured surfaces (smooth vs smooth/rough) using either active touch (surface actively explored with digit tips) or passive touch (surface passively displaced under digit tips). The animal indicated the texture of the surface explored by pulling or pushing a lever with the opposite hand. Out of a total of 194 neurones, 45 had a somatic receptive field (RF; 32, somatic only; 13, somatic and visual) while 52 had no somatic RF (28 discharged specifically with Active Hand Movements, AHM units). While modulation was often observed in active (70%) or passive touch (50%), only a few cells signalled differences in texture (6%); such responses were not specific to the active task and no AHM units showed any relation to texture. The results suggest that neurones in area 7b do not play an important role in these relatively simple tasks of texture discrimination, although a role in the analysis of more complex tactile stimuli cannot be discounted. (Supported by MRC-Canada and FRSQ).

645.10

SOMATOSENSORY RESPONSES TO HUMAN PARIETAL LOBE STIMULATION. F. Richer*, M. Martinez, M. Robert, J.M. Saint-Hilaire. Serv. Neurologie, Hôpital Notre-Dame, Montréal, QC, H2L-4M1.

We investigated the somatosensory perceptions evoked by stimulation of peri-rolandic as well as lateral and medial parietal cortex in 50 epileptic patients undergoing a presurgical evaluation with intracerebral electrodes. Bipolar stimulation trains were delivered in an incremental sequence at medial and/or lateral contact pairs of stereotaxically-implanted multilead electrodes, while monitoring afterdischarge propagation with electrodes in frontal and temporal lobes. Lateral stimulation evoked: 1) contralateral sensations in rolandic sites, 2) sensations on either or both sides in the opercular region, 3) mostly contralateral sensations in posterior parietal regions. Medially we evoked: 4) ipsilateral sensations in cingulate sulcus (around medial area 5), 5) contralateral sensations in posterior cingulate gyrus, and 6) bilateral sensations of changes in body position (levitation) in the region of the subparietal sulcus. These observations suggest the presence of distinct somatosensory regions in the human parietal cortex, some of which may show some correspondence to those identified in the macaque, such as PE, PEci, and PGm medially and PF and PFop laterally (Pandya & Seltzer, 1982).

645.11

AUTOMATED DETECTION OF 2DG/IMMUNOSTAINED NEURONS IN BARREL CORTEX. *L.S. Hibbard*, J.S. McCasland, and T.A. Woolsey, Departments of Neurology and Neurological Surgery, and Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.*

With detailed immunohistochemical and metabolic maps of the barrel cortex, we expect to see a new picture of barrel function in the patterns of neuronal excitation/inhibition and energy use. These maps are montages of contiguous fields (125^2 μm, using a 40X objective with high NA) of immunostained sections and the corresponding autoradiograms, digitized under computer control. To detect neurons in GAD–stained sections, we correlate templates (averaged images of obvious GAD+ and GAD– cells) with the digitized fields. Potential cell locations in the field–images correspond to maxima in the 2–D correlation function $R = F^{-1}\{F(a)F(b)^*\}$, where a and b are the image and template, and F is the Fourier transform operation. Detection is confirmed for features having high–valued normalized correlation coefficients with the templates. By inspection, false positive and false negative detections in GAD–stained sections are <2% of the detected cells. For each section, image collection (1280 digital fields, 320 Mbytes) and cell detection (>10^4 cells per image set) take 5 hours, running unattended under software control. This system can gather vast amounts of data quickly, and apply analyses to it which are both quantitative and exhaustive. By adjusting the templates, this method may be generalized for other histology preparations.

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645.12

CELLULAR MAPS OF METABOLIC ACTIVITY IN ANTIGENICALLY IDENTIFIED NEURONS: A 2-DEOXYGLUCOSE/IMMUNOSTAINING APPROACH TO BARREL FIELD CIRCUITRY. *J.S. McCasland*, L.S. Hibbard, S. Kalmbach and T.A. Woolsey. Department of Neurology and Neurological Surgery, Washington University School of Medicine, Saint Louis, MO. 63110.*

Local circuit axons in barrel cortex do not develop normally without input from the whiskers via the infraorbital nerve (PNAS **89**:1832-1836). This and other evidence suggests that the pattern of connections within and between barrel columns is sculpted by activity, and we reason that the characteristic patterns of metabolic activation in normal behavior (Somat. Mot. Res. **8**:111-116) reflect a dynamic equilibrium disrupted by experimental manipulation. We have developed a new approach to examine cellular patterns of metabolic activation by combining a high resolution 2-deoxyglucose (2DG) technique, glutamate decarboxylase (GAD) immunostaining, and an automated cell detection algorithm (see companion abstract by Hibbard et al.). We compared 2DG labeling densities over GAD+ (presumably inhibitory) and GAD- (mostly spiny stellate and pyramidal) neurons of the awake behaving hamster. GAD+ neurons are heavily 2DG-labeled in every lamina of barrel cortex, more so in layer IV and less in layers II-III. Our data indicate that the ratio of 2DG labeling for GAD+ and GAD- neurons is approximately 2.0 in layer IV, 1.5 in layers V and VI, and 1.3 in superficial layers. These ratios are strongly correlated with overall labeling in their respective laminae, suggesting a dynamic matching between degree of local inhibition and local metabolic activation such that inhibitory influences become more predominant in heavily activated zones. Changes in the pattern of antigenically identified cellular 2DG labeling patterns in the barrel field with different combinations of stimulated whiskers will be evaluated in behaving animals.

Supported by NIH grant NS17763.

645.13

OPTICAL INTRINSIC SIGNAL IMAGING OF SOMATOSENSORY CORTEX IN THE RODENT.

S. Narayan*, E.M. Santori, J.Burton and A.W.Toga , Laboratory of Neuro Imaging, Dept of Neurology, UCLA, Los Angeles CA 90024.

The detection of optical intrinsic signals enables the visualization of dynamic functional architecture in mammalian brain cortex. The response is known to be species and modality specific. Here we demonstrate the application of this method to functional mapping of whisker barrel cortex in the rat.

Male Sprague-Dawley rats were anesthetized (inhaled halothane, followed by urethane 1g/kg i.v.) and stereotactically mounted. Prefabricated brass wells were cemented to the skull, centered over the posteromedial barrel subfield (PMBSF). The cortex was exposed, the well filled with artificial cerebrospinal fluid and sealed with a glass window. Contralateral vibrissae A4 to E4 were pulled for 2 seconds at 4Hz. Synchronized digital images were acquired at 640 nm illumination with a slow scan cooled CCD camera. Stimulated images were divided by control images for timepoints post stimulation, and averaged over 20 to 100 trials. Optical intrinsic signals were observed as a focal decrease in reflectance (magnitude 10^{-3} of control) over stereotactically determined PMBSF cortex. The signal has two distinct spatiotemporal components. The first (diffuse) component begins 1.5-2.0 secs after stimulus onset, peaks at 2.5-3.0 secs, falls away by 4.5-5.0 secs, then exhibits an undershoot lasting approximately 3 secs. The second component (coincident with local vessels) begins at 2-2.5 secs, peaks at 4-4.5 secs and dissipates by 5.5-6 secs.

This work demonstrates that optical intrinsic signals may be used to study functional cortical activity in the rat.

645.14

MAPPING FUNCTIONAL PLASTICITY OF RODENT WHISKER BARREL CORTEX USING OPTICAL INTRINSIC SIGNAL IMAGING

A.W. Toga*, S. Narayan, E.M. Santori, & J. Burton, Laboratory of Neuro Imaging, Dept of Neurology, UCLA School of Medicine, Los Angeles, CA.

The functional anatomy of rat whisker barrel cortex was imaged using optical intrinsic signals. Mechanical and electrical stimulation of the vibrissae or upper lip were varied in intensity, frequency, duration and location to determine the plasticity and robustness of the response. A cranial window was created over the contralateral posteromedial barrel subfield, the cortex illuminated with white light and signals digitized and averaged at 640 nm, 550 nm and 850 nm. Stereotactic measurements were made and bony landmarks identified for subsequent mapping and correlation with 2DG studies and histologically defined 3D digital atlases. The pattern within the focal response was not tightly coupled with the cytochrome oxidase patterns observed in this area. However, its location changed in response to stimulation of different vibrissae. The magnitude and shape of the reflectance change was influenced by stimulus characteristics, anesthetic state of the animal, and integrity of the cranial window. The intrinsic signal response could be divided into separate temporal components, lasting up to 6 secs post-stimulation. The latter half contained changes that included reflectance changes in blood vessels. The vascular component of this response may be influenced by the proximity of functionally active sites to one of two blood vessels. Somatosensory cortex is supplied by the middle cerebral artery and venous drainage is accomplished by the parietal and parietal-temporal branches of the superior cerebral vein.

645.15

INTRINSIC SIGNAL OPTICAL IMAGING IN RAT SOMATOSENSORY CORTEX
J. J. Gelfand, P. M. Gochin, P. Bedenbaugh, C. G. Gross* and G. L. Gerstein, Dept. of Psychology, Princeton University, Princeton NJ 08544

The responses of somatosensory cortex to tactile stimulation of the forepaw were assessed by intrinsic signal optical imaging. The tips of digits 2 or 5 were repeatedly touched with mechanical tappers while CCD photographs were taken of S1 illuminated by an 800 nm light source. The resulting images showed two highlighted areas about 300 μm in diameter and 500 μm apart. Electrical recording in the areas highlighted during stimulation yielded receptive fields appropriate for the stimulated digit and and not the other digit. Penetrations between the highlighted areas had receptive fields on intervening digits. These results demonstrate that intrinsic signal optical images are obtainable in S1 and confirm the functional somatotopy previously reported using electrical recording. Furthermore, the short time required to produce the images and the spatial resolution suggest that optical recording could be used for the study of cortical reorganization in this brain region. Attempts to image activity from the hindpaw, and from adjacent digits of the forepaw yielded only weak and inconsistent signals. Furthermore, we have also been unable to detect acoustic activation of rat auditory cortex although we have replicated results of others in the cat [Frostig et al. 1990], demonstrating orientation stripes in visual cortex. These observations may foreshadow limitations, or at least difficulties which may be encountered in the use of the optical imaging method.

645.16

COMPUTER SIMULATION OF THE DYNAMICS AND PLASTICITY OF SOMATOSENSORY CORTEX
J.Xing* & G.L.Gerstein, Dept. of Physiology, Univ. of Pennsylvania, Philadelphia PA 19104

Recent experimental evidence has clearly demonstrated the functional plasticity of somatosensory cortex in adult animals. A computer model is set up to understand the activity of somatosensory cortex and the fundamental mechanism of the plasticity. The model is a 3-layered feedforward neural network, corresponding to skin, subcortex and cortex. In addition to receiving the input from thalamus, cortical neurons can also be stimulated directly mimicking microstimulation. There are both excitatory and inhibitory lateral connections among cortex neurons. Neuronal activity in the network is simulated by MacGregor's PTNRN10, which produces action potentials and in so doing causes post-synaptic potentials. The excitatory connective strengths are modifiable by general Hebbian rule. Repeated stimulation of the skin layer resulted in the formation of neuronal groups, as has been reported by Pearson. (1987, J. Neurosci.). The sizes of groups are determined by the underlying excitatory connection, irrespective of the training stimuli. The sharpness of groups is largely dependent on the balance between total lateral excitation and inhibition in the cortical network. Groups can not be formed in the absence of distant inhibition. The receptive fields of neurons in the same group have similar locations, but their sizes vary dramatically. Both intensive stimulation of a restricted skin area and simulated cortical microstimulation can result in "regrouping" in the cortex and the subsequent changes in receptive field sizes and locations. However these changes occur at different time scales for the two manipulations. Individual neuron firing patterns and measures of assembly organization are changed by training. NIH-MH46248.

REFLEX FUNCTION II

646.1

DISRUPTION OF SENSORIMOTOR GATING OF THE STARTLE RESPONSE BY RU24969 AND DOI.
T.A. Sipes* and M.A. Geyer. Department of Neuroscience, UCSD, La Jolla Ca 92093.

Prepulse Inhibition (PPI) refers to the attenuation of the startle response (SR) due to the prior presentation of a subthreshold prestimulus and is presumed to reflect sensorimotor gating mechanisms. Sensorimotor gating deficits in schizophrenics can be mimicked in rats with manipulations of a variety of neurotransmitters, including dopaminergic, glutamatergic, GABAergic and cholinergic systems. Based on the report of a disruption of PPI by 8-OH-DPAT (8-hydroxy-2(di-n-propylamino)tetralin), a selective agonist for the 5HT1A receptor, the present studies determined the effects of other selective serotonin agonists on PPI. Male Sprague-Dawley rats were received RU24969 (0.3 - 1.2 mg/kg. s.c.) or DOI (2,5-dimethoxy-4-iodoamphetamine) (0.125 - 0.5 mg/kg, s.c.) 10 min prior to testing with a series of high and low pulse (105, 120dB) and prepulse (75, 85dB) intensities and air puffs (50 psi). RU24969, a 5HT1B/1A agonist, disrupted acoustic-acoustic but not acoustic-tactile PPI, while increasing tactile startle. DOI, a 5HT2/1C agonist, also disrupted PPI, primarily at the lowest prepulse intensities. These findings further implicate a role for serotonin in sensorimotor gating mechanisms.

646.2

SYNAPSES IN VENTROLATERAL PONS (VLP) AND RETICULARIS PONTIS CAUDALIS (RPC) MEDIATE ELECTRICALLY EVOKED STARTLE.
P.W. Frankland* and J.S. Yeomans, Dept. of Psychology, University of Toronto, Toronto M5S 1A1, Canada.

Circuits for the acoustic startle reflex were analyzed by measuring hindlimb EMG latencies, and testing for collision in hindbrain sites in chloral hydrate anaesthetized rats. In medulla and caudal RPC sites, latencies recorded in posterior biceps femoris had a mean value of 4.5 ms and varied by less than 0.2 ms, when two 0.1 ms pulses were delivered at a 1.0 ms interpulse interval at currents 2.5 times threshold. Latencies in other hindlimb muscles were also reliable, but latencies between muscles varied by up to 2.5 ms. The shortest observed latency following medulla stimulation was 3.0 ms in anterior biceps femoris.

As electrodes moved into rostral RPC and VLP, latencies increased by 0.3-0.4 ms. Collision tests between medulla and rostral RPC or VLP resulted in asymmetry with 0.3-0.4 ms collision intervals, suggesting a monosynaptic connection in RPC (Hempel et al., 1990). Between rostral RPC and VLP sites, or between medulla and caudal RPC sites, symmetric collision was observed suggesting connections by axons. Another increase in latency of 0.3-0.4 ms was observed in rostral VLP sites, along with a second increase in collision interval of 0.3-0.4 ms, suggesting that a second monosynaptic connection in rostral VLP near the rostral periolivary area mediates the startle reflex.

(Supported by NSERCC grant A7077 to J.S. Yeomans).

646.3

RELATIONSHIPS BETWEEN THE MODIFICATION OF ACOUSTIC STARTLE REFLEX BEHAVIOR AND VARIATION IN ITS BASELINE STRENGTH IN RODENTS.
G.P. Bowen, J.A. Barlow & J.R. Ison*. Department of Psychology, University of Rochester, Rochester, NY 14627.

The vigor of the acoustic startle reflex is suppressed if weak irrelevant stimuli occur just prior to the moment of reflex elicitation. Here we show how variation in baseline reflex strength, produced by various manipulations, may influence the degree of suppression. When reflex strength is increased because of an increase in the intensity of the eliciting stimulus, then (1) the initial stimulus has a proportionately reduced effect when expressed relative to baseline strength, but (2) a constant absolute effect, measured by subtracting its effect from the amplitude of the baseline response. In contrast, if reflex strength varies because of other factors, namely (a) age of the animal; (b) diurnal rhythmicity; (c) individual differences; or (d) habituation, then the initial stimulus has a constant proportionate effect when expressed as a ratio of the baseline response, and a greater absolute effect, as more is subtracted from large compared to small responses. These data have implications for understanding the theoretical relations between the levels at which these manipulations may be processed, and practical import for interpreting experiments concerned with sensory function. Calculating the impact of a particular test stimulus on startle responses of different strength has become a contentious exercise, and we suggest that relative measures of response suppression are more useful indices of sensory ability.

(Work supported in part by NIH Grants AG-09524 & EY-01319, and the Rochester International Center for Hearing and Speech)

646.4

ANATOMICAL CONNECTIONS MEDIATING THE PINNA COMPONENT OF THE ACOUSTIC STARTLE REFLEX IN THE RAT.
E. G. Meloni & M. Davis*. Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT.

The pinna component of the acoustic startle reflex involves an ipsilateral backward movement of the ear in response to an auditory stimulus. Previous studies have demonstrated that the pinna component of startle shows plasticity similar to that of whole body startle (Cassella & Davis, 1986). By identifying the neural circuit mediating the pinna response, it may be possible to isolate areas where plasticity takes place and eventually determine how these changes are brought about at the cellular level.

Fluoro-Gold injected into the posterior auricular nerve which innervates the muscles responsible for the pinna reflex labelled motoneurons located exclusively in the most medial and ventral division of the facial nucleus. Iontophoretic deposits of Fluoro-Gold in this region produced labelling in three major contralateral areas: (1) the ventral part of the nucleus reticularis pontis caudalis (RPC); (2) the ventrolateral tegmental nucleus (VLTg); and (3) the intermediate nucleus of the lateral lemniscus (ILL) and paralemniscal zone (PL). Afferent connections from each of these areas to the facial motor nucleus were confirmed with the anterograde tracer Fluoro-Ruby.

Electrical stimulation of the ventral RPC and VLTg areas produced a contralateral pinna movement whereas electrolytic lesions of these areas reduced the acoustic pinna reflex. No response occurred to stimulation of the ILL or PL regions and lesions of these areas did not diminish the pinna response.

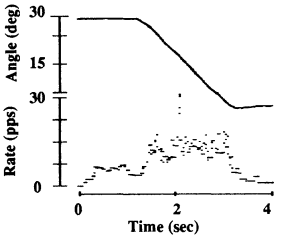
We are currently recording single units in the facial nucleus in freely moving rats. Facial motor neurons that innervate pinna muscles respond to an acoustic stimulus within 5-7 ms. Further studies will record from other nuclei along the pinna reflex circuit.

646.5

CONDITIONAL FEEDBACK OF HUMAN MUSCLE SPINDLE AFFERENTS. S.E. Grill, Å. B. Vallbo, W.Z. Rymer* Human Motor Control Section, NINDS, NIH, Bethesda, MD 20892; Univ Göteborg, Göteborg, Sweden; Rehab Institute of Chicago, Chicago, IL

The presence of parallel intrafusal/extrafusal activation may conceivably enable spindle afferents to function as a model reference system. If such a system were in effect, spindle afferent discharge would remain constant during commanded movements; changes in the feedback signal would be conditional upon an error. We evaluated whether there are situations in which spindle afferents display constancy of discharge during movement by recording microneurographically from single human muscle spindle afferents from finger extensor muscles. Subjects made slow (20 deg/sec) ramp movements against various-sized isotonic loads ranging from 2 to 17 % of maximum voluntary torque (MVT).

For most afferents, the discharge rate increased or decreased at the onset of the shortening movement in a step-like fashion. The rate changed little during the subsequent shortening movement (see fig). This was true for 4 of 5 afferents studied, for at least one isotonic load level ($p<.05$). It was not true, however, for all loads studied for each afferent. These findings suggest that conditional feedback may be operational during slow voluntary finger movements but only under certain loading conditions.



Work performed at Dept Physiology, Univ Umeå, Umeå, Sweden

646.6

IMMOBILIZATION HAS A MINIMAL EFFECT ON MUSCLE AFFERENT RESPONSES TO STRETCH IN A CAT HINDLIMB MUSCLE. M.A. Nordstrom*[1,3], R.M. Enoka[1,2], R.M. Reinking[1], and D.G. Stuart[1]. Depts. of Physiology[1] and Exercise & Sport Sciences[2], Univ. of Arizona, Tucson AZ 85724, and Dept. of Physiology[3], University of Adelaide, Australia.

We studied muscle spindle and tendon organ afferents in cat tibialis posterior muscle after six weeks of right-hindlimb immobilization by splinting. The effects of this protocol on muscle force and fiber cross-sectional area have been reported previously (Nordstrom *et al.*, Neurosci. Abstr. 17:648, 1991; Callister *et al.*, Neurosci. Abstr. 17:649, 1991). Seventy-eight afferents (21 Ia, 34 sp II, 23 Ib) in 12 control and 9 splinted cats were recorded from dorsal-root filaments. Afferent responses were quantified using a ramp-and-hold stretch (3 mm at 5 mm/s with a 2s hold) beginning from two different initial muscle lengths (L_0, optimal for whole muscle twitch & L_0-2 mm). There were no significant effects (ANOVA) of immobilization on axonal conduction velocity, mean or variability of the resting firing rate, initial burst peak frequency, dynamic index or dynamic response. The significant effects of immobilization included: 1) an increased stretch sensitivity of sp II afferents (peak and mean discharge rates during the stretch were 144% and 135% of control, respectively); and 2) increased static length sensitivity of Ib afferents (the static index was 173% of control) and an increase in one index of dynamic sensitivity (148% of control). In all cases, significant differences were only seen with stretch from the shorter initial length. For Ia afferents, there were no significant immobilization effects in any variable. In summary, the effects of immobilization on muscle proprioceptive afferents were relatively minor, and would not appear to pose additional motor control problems for recovery other than the weakness associated with muscle atrophy. Supported by USPHS grants NS 20544, HL 07249, NS 25077, NS 07309 and RR 05675. M.A.N. was a C.J. Martin Fellow of the NH&MRC of Australia.

646.7

REFLEX RESPONSES TO UNEXPECTED LOSS OF FOOT SUPPORT IN INTACT AND CHRONIC SPINAL CATS

K.G.Pearson*, M.Gorassini, G.Hiebert and A.Prochazka.
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Adaptive responses to loss of ground contact during gait were compared in intact and spinalized cats. In normal cats, a walkway equipped with a trap door that opened just prior to hindlimb contact was used. Behavioural responses to this perturbation depended greatly on prior experience and the speed of locomotion. For example, in naive cats, the leg entering the hole remained extended for approximately 200 ms after entry whereas after a few trials, extension was aborted after 80 ms and the cat quickly flexed its leg out of the hole. When the cat trotted across the platform, the contralateral hindlimb commenced its swing phase at the time the ipsilateral foot entered the hole and as a result, this led to a prolonged extension into the hole. Spinalized cats whose forelimbs were supported and hindlimbs walking on a treadmill with a hole cut into one side of the belt exhibited responses to loss of ground contact similar to naive, intact cats. During spontaneous walking, latencies to initiation of flexion were > 200 ms and these responses did not change with repeated exposure to the hole. With concomitant perineal stimulation, however, the latencies to initiation of flexion were reduced, but not to the minimal values seen after a few stimulus presentations in intact cats. This indicates that the fast flexion responses in experienced intact animals are supraspinally initiated and dependent on anticipatory set.

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646.8

A METHOD FOR ELICITING MONOSYNAPTIC REFLEXES IN THE NORMAL CAT. A. Prochazka*, P. Whelan, J.L. Taylor & K.G. Pearson
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H-reflex studies have provided valuable information on monosynaptic reflex (MR) gain during various locomotor tasks[1]. Potentially, MR testing in the cat could provide valuable information on reflex gain in a variety of normal and disordered movements, but so far it has been difficult to elicit stable MR's in the freely moving animal. Our method is derived from the classical work of Lloyd[2], who showed that MR's could be produced by stimulation of the dorsal root (DR) in acute spinal cats. We have found in 4 intact cats that short-latency responses can be evoked in various hindlimb muscles by stimulating at low amplitude via implanted DR electrodes[3]. In a decerebrate cat we found that amplitudes of DR-evoked MR's (DRMR's) rose and fell in parallel with MR's evoked by conventional LG-SOL nerve stimulation. Electrodes on the sciatic nerve and on the dura mater overlying the L5 dorsal spinal quadrant were used to monitor the afferent volleys. Latencies were consistent with monosynaptic transmission around the reflex arc. We conclude that low-threshold, DR-evoked responses at 5-6 ms latency are MR's and that this new approach will allow MR gain to be studied simultaneously in various muscles under a variety of normal and pathological conditions.

1) Capaday,C, Stein, R.B. (1986). J. Neurosci. 6, 1308-1313.
2) Lloyd, D.P.C. (1943). J. Neurophysiol. 6, 317-326.
3) Prochazka, A. et al. (1977). J. Physiol., 268, 423-448.

Funded by Canadian MRC & NCE, Australian NHMRC & Alberta Heritage Foundation for Medical Research

646.9

QUANTITATIVE ASSESSMENT OF INTERSEGMENTAL REFLEXES BETWEEN THE TAIL AND HINDLIMB IN THE AWAKE CAT. R. M. Friedman*, C. J. Vierck, Jr., and L. A. Ritz. Departments of Neuroscience and Neurosurgery, University of Florida College of Medicine, Gainesville, FL 32610.

We have previously investigated the hyperreflexia observed in segmental reflexes of the tail of cats after a chronic sacrocaudal transection (Friedman et al., 1990, 1991). Here we report on observations of intersegmental modulation of reflexes of the tail and hindlimb. Normal cats were conditioned to receive innocuous electrocutaneous stimulation to the tail and then the hindlimb (or visa-versa). Interstimulus intervals (ISIs) in the condition-test paradigm were 20, 50, 100, 250, 500, and 1000 msec. Quantitative assessments of reflex force were performed by tethering the tail and hindlimbs to strain gauges. At short ISIs we observed facilitation, while at intermediate intervals there was a decrease in amplitude of the test reflex. In addition, conditioning stimulation affected the amplitude of hindlimb reflexes greater than that of the tail. With this approach, we will be able to characterize segmental and intersegmental reflexes prior to and after specific lesions of spinal cord. Supported by grant NS27511.

646.10

IPSI- AND CONTRALATERAL AFFERENT INTERACTIONS IN CAT SACROCAUDAL MOTONEURONS. Louis A. Ritz* and Robert M. Friedman. Depts. of Neuroscience and Neurosurgery, University of Florida, Gainesville, Florida 32610

Anatomical evidence from our laboratory has demonstrated that tail Ia fibers project bilaterally and that sacrocaudal motoneurons have bilateral dendritic trees. Sacrocaudal motoneurons, innervating tail muscles, might receive monosynaptic input from contralateral dorsal roots. Intracellular recording techniques were used to investigate contralateral influences on sacrocaudal motoneurons.

In most cases, contralateral input was weakly excitatory, at a latency of 0.7-1 msec longer than that of ipsilateral input. When contralateral and ipsilateral input were combined, there was usually a late augmentation of the compound EPSP, compared to that produced by ipsilateral input alone; occasionally, there was a diminution of the compound EPSP at high stimulus rates. In other cases, the contralateral input inhibited ipsilateral responses at delays of 5 msec or longer. We have thus far found no conclusive physiological evidence for monosynaptic input from contralateral dorsal roots to sacrocaudal motoneurons. Supported by NS27511.

646.11

INHIBITORY INFLUENCES OF PERIPHERAL C FIBERS ON THE FLEXION WITHDRAWAL REFLEX IN SPINAL CATS. J.A. McMillan*, J.E. Quisno and J.L. Small. Biology Department and WAMI Program, Montana State Univ., Bozeman, MT 59717

We reported earlier (McMillan et al., 1989, Soc. Neurosci. Abs. 15:918) that the peripheral C fibers contribute little to the flexion reflex (FR) in spinal cats. We report here C fibers can in fact inhibit the reflex.

Experiments were performed on cats initially decerebrated under ketamine anesthesia. The FR, evoked by stimulating the left sciatic nerve at 10 Hz, was monitored by recording isometric tension from the left semitendinosis. To evaluated influences of myelinated vs unmyelinated fibers we added C fibers to, or removing them from, the afferent volleys by adjusting the intensity of the stimuli.

C fibers were consistently excitatory on the FR in the decerebrate state. After cutting the spinal cord (T12-L1), the C fibers typically had either an initial excitatory and subsequent inhibitory effect or just a pure inhibitory effect. In only 1 of 10 cats did C fibers have a pure excitatory effect in the spinal state.

These observations suggest a population of interneurons which are inhibitory on the FR. We propose a model in which these neurons (i) receive more excitatory inputs from C vs myelinated afferents and (ii) are more strongly inhibited by descending inputs than are the interneurons which have excitatory influences on the FR. (Supported by NSF BNS 86-1948, NSF EPSCoR RII-3921978 and NIH 5SO6GM08218-09).

646.12

THE STRENGTH OF RECURRENT INHIBITION BETWEEN LATERAL AND MEDIAL GASTROCNEMIUS MOTONEURONS IN THE CAT M.L. McCurdy* and T.M. Hamm. Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

The strength of recurrent inhibition is reported to be greatest between motor pools that innervate muscles with common periods of activity, within homonymous motor pools, and between motor pools or motoneurons in close proximity (Eccles et al.,1961; Hamm et al., 1987; Hamm, 1991; McCurdy and Hamm, 1991). We evaluated these findings by measuring the strength of recurrent inhibitory post synaptic potentials (RIPSPs) in ischemic decapitate cats by impaling pairs of homonymous or heteronymous motoneurons that innervated the medial (MG) or lateral gastrocnemius (LG) muscles, which are muscles with common activity patterns. RIPSPs were recorded in one motoneuron in response to stimulation of the other motoneuron by injecting brief current pulses.

Results indicate that proximity of these motoneurons was a stronger variable than motoneuron specie in determining the strength of recurrent inhibition of gastrocnemius motoneurons. RIPSPs recorded in homonymous LG, but not MG, pairs and those recorded in MG, but not LG, motoneurons of heteronymous pairs had significantly greater RIPSPs when motoneurons were separated by 1 mm or less than when separated by greater distances. Homonymous pairs of Mg and Lg motoneurons did not have significantly greater RIPSPs in comparison to heteronymous pairs. These results suggest that the pattern of recurrent inhibition within and between the MG and LG motor pools is not symmetric.

Additional analysis revealed that homonymous pairs of motoneurons innervating the muscle anterior-middle biceps femoris had significantly greater RIPSPs than homonymous pairs of MG, but not LG, motoneurons. This result suggests that the strength of recurrent inhibition in homonymous pools is not homogeneous for all species of motoneurons. Supported by NS22454, NS07309 and GM08400.

646.13

CONDUCTANCE CHANGES PRODUCED BY RECURRENT INHIBITION IN CAT SPINAL MOTONEURONS. T.M. Hamm* and M.L. McCurdy. Div. of Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013.

Both the magnitude and location of changes in conductance during synaptic inhibition can be significant factors in the inhibitory effect (e.g., Rall, 1964; Koch et al., 1983). Previous studies have been unable to detect a conductance increase associated with recurrent inhibition of spinal motoneurons (Smith et al., 1967; Friedman et al., 1981). However, Lindsay and Binder (1991) demonstrated small changes in motoneuron input resistance during sustained recurrent inhibition. Since synapses from Renshaw cells to motoneurons are located on proximal dendrites of motoneurons (Burke et al., 1971; Fyffe, 1991), we thought it important to evaluate the conductance changes associated with recurrent inhibition using a method that determines not only conductance magnitude but also synaptic location. To do this, we have determined motoneuron impedance with and without recurrent inhibition produced by repetitive stimulation of muscle nerves. The preparation used has been the pentobarbital-anesthetized cat with sectioned dorsal roots. Intracellular recordings were made of the voltage response to the injection of a mixture of sinusoidal currents (2.5 - 500 Hz), which provides information on the location of the conductance change (Fox, 1985). Current injection and voltage recording were accomplished with a single glass microelectrode using a discontinuous current clamp. Sets of voltage and current records were then subjected to Fourier analysis to determine power spectra.

Our preliminary data show a small decrease in the voltage response in trials with recurrent inhibition, indicating an increased conductance. The change in the voltage response is greater at lower frequencies (< 300 Hz), consistent with the dendritic location of the synapses in this pathway. These results indicate that the inhibitory effects of recurrent inhibition are attributable to conductance changes in addition to hyperpolarization. Supported by NS22454, NS07309 and GM08400.

646.14

CUTANEOUS AFFERENT STIMULATION AND MOTONEURON RECRUITMENT IN THE MEDIAL GASTROCNEMIUS OF THE DECEREBRATE CAT. B.D. Clark*, S.M. Dacko and T.C. Cope. Dept. Physiol & Biophys, Hahnemann Univ., Phila. PA 19102

It is often suggested that Henneman's size principle of recruitment is violated by certain cutaneous inputs which can preferentially recruit large, fast-twitch (F) motor units over small, slow-twitch (S) motor units supplying the same muscle. In the cat, evidence for this view rests on a few cases described in a single study (K. Kanda et al., *Exp. Br. Res.* 29: 55) in which motor units were characterized only by their axonal conduction velocities. In contrast, in our previous report (Cope and Clark, *J. Neurophysiol.* 66: 1127) we found both stretch of the medial gastrocnemius (MG) and electrical stimulation of the caudal cutaneous sural nerve (CCS) to recruit motoneurons in accord with the size principle. The present study describes our attempt to resolve this disagreement. Using simultaneous intra-axonal recording from pairs of motoneurons, we assessed recruitment of physiologically characterized motor units in the MG under stimulation conditions that replicated as closely as possible those of Kanda et al. In agreement with our earlier findings, the relative excitability of the units in 27 out of 27 pairs (including 5 pairs containing one S and one F unit) was the same for the tonic vibration reflex (TVR), pinch of the skin overlying the ankle, or their combination. Furthermore, there was little evidence of inhibition of the firing of small motor units by cutaneous sources; only 1 of 9 type S units and 1 other unit of unknown type showed any slowing from skin pinch or electrical stimulation of the CCS delivered during TVR, regardless of whether electromyograms from the soleus showed that muscle to be excited or inhibited. For the two units that were slowed, this effect appeared only in some of the trials, lasted only for 100-400 msec, and was followed by an increase to firing rates equal to or greater than they exhibited for TVR alone. We conclude that differential activation of motor units by cutaneous afferents in this preparation is rare, variable and, when it occurs, transient. (Supported by NIH NS21023)

646.15

THE USE OF NATURAL SENSORY SIGNALS IN FUNCTIONAL ELECTRICAL STIMULATION (FES) SYSTEMS. M. Haugland*, T. Sinkjær, J.A. Hoffer, J. Haase. Department of Medical Informatics and Image Analysis, Aalborg University, Fredrik Bajersvej 7D, DK-9220 Aalborg, DENMARK.

In an experimental cat model, we have previously demonstrated that the tactile sensory information recorded from a whole nerve cuff electrode contains reproducible information about changes in a force applied on the skin. In conformity with the human precision grip, where updating of the motor program during a small slip is based on the triggered phasic activity in glabrous skin mechanoreceptors, an event-driven FES system automatically corrected the "motor" output to the controlled muscles, whenever an object started to slip.

The aim of the present study was to show that it is possible to extract signals from whole nerve cuff electrode recordings in a human cutaneous nerve which contains similar important information as demonstrated in the FES system of the animal model.

Whole nerve cuff electrodes were implanted on the sural nerve of three human subjects in general anaesthesia. All subjects gave their consents and the study was approved by the Local Ethical Committee. The dorsal and lateral parts of the foot were mechanically stimulated using a hand-held probe manipulated by the experimenter. The human sural nerve responses to perpendicular and lateral force input on the skin were nearly similar to the tibial nerve responses found in cat experiments. In both specimens, a large phasic response was detected when a step force was applied on the skin or when the probe slipped along the skin. These findings suggest that FES systems in paralysed humans can be controlled from the natural tactile sensory information in the same manner as shown in the cat experiments.

646.16

SELECTIVE RECRUITMENT WITHIN TRICEPS SURAE OF THE DECEREBRATE CAT ALTERS THE DIRECTION OF ANKLE TORQUE PROFILES. S.J. Bonasera, J.H. Lawrence, III, and T.R. Nichols*. Department of Physiology, Emory University Atlanta, GA 30322.

The feline gastrocnemius (G) inserts in a complex manner onto calcaneus. The lateral head (LG) forms a tendon whose insertion is parallel to the long axis of the foot. By contrast, a portion of the medial head (MG) tendon crosses the LG tendon to insert on the lateral aspect of calcaneus. Thus, differential activation of the heads of G can potentially generate torques in different directions. We placed a three-dimensional torque transducer at the ankle joint of unanesthetized decerebrate cats and employed peripheral nerve stimulation to evoke the flexion reflex, crossed-extension reflex (XER), and the sural nerve reflex (SNR). Muscle denervation and successive tenotomy isolated muscle groups of interest. XER and SNR both generated torques with large plantarflexion/toe-out components, but SNR generated torques with consistently higher proportions of toe-out compared to matched XER responses. These data are consistent with prior observations demonstrating selective recruitment of MG during SNR (Kanda et al., *Exp. Brain Res.* 29:57-74, 1977). We have already demonstrated that muscle nerve activation of MG produces larger toe-out torques compared with muscle nerve activations of LG and soleus (S). We conclude that there are interneuronal mechanisms subserving the control of three-dimensional ankle torque through differential activation of MG and LG. (Supported by NIH Grant NS20855).

646.17

COMPUTER SIMULATIONS OF FORCE-LENGTH-VELOCITY FUNCTIONS OF A HETEROGENEOUS POPULATION OF MOTOR UNITS. C.J. Heckman* and K. Paul, Physiology, Northwestern Univ. and VA, Lakeside Hospital, Chicago, IL 60611.

The Hill model of muscle is widely used for theoretical studies of limb movements. It assumes that the shapes of the muscle's force-length (FL) and force-velocity (FV) functions do not vary as a function of neural drive. To test whether the FL and FV functions of a heterogeneous population of motor units undergoing normal recruitment and rate modulation conform to this assumption, data on single motor unit FL and FV functions in the cat medial gastrocnemius (MG) muscle were coupled to realistic computer simulations of the steady-state recruitment and rate outputs of the MG motoneuron pool.

The simulation results showed that, at low to moderate input levels, the population FL and FV functions were both much steeper than predicted by the Hill model. The FL steepness was due to the net effects of rate-dependent shifts in optimal lengths of each of the single unit FL functions. The FV steepness occurred because the FV slopes of type S units were steeper than those of F units. These basic results were not sensitive to changes in the input organization to the motoneurons nor to nonlinear summation of motor unit forces.

In conclusion, neither the Hill model nor whole muscle analyses provide an accurate description of intrinsic muscle FL and FV functions. The simulations are being adapted to include steady-state reflex actions to study the contributions of intrinsic and reflex mechanisms underlying the equilibrium point hypothesis.

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646.18

DIRECTIONAL SENSITIVITY OF DSCT RESPONSES TO HINDLIMB MOVEMENT IN THE CAT. G. Bosco, R. Poppele* Dept of Physiology, University of Minnesota, Minneapolis MN 55455

A large polysynaptic convergence of afferent input onto DSCT cells suggests they may encode information about the whole limb rather than single joints or muscles. We tested this possibility by recording from DSCT units during passive movements of the hindlimb in anesthetized cats.

From a normal standing position, limb configuration (length and sagittal orientation) was manipulated by passively displacing the foot. Spontaneous discharge rates depended on a specific configuration in 70% of the cells tested. There was a maximum discharge rate associated with a single configuration corresponding to a specific foot placement with respect to the normal position. Such tuning was observed for all placement directions except those oriented forward and upward.

Transient discharge patterns were observed during displacements in 95% of the cells and 76% of them exhibited a tuning for a single direction which was either forward and downward 30 deg from horizontal or opposite. Only half of the cells exhibiting both static and dynamic tuning showed the same directional sensitivity for both.

The results illustrate the capability of DSCT cells to encode the configuration of the whole limb and direction of movements.

Supported by NIH Grant NS21143 and Human Frontiers Science Program.

CONTROL OF POSTURE AND MOVEMENT: ARM MOVEMENT II

647.1

MOVEMENTS TO APPARENT AND TRUE MOTION TARGETS. N.F. Port*, G. Pellizzer, and A.P. Georgopoulos. Brain Sciences Center, VAMC, Minneapolis, MN 55417; and The Graduate Program in Neuroscience, Univ. of Minnesota, Minneapolis, MN, 55455.

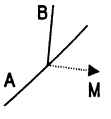
Apparent motion is a well known perceptual phenomenon in which visual objects appear to be moving when physically they are a series of still frames. Apparent and real motion were used to assess sensory-motor performance in intercepting targets. Normal human subjects were asked to intercept targets traveling in a square by moving a pointer from the center point to the midpoint of a target's path. In the true motion condition, subjects intercepted a target moving smoothly in a square around the center point. In the apparent motion condition, the target appeared only at the corner points of the square, but the subjects were required to intercept the midpoint of the "apparent moving" target. Target velocities were varied across blocks ranging from 1 deg/s to 9 deg/s.

Subjects successfully intercepted the target in both conditions. Interception of the apparent moving target was slightly but systematically worse than that of the real target. The error in interception increased in an approximately linear fashion with velocity for both types of motion. A similar increase was observed in the variance of the error. These results show that the motor system can successfully utilize information from apparent motion and that visual-motor performance is very similar under conditions of real or apparent motion. (Supported by 1-PSMH48185-01).

647.2

MOVING TO THE SYMMETRICAL DIRECTION FROM A VISUAL STIMULUS. G. Pellizzer[1]*, G. Léone[2], and A.P. Georgopoulos[1]. [1]Brain Sciences Center, VAMC, Minneapolis, MN 55417; Dept. of Physiology, Univ. of Minnesota, Minneapolis, MN 55455; and [2]Lab. de Physiologie Neurosensorielle, CNRS, 75270 Paris, France.

Bilateral symmetry was used to study perceptual mechanisms since the pioneer work of Mach nearly a century ago. Basically, the perception of bilateral symmetry is more salient when the axis of symmetry is vertical, less so when the axis is horizontal, and the least salient when the axis is oblique. We studied the processing of symmetry when the motor system is involved. For this purpose, normal human subjects were tested in a series of experiments in which the stimuli consisted of a red line (axis of symmetry) and a black line starting from the center of the red line and at an angle from it. The orientation of the axis and the angle of the black line were varied. Subjects were asked to make an arm movement (M) in the symmetric direction from the black line (B) relatively to the axis of symmetry (A). We measured the reaction time (RT) and the spatial accuracy. The perception of symmetry was tested in two additional psychophysical tasks. We found that movement RT increased with the angle of the black line. Moreover, the rate of increase varied with the orientation of the axis of symmetry, and was lowest when the axis was vertical. These results suggest that the intended movement direction is mentally rotated from the axis of symmetry toward the correct direction, and that the rate of rotation depends on the orientation of the axis of symmetry. Similar effects were obtained in the perceptual tasks. (Supported by NIH and HFSP).



647.3

ROLE OF PROPRIOCEPTIVE INPUT IN THE CONTROL OF INTERACTION TORQUES DURING MULTIJOINT ARM MOVEMENTS. R.L.Sainburg, M.F.Ghilardi, F.Ferraccl, H.Polzner, C.Ghez*. CMBN, Rutgers Univ., Newark, NJ, 07120; Ctr. for Neurobiol. & Behav., Columbia Univ. & NYS Psych. Inst., New York, NY 10032.

We have previously shown that deafferentation disrupts the temporal coordination of multijoint arm movements (Neurosci. Abs. 17:553). We now ask whether this disruption results from failure to control joint interaction torques that develop from motion of mechanically coupled limb segments. We studied horizontal movements in normal subjects and patients with large-fiber sensory neuropathy. Subjects were to trace a series of straight paths in different directions from a central starting position on a digitizing tablet. They were to make single overlapping outward and inward movements reversing direction without stopping. Elbow and shoulder angular displacement and EMGs of elbow flexors and extensors were recorded; hand paths and joint torques were calculated. Controls produced straight paths, with bell-shaped elbow and shoulder joint displacements which reversed direction simultaneously. The patterns of elbow muscle activity varied systematically with movement direction, acting to accelerate the limb or to counter the interaction torques produced by motion of the upper arm. The paths produced by deafferented patients were severely distorted; joint displacement profiles were assymetric and direction reversals at each joint were temporally decoupled.The distortions varied with the direction of movement and reflected the magnitudes of the interaction torques at each joint. Muscle activation patterns did not have a consistent temporal structure and were not matched to the differences in interaction torques. We conclude that proprioceptive input is critical to control the interaction torques which arise during multijoint movements. Supported by NS 227715, NS 25149, McKnight Fnd.

647.4

SELECTIVE EFFECTS OF MUSCIMOL MICROINJECTIONS INTO CEREBELLAR NUCLEI IN CATS PERFORMING BOTH A LOCOMOTOR AND A REACHING TASK. M.S. Milak*, V. Bracha, F. Kolb, J.D. McAlduff, J.R. Bloedel, Barrow Neurological Institute, Phoenix, AZ 85013.

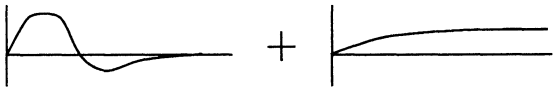
These experiments were designed to test the hypothesis that the individual cerebellar nuclei play different roles in regulating the performance of two different tasks. Each animal was trained to walk on a treadmill while avoiding a bar interjected into each swing phase and to perform a reaching task in which a manipulandum was moved through a grooved plexiglass template in a sequence of 2 straight movements. The effects of muscimol (400 ng) injected into individual cerebellar nuclei ipsilateral to the performing extremity were assessed during both tasks. The EMG was recorded from forelimb muscles, and the kinematics of the same extremity was assessed using an active LED system.

Microinjections in the dentate and fastigial nuclei affected the coordination of the locomotor cycle. Injections in the anterior interposed nucleus (AIN) resulted in an inability of the animal to perform a flexion adequate to avoid the bar, thereby forcing the animal to adapt a different strategy to avoid the perturbation. In the reaching task, dentate injections resulted in a distinct tremor before contact with the manipulandum and an apparent difficulty in smoothly directing the sequence of movements. Injections in the AIN caused marked ataxia before bar contact as well as changes in posture before the initial reach was performed. Even though movements were impaired following these injections, the movement sequences could still be performed and actually improved with practice. NIH Grant R01 NS21958.

647.5

MUSCLE ACTIVATION WAVEFORMS DURING ARM MOVEMENTS OF VARYING DISTANCE. C. Buneo*, J. F. Soechting, and M. Flanders. Dept. of Physiology, Univ. of Minnesota, Minneapolis, MN 55455.

We have previously shown that during arm movements of fixed distance and varying speed, the amplitude scaling of muscle activation waveforms can be described by a summation of two components: a phasic component that scales with speed (or movement time) and a tonic component that remains relatively constant. In the present study activation waveforms from 7-9 shoulder and /or elbow muscles were examined as human subjects made pointing movements of varying distance and speed to stationary targets aligned in the sagittal plane. All movements were made from a fixed initial position. Movement times ranged from 300-1500 ms.



Observation of the electromyographic (EMG) waveforms revealed that the amplitude of the tonic component was dependent upon the distance of the movement. The amplitude of the phasic component was related more to movement time than to the speed of movement: shorter movement times were associated with more phasic EMG waveforms.

647.6

CHARACTERISTICS OF ARM MOVEMENTS IN NORMAL SUBJECTS AND SUBJECTS WITH UNILATERAL BRAIN LESION

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The purpose of this study was to characterize the motor control of arm movement during a tapping task in normal subjects and subjects with unilateral brain lesion. A method for analysis was developed to characterize differences between the groups. Data were analyzed from five normal subjects and five subjects with unilateral brain lesion. Subjects were instructed to tap vertically on a target with a stylus. Six trials were videotaped and three trials were digitized at 60 Hz. Variables calculated from the digitized data of a marker on the stylus were: number of tap cycles; mean latent, event, and cycle periods; vertical displacement and velocity. An FFT was performed for each trial, and vertical velocity/position phase planes were generated. Analysis of normal data revealed consistent cycle, event, and latent periods within and across trials with the greatest variability in the latent periods, and for the non-dominant arm. Latent period doublings were more common in the non-dominant arm than the dominant arm, and observed more frequently within the first few cycles of the trial. Analysis of the stroke data revealed increased variability of all variables within and across trials, increased cycle periods, decreased mean velocity, a broader distribution of the frequencies in the power spectrum, and an increased range of vertical amplitude for the hemiparetic limb compared to the contralateral limb. These analyses appear to be sensitive indicators of motor control differences between limbs and for deficits in subjects with unilateral brain lesion.

647.7

KINEMATIC ANALYSIS OF PLANAR TWO-JOINT ARM MOVEMENTS IN HEMIPARETIC STROKE. R.F. Beer*, J.P.A. Dewald and W.Z. Rymer. Depts. of Biomedical Engineering and Physiology, Northwestern University, Chicago, IL 60611

There have been relatively few quantitative studies of the disturbed motor performance associated with hemiparetic stroke. Accordingly, we have conducted a preliminary investigation of the kinematics associated with performance of planar two-joint arm movements by a number of moderately impaired subjects.

Subjects were seated in a dental chair in front of a horizontal surface upon which thirteen targets were indicated - 12 located equidistantly along the circumference of a circle and one central marker which served as the beginning point for all movements. A set of straps were used to immobilize the torso and shoulder girdle while the wrist and finger joints were immobilized using a fiberglass cast. Subjects were instructed to move as fast as possible from the central starting point to a designated target without regard to movement accuracy. The OPTOTRAK/3010 motion analysis system was used to track the position of an IRED located distally on the hand. EMGs were recorded from the major flexors and extensors of the elbow and shoulder using surface electrodes. Two sets of experiments were conducted. In the first set, subjects were required to actively generate the anti-gravity torques necessary to maintain the arm in a horizontal plane. In a second set of experiments the protocol was repeated with the upper limb passively supported.

For unsupported movements, trajectories were most disturbed in directions involving shoulder flexion and elbow extension, and hence, increased gravitational torques at the shoulder. Angular velocity profiles were distinctly asymmetric, with a prolonged deceleration phase. Most striking was the positive effect provided by arm support. Trajectories became nearly linear, velocity profiles bell-shaped, with as much as a 300% increase in peak joint velocities. Our results suggest that moderately impaired subjects retained a significant residual capacity to plan and execute goal-directed movements. We are currently examining various hypotheses that may explain the radical deterioration in movements requiring stabilization against gravitational loads.

This work was supported by NS 19331 to WZR.

647.8

THE CONTRIBUTION OF VISUAL AND EYE MOTION SIGNALS IN DIRECTING THE ARM TO MOVING TARGETS. P. van Donkelaar, R.G. Lee* and R.S. Gellman. Dept. of Clinical Neurosciences, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

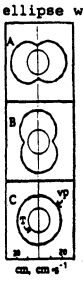
Normal human subjects were required to track a moving target with their extended index finger either (i) with full vision of the arm and unrestricted movement of the eyes, (ii) without vision of the arm, or (iii) while fixating a stationary target. The subject initially pointed at the target when it appeared at its starting position. After a variable delay the target started moving to the right and the subject was required to catch up to and follow it as accurately as possible. In each condition target velocity was varied randomly from trial to trial. Under normal conditions subjects were able to catch up to the target and match its velocity. Without vision of the arm subjects did not catch up to the target, but did match target velocity. However, when the arm was visible for the first 100-400 ms of the response the accuracy increased.

When subjects were required to fixate they caught up to the target, but then produced a steady-state hand velocity which was greater than target velocity. This is consistent with studies which demonstrate that target velocity is perceived as greater during fixation. Together these results suggest that extraretinal signals concerned with eye motion are needed to produce an appropriate hand velocity. However, one needs to see the position of the limb relative to the target to produce an appropriate hand position.

647.9

VELOCITY OF MOTION INFLUENCES THE PERCEPTION OF HAND TRAJECTORIES IN THE ABSENCE OF VISION. A.H.Fagg[1], S.I.Helms Tillery[2] and C.A.Terzuolo[2]*. [1] Center Neural Eng. Dept. Comp. Sci., Univ. Southern California, Los Angeles 90089 and [2]Dept. of Physiology, Univ. Minnesota, Minneapolis 55455

During naturally executed movements the radius of curvature of the trajectory (T) and the tangential velocity are related by a power law ($V_{(t)} = kR_{(t)}^{1/3}$). Thus one can ask if the recognition of curvilinear Ts can be altered by imposing different velocity profiles (vps). A robot arm was used to transport repetitively the hand along a circular T which engaged primarily shoulder and elbow joints. Three vps were used corresponding to a circle (C) or an ellipse with either an horizontal (B) or vertical (A) major axis. Data from 16 subjects show that vp significantly affects the identification of T as a circle ($F(2.45) = 22.1$, $p < .001$), a horizontal ellipse ($F(2.45) = 38.59$, $p < .001$) or a vertical ellipse ($F(2.45) = 118.19$, $p < .001$). In addition, perception of tilted ellipses was frequent for conditions A and B. This illusion, which during passive movements depends exclusively on kinesthetic inputs, is taken to suggest that $V_{(t)}$ contributes to the perception of curvilinear Ts also during self-initiated movements.



Supported by a Human Frontier Science Program Grant.

647.10

STRATEGY SELECTION TRIGGERS MOVEMENT SCALING. V. B. Brooks*[1], F. Hilperath[2], H.-G. Ross[3], M. Brooks[4] and H.-J. Freund[2]. Dept. of Physiology[1], Univ. of Western Ontario, London N6A 5C1, Canada. Dept. of Neurology[2]; Physiology[3], Heinrich Heine Univ. Düsseldorf, FRG. Inst. for Information Technology[4], National Res. Council of Canada, Ottawa K1A 0R6.

A novel paradigm for human subjects learning a task using goal-directed hand movements showed that a strategy: *what* to do, was learned first (phase 1); and then tactics: *how* to make it successful (phase 2). Phase 1 included cognition of the correct strategy: the movement pattern; and phase 2 consisted of calibration of strategy use by scaling movement amplitudes and timing. Some subjects could declare *that* they had found the right strategy after one or only a few trials, before they had achieved successful scaling.

The paradigm required subjects to guide a display cursor from a start-box to a target-box by moving a pen on a digitizing board. Hand positions modulated cursor velocities ('rate control'). Thus correct strategy was to start and stop the cursor with two oppositely directed hand movements; and correct tactics required their amplitudes to be close enough to each other to stop the cursor in target, within the time set by the paradigm. Sessions of 200 trials took 25 min.

Phase 1 began with naivety and ended with strategy selection [measured as task performance with no more than 2 consecutive non-strategy trials], attained by different subjects in up to 111 trials. End of phase 1 triggered phase 2, the highly visible movement rescaling, that ended with stable ratios of stop and start move amplitudes [ratios sufficiently close to 1 in 9 consecutive trials]. Rescaling (phase 2) was completed by seven subjects using the correct strategy in 7 to14 trials (in a total of 7 - 25 trials); and by three subjects with the strategy in 34 to 56 trials (in 44 - 61 total). The number of trials used to attain stable amplitudes for the correct pattern strategy is in the range of trials reported for adaptive scaling attributed to cerebellar function.

This simple kind of test with easy read-out could be used for recording and imaging brain activity during visuomotor learning.

647.11

COORDINATION OF SINGLE AND DOUBLE JOINT MUSCLES OF THE ELBOW. L.E. Sergio* and D.J. Ostry. McGill University, Montreal, PQ, Canada H3A 1B1

In previous work with A.G. Feldman and J.R. Flanagan we have suggested that central commands specify the equilibrium point in multi-joint movements by controlling many muscles in concert. In particular, for both arm and jaw movements we have proposed central commands which control motion in different degrees of freedom as well as the level of coactivation without motion. The basic problem is that since muscles do not - in general - act in individual degrees of freedom central commands must be coordinated to produce motions in separate degrees of freedom. In this paper we report an empirical study of the coordination of central control signals necessary to produce movements about the elbow involving flexion alone, supination alone, and combinations of the two. The work was carried out by examining the kinematic and electromyographic patterns associated with 3D arm movements. EMG was recorded from eight single and double joint elbow muscles as subjects made pronation / supination and flexion / extension movements of different magnitudes. Shoulder elevation and orientation were also varied. Kinematic patterns were recorded using WATSMART. The data presentation focuses on the relationships between kinematics and EMG activity associated with motion in more than one degree of freedom.

647.12

CONTROL STRATEGIES FOR AIMED ARM MOVEMENTS IN 3-DIMENSIONAL SPACE O.Bock and K.Arnold (SPON: European Neuroscience Assoc.). Human Perf. Lab, Inst. f. Space&Terrestrial Sci., York University, Downsview, Ont. M3J 1P3, CDN

When humans point without seeing their arm, successive errors tend to accummulate, which suggests that pointing is amplitude- rather than position- controlled[1]. We now compare trends for error accummulation and for error correction for movements with varying directions.

Six humans pointed, without seeing their arm, at mirror-viewed targets. The targets appeared sequentially in a frontal plane and required direction changes between successive movements of 0, 45, 90, 135 or 180 deg. Pointing accuracy was registered by the Watsmart® system, and we calculated the linear regression between successive pointing errors. Positive correlation R indicated error accummulation; the regression slope S indicated the relative roles of accumulation (S=1) and correction (S=0).

We found that R>0 throughout the sequences (mean: 0.53, $p<0.01$), and that R was significantly smaller for movements preceded by a direction change of more than 45 deg (0.37 versus 0.71, $p<0.01$). We also found that S>0 (mean: 0.60, $p<0.01$) and that S depended on direction changes in much the same way as R (0.42 versus 0.75, $p<0.01$)

We conclude that error accummulation persists, albeit reduced, across changes of movement direction, and that a complementary trend for error correction exists. Correction is probably due to proprioceptive feedback since it is absent in deafferented subjects[2].

[1] Bock&Eckmiller, Exp Brain Res 62 (1986); Bock et al., Behav Brain Res 40 (1990); [2]Larue et al., Abstr 3d IBRO World Congr (1991) P39.18

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647.13

TIMING AND AMPLITUDE CONTROL OF REACHING AND GRASPING. P. Cordo* and M. Schieppati. Institute of Human Physiology II, University of Milan, Italy

Reaching and grasping is a multijoint movement involving a sequence of actions that include transporting the arm, orienting the hand, and opening and closing the fingers. This movement sequence is controlled by many of the muscles of the shoulder, arm and hand. The purpose of the experiment reported here was to determine what principles underlie the coordination of activity in these muscles.

Normal human subjects, seated at a table, reached for an L-shaped handle located at 1 of 3 heights, with 1 of 3 orientations with respect to the vertical plane. Subjects grasped the handle with one of these 9 combinations of height and hand orientation in contiguous blocks of 10 repetitions. The movement time to the handle was constrained by playing a series of tones through headphones. We recorded the activity of up to 7 muscles with surface and wire electrodes: upper trapezius, anterior deltoid, biceps brachii, pronator teres, supinator, flexor digitorum communis, and extensor digitorum superficialis.

We made the following observations with these reaching and grasping movements: 1) different muscles are involved in the control of reaching height, hand orientation, and hand opening and closing, but some muscles can control more than one of these kinematic variables, 2) the control of a given variable can be via the *onset timing* of activity or via the *amplitude* of activity, and 3) changes in the level of muscle activity occur at discrete, periodic times during the movement.

647.14

FAST GOAL-DIRECTED ARM MOVEMENTS CAN BE PERFORMED WITHOUT THE PERFORMER'S KNOWLEDGE. A.C. Sittig and J.J. Denier van der Gon (SPON: European Neuroscience Association). University of Utrecht, Princetonplein 5, NL-3584 CC Utrecht, The Netherlands.

Muscle tendon vibration is known to excite muscle spindles, the primary source of information for the perception of limb position in the absence of vision. Vibration of the biceps of a stationary or slowly moving arm causes the perception of elbow position to be biased towards extension. During slow tracking tasks undue flexion of the elbow results. Contrarily, in fast step-tracking tasks correct positioning is achieved despite vibration, even if the arm was unduly flexed before the step as a result of matching arm and initial target position. We now asked if we could make subjects perform fast goal-directed movements when they thought their arm was already at the target position. Four subjects tracked slowly moving targets. A mirror covered the arm. The image of a light above the mirror formed the target such that the hand and the target moved in the same plane below the mirror. Biceps vibration induced undue flexion. At times subjects were asked to grab the target with their hand. All subjects then performed fast and often substantial elbow extension movements reaching the target and, when asked about this, claimed they had not made any such fast movements. Electromyograms recorded from biceps and triceps show that the muscles were activated as if the subject were performing a voluntary fast movement. We conclude that the programming of fast movements does not depend on the conscious decision to perform such a movement, and that the performance of a fast movement in itself does not give rise to the perception of movement.

647.15

A COMPUTATIONAL MODEL FOR OPTIMAL PLANNING AND CONTROL OF LIMB MOVEMENTS. N. Lan* and P. E. Crago. Applied Neural Control Lab, Dept. of Biomedical Engineering, Case Western Reserve Univ., Cleveland, OH 44106, and Center for Biomedical Engineering, University of Kentucky, Lexington, KY 40506.

In this abstract we present a computational model for generating limb movement trajectory, muscle control signals, and the associated joint stiffness and equilibrium states. The model includes a central planning algorithm derived from a minimum effort criterion, a spinal integration circuit involving reciprocal inhibition, as well as the musculoskeletal dynamics. The central nervous system (CNS) initiates the movement by issuing commands representing movement direction, amplitude and the height of an excitation pulse. Movement planning and control are eventually described as a dynamic optimization problem, minimizing the centrally perceived effort functional, and are solved by a numerical paradigm of nonlinear optimization.

Simulation results indicate that a movement may be performed with excitation pulses of a range of heights. The gain of reciprocal inhibition appears to modulate mainly the extent of reciprocal control of the antagonists, and has relatively less influence to movement kinematics, as compared with that of the pulse height. The stereotyped tri-phasic muscle control signals are shown to be characteristic of the minimum effort criterion. The joint stiffness increases during the movement as a consequence of the tri-phasic muscle control pattern. The equilibrium trajectory displays an "N" shaped alternating profile about the movement trajectory. It is shown that when the velocity profiles of movements are scaled with each other, invariant kinematic property emerges, and the height of the excitation pulses is well correlated to the speed of movements, suggesting a speed control strategy by pulse height modulation. The model is able to reconcile many lines of experimental findings about single joint voluntary movements. It can also be extended to multi-joint movements.

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648.1

MIDLINE PLANE ATTRACTION IN THE LOCOMOTION OF NORMAL AND DOPAMINE STIMULANT TREATED RATS. I. Golani, H. Einat and P. Teitelbaum*. Dept. of Zoology, Tel Aviv University, Ramat Aviv, Israel and Dept. of Psychology, University of Florida, Gainesville, Florida, U.S.A.

In our work we search for "natural" frames of reference and collective variables, presumably used by the brain in the coordination of movement. The rat's midline plane, which divides body-related space into two symmetrical hemispheres, was used as a reference plane for the scoring of five measures of locomotor behavior. A comparison of the behavior of rats with saline and three dopamine stimulants revealed three distinct locomotor profiles which differed in the strength of attraction of the anterior body parts to the midline plane. With saline and apomorphine rats showed a similar rather strong attraction of the torso and the head to this plane. Quinpirole greatly enhanced and amphetamine first enhanced and then greatly reduced this attraction. This suggests that the midline plane is used by rats as a reference plane in the coordination of trunk movements. The head's angular displacement from this plane could be a collective variable used in the coordination of locomotor behavior.

648.2

RECIPROCAL INHIBITION (RI) DURING VOLUNTARY DORSIFLEXION OF THE ANKLE: A LINEAR RELATIONSHIP. L.E. TREMBLAY* OTTAWA UNIV. CANADA J.L. PÉPIN AND P.J. DELWAIDE AND LIEGE UNIVIERSITY, LIEGE 4000, BELGIUM.

RI is believed to play a crucial functional role in motor control with spinal inhibitory interneurons (IN 1a) being clearly implicated. Tanaka (1974) found that RI was scarcely detectable in normal subjects at rest. He has shown that the 1a inhibitory pathways to the triceps surale muscle become active during voluntary dorsiflexion of the ankle. The purpose of this study was to quantify the effect of voluntary isometric contraction in dorsiflexion on the H-soleus reflex. Twenty nine normal subjects (30 $\pm$ 8 years) were studied. Hoffman's H-reflexes in the soleus (S) was used to assess the changes in (RI) evaluated by electrical stimulation of the antagonist muscle (tibialis anterior) (TA) via the common peroneal nerve (CPN) using five condition levels; at rest, slight contraction, 1kg, 2kg and 4kg resistance in dorsiflexion. Subjects were given auditory and visual feedback of the EMG to help maintain a steady voluntary contraction of the TA muscle. The electrogoniometer was used to ensure the isometric condition. Stimulation of the CPN demonstrated a 9.2 $\pm$ 7% ($P < 0.01$) augmentation in RI in (S) at rest and 26.6 $\pm$ 17%, 46 $\pm$ 16%, 61% $\pm$ 10% and 58 $\pm$ 12.5% respectively for slight contraction, 1, 2, 4kg respectively. The relationship is linear until 4kg. The regression curve is $y = 1.2x + 11.96$ ($r=.94$). A voluntary contraction of the TA muscle is one the best methods of RI reinforcement. The possible implications of Renshaw cell discharge and presynaptic inhibition will be discusssed.

648.3

EFFECTS OF ANKLE EXTENSOR CONTRACTIONS ON CAT LUMBAR MOTONEURONS . L. Jami*, D. Zytnicki, J. Lafleur and G. Horcholle-Bossavit CNRS URA 1448, Collège de France, Paris, France.

The effects of muscle afferent inputs generated by contractions of gastrocnemius medialis (GM) were recorded in ipsilateral lumbar motoneurons of chloralose-anaesthetized cats. Contractions were obtained by stimulating a cut branch of the muscle nerve.

In homonymous and synergic motoneurons, GM contraction evoked a quickly declining Ib inhibition in spite of a persistent Ib input (1). Recordings of contraction-induced PAD in Ib terminals suggested that pre-synaptic inhibition of Ib effects accounts for the decline of autogenetic Ib inhibition (2).

Declining inhibitions elicited by GM contractions were also observed in various other species of motoneurons. On repetitive electrical stimulation of GM nerve, the strength required to elicit declining inhibitions similar to those evoked by GM contraction in non-synergic motoneurons was 5-8 times group I threshold, recruiting group II in addition to group I fibers. These observations suggest a significant contribution of group II afferents to the effects elicited by GM contractions in non-synergic lumbar motoneurons. The mechanism causing the decline of the contraction-induced effects might involve pre-synaptic inhibition of group II fibers.

In addition to tendon organs and spindle primaries, group II muscle afferents also contribute information about ankle extensor contractions, in the form of a negative feedback distributed to a variety of lumbar motoneurons and quickly filtered out, leaving motor units available for recruitment as may be required by motor coordination.

(1) Zytnicki et al. (1990) J. Neurophysiol. 64:1380-1389
(2) Lafleur et al. (1992) J. Physiol. Lond. 445 : 345-354

648.4

OSCILLATIONS OF EMG BURST AMPLITUDE IN NORMAL AND SPINAL CATS DURING TREADMILL LOCOMOTION. J. A. Hodgson, R. R. Roy, C. P. de Guzman, R. de Leon, A. Garfinkel* and V. R. Edgerton. Brain Research Institute and Department of Physiological Science, UCLA, Los Angeles, CA 90024.

Recordings of EMG activity from locomoting spinal cats in our laboratory have indicated exaggerated levels of oscillations in amplitude of EMG bursts (Lovely et al. Brain Res 514:206, 1990). In most cases, the bursts were divided into short 'packets' of activity separated by periods of silence. This contrasts with examples of EMG bursts in spinal cats from other laboratories where such clear oscillations were not apparent (Barbeau and Rossignol Brain Res 412:84, 1987; Forssberg et al. Brain Res 50:184, 1973). One significant difference in our treatment of the animals was that they were left to recover for one month before locomotor training was initiated. In more recent experiments, we have initiated training one week after spinalization and found much less oscillation in the amplitude of EMG bursts following spinalization. This observation suggests that the timing of therapeutic interventions in the treatment of spinal injuries may affect the outcome of those therapies. We have also analyzed the frequencies of EMG oscillations in control and spinal cats and in two instances compared tremor before and after spinalization in the same animal. The frequency of EMG oscillation in all spinal cats was approximately 25 Hz, compared to 50 Hz in controls. Similar results were found in the two cats studied before and after spinalization. Together these data suggest that descending pathways influence the spinal circuitry involved in generating tremor. (Supported by NIH Grant NS16333)

648.5

GAIT ASSISTANCE WITH FES CONTROLLED BY THE CUTANEOUS ENG. K. Kallesøe[1], M. Haugland[2] and J.A. Hoffer[1]*. 1) School of Kinesiology, Simon Fraser University, Burnaby, B.C. V5A 1S6, Canada and 2) Department of Medical Informatics and Image Analysis, Aalborg University, Aalborg, Denmark.

The activity of skin mechanoceptors recorded by cuff electrodes implanted on sensory nerves can provide useful information on the forces applied on the skin. Last year we reported an application in an anesthetized animal model, in which the cutaneous electroneurogram (ENG) was used for closed-loop control of functional electrical stimulation (FES) of paralyzed muscles in order to restore a precision grip reflex (Hoffer & Haugland, SN Abstr. 17:1031). We now present an application in the conscious, walking animal, in which the cutaneous ENG was used to control FES in order to assist gait.

The cat hindlimb served as a model to test this application. A tripolar nerve cuff electrode (30 mm long, 2.5 mm ID) was implanted on the tibial nerve near the ankle, in order to record activity generated largely by footpad mechanoceptor afferents. Bipolar stimulating electrodes were implanted in each head of triceps and plantaris. For each muscle, a recruitment curve was determined under anesthesia by stimulating at 25 Hz with constant current pulses of variable duration ($\leq$255 µs). Pulse amplitude was set to the minimum value needed to obtain maximal force using 255 µs pulses.

The tibial ENG recorded during gait included a sharp burst at footfall and modulated activity during stance. A "ground contact" signal was derived from the rectified, filtered ENG and served as control signal for a 4-channel stimulator that activated each muscle at 25 Hz (100 Hz total) as long as the foot was in contact with the ground. A reliable control signal was obtained from the tibial ENG during walking, even in the presence of FES of muscles that surrounded the nerve cuff. An interleaved sampling method was used to reject simulation artifacts that contaminated the nerve signal. At low levels of stimulation (<30% of maximal), FES did not interfere with the cat's normal gait. At 30-40% of maximal, the cat occasionally shook the stimulated limb during the swing phase. At 40-75% of maximal stimulation, the paw shakes became more vigorous and the ENG signal during stance was more irregular. This caused discontinuities in the stimulation, but the cat continued to walk regularly, with no sign of pain or discomfort.

Since this approach is feasible in both anesthetized and normally active animals, it promises to be clinically applicable to paraplegic as well as to hemiplegic humans.

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648.6

STRYCHNINE- INDUCED MODULATION OF EMG OF HINDLIMB FLEXORS AND EXTENSORS DURING STEPPING IN CHRONIC ADULT SPINAL CATS. C. P. de Guzman, J. A. Hodgson, R. D. de Leon, R. R. Roy and V. R. Edgerton*. Department of Physiological Science and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Strychnine initiated full weight-bearing stepping in spinalized cats that were unable to walk and resulted in a more robust stepping pattern in those that were trained to walk (de Guzman et al. Soc Neurosci Abstr 17:1577, 1991). EMG activity of hindlimb flexors (semitendinosus, iliopsoas, tibialis anterior) and extensors (vastus lateralis, soleus-Sol, medial gastrocnemius) were examined in 4 adult spinalized cats (T12-T13) before and after the administration of 2 doses of strychnine (0.03 and 0.10 mg/kg, i.p.) to determine if the improvements in locomotion were a result of a direct effect of strychnine on motor pools or due to changes in other spinal mechanisms. Two cats were trained to walk on the treadmill at varying speeds (0.2-1.0 m/s) and 2 were trained to stand. Training (30 min/day, 5 days/week) began 1 week after spinalization. At 3 months post-spinalization the treadmill-trained cats walked at all speeds. Standing-trained cats rarely exhibited coordinated cyclic activity and were unable to walk at any speed throughout the entire training period. Averaged EMG waveforms for each muscle were generated from 5-12 steps. In treadmill-trained cats strychnine had only a minor influence on the EMG patterns observed after spinalization. Burst durations were similar before and after strychnine administration. Mean amplitudes in Sol increased slightly after strychnine and was dose-dependent. The standing-trained cats were able to walk after strychnine administration and higher doses increased the Sol mean amplitude in one of two cats. These data indicate a selective effect of strychnine on the amplitude of specific muscles and a maintenance of the EMG patterns in both flexors and extensors after spinalization suggesting that the major influence of strychnine is on premotoneuronal networks that generate cyclic activity in the spinal cord rather than a direct effect on motoneuronal pools. (Supported by NIH Grant NS16333)

648.7

EFFECTS OF SPINALIZATION ON EMG WAVEFORMS IN CAT FLEXOR AND EXTENSOR MUSCLES. R. D. de Leon, C. P. de Guzman, J. A. Hodgson, R. R. Roy, B. Jiang* and V. R. Edgerton. Department of Physiological Science and Brain Research Institute, UCLA, L.A., CA 90024.

Cats spinalized as adults can regain effective full weight-supported stepping of their hindlimbs when properly trained over a period of weeks to step on a treadmill (Lovely et al. Brain Res 514:206, 1990). Although it is known that the stepping pattern in these spinalized cats can accommodate varying speeds, loads and other sensory perturbations, details of the EMG during stepping that reflect supraspinal compared to spinal control has not been defined. In the present study, EMG from selected flexors and extensors in 4 adult cats were recorded during bipedal stepping before and 1 or 3 months following spinalization (T12-T13). Two spinal cats were trained to walk on a treadmill at speeds ranging from 0.2 to 1.0 m/sec and 2 cats were trained to stand. Each cat was trained for 30 min/day, 5 days/week. Training began 1 week after spinalization, at which time none of the cats was able to execute weight-supported stepping. The 2 cats trained to walk were able to step with full weight support after 3 weeks of training. The standing-trained cats were unable to walk throughout the entire training period. Following an acute administration of strychnine (0.03 mg/kg) 3 months after spinalization, however, these 2 cats could generate full weight-supported stepping (see accompanying poster, de Guzman et al.). Amplitudes within each burst of EMG activity were averaged over 5-15 consecutive step cycles. Double bursts per cycle were observed consistently in the semitendinosus and iliopsoas before spinalization while one of these bursts became much less prominent following spinalization. Packets of EMG bursts were observed frequently in the flexors, particularly in the tibialis anterior, after, but not before, spinalization. Generally, the EMG waveforms of extensors (vastus lateralis, soleus, medial gastrocnemius and gluteus medius) were similar pre- and postspinalization. These data suggest that the neural networks of the lumbar spinal cord that control the extensors can compensate more completely for the absence of supraspinal control or that in the absence of compensation, the flexor motor pools are more dependent on supraspinal control than extensors. (Supported by NIH Grant NS16333)

648.8

GENERATING HUMAN LOCOMOTOR ACTIVITY PATTERNS USING AN ARTIFICIAL NEURAL NETWORK MODEL.
S.D.Prentice* and A.E.Patla. Dept. of Kinesiology, Univ. of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

A neural network model was used to map out a relationship between desired locomotor trajectories and the necessary muscle activations. The proposed network incorporates a fully connected feed-forward model receiving twelve inputs, ten hidden units and four output units. The inputs include the vertical and horizontal displacement trajectories for the hip and toe, the hip and knee angles, and the time derivatives of these six inputs. The output consists of the muscle activation time histories of the major extensors and flexors of the lower limb - soleus, tibialis anterior, biceps femoris and rectus femoris. The bias values and connection weights of the network were learned using the back-propagation rule, using the NeuralWare software. A robust model must be able to control walking over uneven terrains. Therefore, data from subjects walking over obstacles of different heights were used to train and test the network. The test data set was presented at specific intervals during training to monitor the model's ability to generalize to novel data. The muscle activation patterns produced by the resulting model upon presentation of the training data closely matched those obtained experimentally. Presentation of the novel data also closely resembled the actual activation time histories. The performance of the model was quantified by calculating the correlation and root mean squared error between the actual and predicted curves. All muscles of the training data had correlations greater than 0.9 and RMS errors less than 0.1. Most of the muscles in the test data values had correlations greater than 0.8 and RMS errors less than 0.3.

648.9

DIFFERENTIAL EFFECTS IN PATHWAYS INTERCONNECTING KNEE AND ANKLE EXTENSORS IN MAN. R. Pelletier, R. Forget, D. Bourbonnais*. Centre de Recherche, Institut de Réadaptation de Montréal et l'Ecole de réadaptation, Faculté de Médecine, Université de Montréal.

To study the specificity in spinal pathways interconnecting heteronymous muscles a conditionning H-reflex technique was used to investigate, at rest, the pathways interposed between the soleus (SOL) and the vasto-crureus (VC) muscles. Fourteen normal human subjects (x=27 ± 1.37 years) participated in the study. Two experimental conditions were investigated: (1) conditioning stimulation applied to the femoral nerve (FN) and a test stimulation applied to the the posterior tibial nerve (PTN), and (2) conditionning stimulation applied to the PTN and a test stimulation applied to the FN. Under both experimental conditions the intensity of stimulation was adjusted so that conditionning stim. was at motor threshold and the test stim. at Hmax/2. The results for both experimental conditions was an initial short lasting facilitation (≈ 5 ms) followed by a relatively long lasting (≈ 50 ms) inhibition. However this inhibition was much stronger in the FN-sol experimental condition. In this situation (FN-SOL) the Conditionning-Test intervals (C-T int) had a significant effect on the conditionned H-reflex values (p=.0001) and these conditionned values were found to be significantly different from their control values (p=.0005). In the second experimental condition (PTN-VC), although the C-T int had an effect on the conditionned values (p=.004) no difference between the conditionned and control values were demonstrated (p=.8456). Different conditionning stimulation intensities of the FN were investigated at the C-T int where the strongest inhibition of the soleus muscle was present. The inhibition appeared with a stimulation strength of 1.0 x Reflex Threshold and increased in concordance with the amplitude of the H-reflex response in the conditionned muscle. Spinal mechanisms regulating inhibition between extensor muscles may facilitate the task of supraspinal structures in the regulation of heteronymous muscles important to maintain an erect posture in stance and gait. (R. Forget and D. Bourbonnais are funded by the FRSQ)

648.10

THE EFFECT OF EARLY LOCOMOTOR TRAINING WITH CLONIDINE ON THE RECOVERY OF LOCOMOTION IN ADULT SPINAL CATS.
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Our previous studies have shown that, after spinal transection at T13 in cats, locomotor recovery with plantar foot placement and weight support of the hindquarters takes between 2 to 3 weeks. We have also shown that injection of noradrenergic drugs (clonidine and L-Dopa) together with perineal stimulation can produce, in the first week post-transection, a coordinated locomotor pattern on the treadmill for several hours. We have taken advantage of this possibility to train cats on a treadmill after daily injection of clonidine (150 to 225 ug/kg i.p.) in the first post-transection week and study the effects of training (60-90 mins/day) on the recovery of spontaneous locomotion. Electromyographic (EMG) activity synchronized to video images of the hindlimbs were recorded before and after clonidine injection. From the 3rd to the 9th day post-transection, there was a gradual increase in the duration of the step cycle for the same treadmill speed accompanied by a corresponding gradual increase in the duration of extensor EMG activity and a gradual decrease in the duration of flexor muscles activity. The increase in total angular excursion of the hip, knee and ankle joints was also evident. Concomitant changes in kinematics included a brisk transition from swing to stance, hyperextension of the ankle during late stance, and synchronous flexion of hip, knee, and ankle during early swing. It is noticeable that, from the 3rd to the 7th day, the effect of clonidine given on one day was not carried over the next day i.e. the spontaneous locomotion before clonidine injection was not improved. The 3 cats were able to elicit a locomotor pattern without clonidine injection by the 8th, the 9th and the 11th day respectively. These observations suggest that early locomotor training with clonidine accelerates the recovery of locomotion after spinalization. (Supported by the NCE, the MRC and the FCAR).

648.11

EFFECTS OF MOVEMENT INITIATION CONDITIONS ON POSTURAL AND TASK EMG ACTIVITY DURING INITIATION OF RAPID SHOULDER FLEXION. L.D. Abraham, D. Kalakanis, and A.M. Baylor*. Kinesiology & Health Education and Institute for Neuroscience, University of Texas, Austin, TX 78712.

In order to study in detail the relationship between patterns of postural and voluntary muscle activation, kinematic, dynamic, and electromyographic data were collected from normal adult male subjects performing bilateral rapid shoulder flexions while freely standing. Experimental conditions varied temporal constraints on initiation by including self-paced (SP), reaction-time (RT), and coincidence-anticipation (CA) paradigms. Data were collected with a two-camera video system, a force plate, and twelve channels of surface EMG. The data were examined to identify variations in performance which could suggest the extent to which postural and task muscle activation patterns played a fixed role in execution of the task. We report here data from subjects who displayed consistent arm movement kinematics in all three conditions. RT data were clearly different from SP and CA data. Ankle muscles were less involved in movement initiation in the RT condition, evidenced by later and less consistent bursts of activity. Activation patterns of other muscles were also different in RT vs SP and CA conditions, though in subject-specific ways. These data suggest that patterns of anticipatory postural activity for similar arm movements may be highly sensitive to temporal constraints on movement initiation.

648.12

INFANT STEPPING: A LONGITUDINAL STUDY OF LOCOMOTOR DEVELOPMENT. **M.C. O'Sullivan*, E.L. Leonard, B.G. Farley and J.R. Bloedel. Dept. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.**

Studies were undertaken in 10 normal infants at ages 12, 18 and 24 weeks to investigate the characteristics of the early changes in the kinematics of stepping prior to the development of independent locomotion. Infants were supported over a treadmill moving at 0.06m/s and light emitting diodes were placed over critical points on the lower limb. Three classes of lower limb movements were identified: alternate steps, hops and jumps. The active phase of alternate steps was analyzed. Dependent variables included percentage of alternate steps, step duration, height, length, angular displacements of hip, knee and ankle, limb velocity and acceleration. With age, significantly increasing values were obtained for step length, step height, time of maximum ankle flexion and maximum velocity of the leg during gait with smoothing of the velocity profile. These data suggest that in preambulatory infants between 3 and 6 months of age, stepping acquires characteristics which contribute to the development of independent bipedal locomotion. **(Supported by Az Dis Res Cont Com and NIH RO1NS-21958).**

648.13

THE ROLE OF SOMATOSENSORY INFORMATION IN A CONSTRAINED LOCOMOTOR TASK. S.M. Henry, J.M. Held,* B.P. Vietje and J. Wells. Dept. of Anatomy and Neurobiology, Univ. of Vermont College of Medicine, Burlington, VT 05445.

Sensory information from the body is distributed and processed by different central nervous system structures which are involved in both sensory perception as well as motor activity. The present study examined the role of the dorsal column nuclei and the ventral posterolateral (VPL) nucleus of the thalamus in a constrained locomotor task. Rats were trained to traverse an elevated 1" bar for a reward. The time it took to run across the bar was used as a measure of goal directed behavior. The trajectory of the hindlimb during the swing cycle was quantified from videotape. The lesion groups were 1) right gracile nucleus, 2) bilateral gracile nucleus, 3) right gracile nucleus and left VPL and 4) bilateral VPL. The animals were tested on the bar running task for 50 days post-lesion and filmed periodically. Two measures of loss and recovery of function were used: 1) the running time and 2) the movement topology of the hindlimb swing cycle. Only the bilateral VPL lesioned animals were significantly impaired in run times during week 1 post-lesion. However, all groups demonstrated an impairment in movement topology even on the day they returned to their pre-lesion run times. This impairment in movement did not recover. This result is similar to sensorimotor cortex lesions and suggests that central processing of tactile information is important in constrained locomotion.

648.14

UNIQUE EMG PATTERNS FOR FDL AND EDL DURING FORWARD AND BACKWARD WALKING. T.V. Trank* and J.L. Smith, Dept. of Physiological Science, UCLA, Los Angeles, CA, 90024-1568.

We have previously described kinematics and related EMG of the hip, knee, and ankle joints during forward (FWD) and backward (BWD) treadmill walking. However, the action and muscle activity at the toe (metatarsophalangeal joint) was not addressed. Our methods for assessing EMG and kinematic data in the cat are published (Buford and Smith, *J Neurophysiol* 64:756-766, 1990).

The flexor hallicus longus (FHL) exhibited activity typical of an extensor muscle in both forms of walking, being active prior to paw contact (PC) and remaining active through 75% of stance. The extensor digitorium longus (EDL) tended to have a biphasic burst during swing. In FWD walking, the first burst was related to the F-E1 toe-joint reversal that occurred 30 ms after paw off (PO). The second EDL burst, coactive with the onset of FHL, was associated with deceleration of toe extension in preparation for PC. Overall, EDL activity appeared to control toe extension throughout swing. During BWD walking, the EDL also exhibited biphasic activity. However, the onset of the first burst occurred before PO and coincided with slight toe extension as the paw prepared for lift off. The second EDL burst centered around PC and was related to the F-E1 reversal at the end of swing. In FWD walking, the flexor digitorium longus (FDL) had a brief burst (15-45 ms) that occurred prior to PO; this burst preceded the onset of rapid toe flexion that ended 30 ms after PO. 'Facultative' activity during stance was evident in both forms of walking. In BWD walking both the swing burst and its associated toe flexion were absent. Instead, a brief burst unrelated to 'facultative' activity was occasionally recorded just after PC coincident with FHL activity.

Like previous muscles studied (Buford and Smith 1990), FHL and EDL maintained their basic flexor or extensor synergies. But for the first time, we have described a muscle that does not. Under fictive preparations, the CPG programs a short FDL burst coincident with the flexor synergy (Fleshman et al. 1984); this burst does not occur during backward walking and may be suppressed by supraspinal input or sensory feedback associated with BWD walking. Supported by NIH NS 19864.

648.15

MUTABLE ACTIVATION OF BIFUNCTIONAL THIGH MUSCLES DURING FORWARD AND BACKWARD WALKING. C.A. Pratt*, J.A. Buford and J.L. Smith. Dept. of Physiological Science, UCLA, Los Angeles, CA 90024-1586.

In contrast to unifunctional muscles, the bifunctional semitendinosus (ST) has a distinctly different EMG pattern during the swing phase of forward (FWD) and backward (BWD) walking, suggesting that the activity of bifunctional muscles may be more mutable and adjusted by proprioceptive feedback to match the different limb kinematics associated with the two forms of walking (Buford et al. II. *J. Neurophysiol.* 64, 1990). To explore this possibility, the activity of two hip flexor-knee *extensor* muscles (anterior sartorius, SAa, and rectus femoris, RF) and a hip flexor-knee *flexor* (medial sartorius, SAm) were recorded along with limb kinematics (ciné film) in 3 cats trained to walk FWD and BWD on a motorized treadmill.

Both of the hip flexor-knee extensors had significantly different EMG patterns during BWD as compared to FWD walking. The changes in pattern were different among the muscles and appeared to reflect relative differences in their actions at the hip and knee. The reversal from flexion to extension occurs *later* at the knee during BWD swing (0.22 of the normalized step cycle; paw off at 0.0) than in FWD swing (0.10), but *earlier* at the hip (BWD = 0.12, FWD = 0.30). During FWD swing, activity in all three muscles ended at 0.29 and, thus, appeared to be related to their *hip* flexor action. In contrast, during BWD swing, SAa and RF activity (EMG offset = 0.04) was related to hip flexion, but SAm activity ended at 0.29 and was more related to *knee* flexion. FWD and BWD walking also differ in that the hip flexes during BWD stance. Typically, SAm was not active during either FWD or BWD stance. SAa and RF were both active during late FWD stance (0.68-0.86), but the two knee extensors were differentially activated during BWD stance: RF was active throughout knee extension (0.22-0.98), but SAa was active just briefly (0.26-0.47) around paw contact (0.35). In both FWD and BWD stance, SAa activity coincided with the reversal of hip extension to flexion. The temporal shifts in SAa activity were linked to the timing of hip kinematics and allowed SAa to contribute to deceleration of hip extension in both FWD and BWD stance. Supported by NIH NS 19864.

648.16

EFFECT OF SURFACE CHARACTERISTICS ON GAIT MODIFICATION IN INDIVIDUALS WITH AGE-RELATED MACULAR DEGENERATION. S.J.Spaulding*, A.E.Patla, S.Rietdyk, J.Flanagan & D.Elliott, Kinesiology Dept. & Sch. of Optometry, U. of Waterloo, Waterloo, Ont., Canada, N2L 3G1.

The possible strategies used by individuals with reduced central vision for travel over various surfaces under different ambient light levels were examined. Age-related macular degeneration (ARMD) subjects (N=7) and age-matched controls (56-86 years) walked over different surfaces (uneven, compliant and shiny) imbedded halfway into a 12 meter walkway under high light (2300 lux) & low light (5 lux). Gait adaptations prior to the surfaces were characterized by changes in impulse parameters under the contralateral limb, ipsilateral limb (landing first on altered surface) muscle activity during functionally relevant phases, and ipsilateral kinematic changes. Differences between the two groups were as follows: Normal subjects used similar vertical braking impulses regardless of the surface, whereas the ARMD subjects used a greater braking impulse in the single support phase for the uneven and shiny surfaces; and ARMD subjects had lower heel and toe velocity and increased vertical displacement as the foot cleared the edge of the surface. Level of lighting had no effect on gait. The ARMD group used both contra and ipsilateral strategies to reduce foot velocity, prior to landing on the altered surface. Both groups proactively altered their gait; increased toe elevation over the surface, increased contralateral horizontal braking and increased contralateral stance time suggest that subjects perceived that more caution was required for approaching the uneven surfaces. Decreased head angle suggests that subjects were monitoring the altered surface. Changes in gait parameters from the first to the tenth trial within a surface suggests that subjects adapt relatively quickly to the altered terrain. Supported by NHRDP.

648.17

THE EFFECTS OF TERRAIN DIFFICULTY ON CHARACTERISTICS OF VOLUNTARY VISUAL SAMPLING OF THE ENVIRONMENT DURING LOCOMOTION. A.E. Patla*, C. Martin, R. Holden & Sa. Prentice. Dept. of Kinesiology, Univ. of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

Young subjects (N=16) were instructed to walk over travel paths (9.1 m long) of varying difficulties while wearing opaque liquid crystal eyeglasses, and pressing a switch to make the glasses transparent when they needed to sample the environment. Their movement time (MT) & following visual sampling characteristics were recorded: number of visual samples (#SAMP); total duration of visual samples (TDVIS); average (ADVIS) & variability of visual sample duration (SDVIS); average (AISI) & variability of intersample time (SISI). A 2 (straight or winding path) x 2 (even or uneven step length requirement) x 2 (one or two obstacles in path) repeated measures ANOVA revealed the following results. #SAMP decreased in presence of obstacles coupled with even step length (6.8 vs 7.7); TDVIS was higher for winding compared to straight path (3.68s vs 3.06s) & higher for uneven step length condition compared to even step length (3.46s vs 2.90s); SISI was higher when obstacles were present (0.43s vs 0.29s). When a hole was included in the travel path affecting the consequence of error in foot placement, #SAMP increased (8.3 vs 7.0) along with higher TDVIS (3.73s vs 2.83s), & AISI reduced (1.23s vs 1.46s). When subjects were not constrained to place their feet on specific location, TDVIS (2.45s to 0.88s) & #SAMP (5 to 1.4) reduced dramatically. MT was similar for all conditions (9.38s). Inclusion of a large barrier in the winding path to influence preview region increased MT (11.91s vs 9.34s), AISI (1.76s vs 1.36s), & SISI (0.57s vs 0.29s). These results provide insights into how intermittent visual sampling of the environment is normally used for navigation. (Supported by a grant from NSERC, Canada.)

648.18

EFFECT OF LESIONS OF THE DORSAL COLUMNS AND DORSOLATERAL FUNICULI ON LOCOMOTION IN ADULT CATS. W. Jiang* and T. Drew Dept. of Physiology, Université de Montréal, Canada, H3C 3J7.

Experiments to determine the relative importance of different descending systems for the adaptive control of locomotion were performed in two cats which were chronically implanted for recording electromyographic (EMG) activity from muscles of the fore- and hindlimbs. Following control studies, a bilateral lesion of the spinal cord was made under general anaesthesia at T13 which completely interrupted the dorsal columns (DC) and extended to differing degrees into the dorsolateral funiculi (DLF). In one cat, with a relatively small lesion of the DLF, locomotion recovered within one week, and the cat was readily able to adapt its locomotion to walk at different speeds and at different treadmill inclinations (both roll and tilt). In the other cat, with a larger DLF lesion, locomotion recovered more slowly over a period of 4-6 weeks, and even after this time there was often a tendency for the cat to place the hindpaw on its dorsal surface. Nevertheless, this cat could also walk at different speeds and adapt its locomotion to inclined planes. Both cats, however, showed long-lasting deficits in their capacity to modify their hindlimb gait in order to step over obstacles attached to a treadmill belt or to walk along a horizontal ladder. These preliminary results suggest that whereas pathways contained within the ventrolateral and ventral spinal cord are sufficient for normal locomotion, pathways within the DC and/or DLF seem to be essential for anticipatory control. Supported by the FRSQ, the NCE, and the Rick Hansen Man in Motion Legacy Fund.

649.1

DISCORDANT EXPRESSION OF ACETYLCHOLINESTERASE ACTIVITY AND mRNA LEVELS IN SINGLE NEUROMUSCULAR JUNCTIONS OF NORMAL MUSCLE. R.K. Lee, B.J. Jasmin and R.L. Rotundo*. Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, FL 33101.

Acetylcholinesterase (AChE) is highly concentrated at the vertebrate neuromuscular synapse. Among the mechanisms that could account for this selective accumulation of AChE molecules is localized transcription of the AChE gene in postsynaptic sarcoplasmic nuclei. One prediction of this model is that AChE enzyme at the neuromuscular junction (NMJ) would mirror the levels of AChE transcripts. To examine this, we used a PCR-based assay to determine AChE mRNA copy number and a micro-assay for determining AChE activity in samples containing single isolated NMJs. AChE mRNA is an intermediate message at the NMJ compared to our internal standard used in competitive PCR assays (pACQNT) and to α-actin mRNA levels measured in the same samples. AChE message levels in non-innervated regions of the muscle fibers are either undetectable or very low, making the AChE mRNA a rare transcript in these fiber regions. Analysis of more than 50 individual NMJs shows that the AChE transcript levels are highly variable (more than 20 fold range) yet were detected in only 36% of our single NMJ samples. In contrast, analysis of AChE activity in single NMJs showed that enzyme levels were remarkably constant (12.23 ± 1.4 pmoles of ACh hydrolyzed/ min/NMJ; mean ± S.D.). These results show that AChE mRNA is compartmentalized at the NMJ and provide a mechanism for regulating the abundance of this synaptic protein at sites of nerve-muscle contact. Furthermore, the observed variability in AChE transcript levels compared to enzyme activity suggests that transcription of this synaptic protein gene may occur intermittently rather than constitutively possibly reflecting a regulated transcriptional control mechanism linked to muscle activity. Supported by NIH and MDA grants to R.L.R.

649.2

ACETYLCHOLINESTERASE mRNA LEVELS INCREASE IN PARALLEL WITH ENZYME ACTIVITY IN OVERLOADED SKELETAL MUSCLE. B.J. Jasmin*, R.K. Lee and R.L. Rotundo. Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, FL 33101.

Normal expression of acetylcholinesterase (AChE) in vertebrate skeletal muscle depends upon the presence of the nerve as well as normal contractile activity. In general, denervation of skeletal muscles results in disappearance of the collagen-tailed AChE form whereas muscle overload induces significant increases in specific AChE oligomeric forms. These observations indicate that expression of AChE can be modulated according to the activity state of the muscle. As a first step towards studying the molecular mechanisms underlying the activity-induced AChE plasticity in muscle we subjected adult quails to an overload model that results in a dramatic enlargement of the anterior latissimus dorsi (ALD) muscle. Birds had a weight corresponding to 10% of their body mass attached to one wing, the contralateral side serving as control. Seven days later, control and overloaded ALD muscles were excised, weighed and, protein or RNA extracted. Overloaded ALD muscles showed an 80% increase in wet weight compared to contralateral muscles. Enlarged ALDs showed an average 130% increase in total AChE activity per muscle compared to controls. Sedimentation analyses revealed an increase in all AChE oligomeric forms with A12, G4, G2 and G1 displaying 32%, 122%, 230% and 210% increases, respectively. Quantitative RT-PCR and Northern blot analyses showed that AChE mRNA levels more than doubled over control values thereby paralleling the increase in AChE activity. These results indicate that regulation of transcript levels may play an important role in dictating AChE activity in skeletal muscle fibers. Furthermore, this overload model will prove useful for testing the proposal that AChE gene transcription occurs intermittently and is regulated by the functional demands placed upon the muscle. Supported by NIH and MDA grants to R.L.R.

649.3

COORDINATION OF MYOSIN HEAVY CHAIN AND SARCOPLASMIC RETICULUM CALCIUM ATPASE ISOFORMS IN CAT SOLEUS FIBERS SIX MONTHS AFTER SPINAL TRANSECTION. R.J. Talmadge, M. Wang, and R.R. Roy*. Department of Physiological Science and Brain Research Institute, UCLA, L.A., CA 90024.

The coordination of isoform expression of proteins responsible for both contraction (myosin heavy chain, MHC) and relaxation (sarcoplasmic reticulum ATPase, SR-ATPase) was evaluated in the soleus of cats undergoing a slow to fast transition, induced by spinal transection at T_{12}-T_{13}. Three groups of adult cats were used: 1) control ; 2) spinal transected (ST); and 3) spinal transected with daily weight support exercise (ST&WS). Serial cross sections were processed for immuno-histochemistry using monoclonal antibodies to MHC and SR-ATPase isoforms. Fibers with the following MHC compositions could be determined: Type I, I & IIa, IIa, IIb, and Embryonic/Neonatal (Emb/Neo). In controls, 98% of all fibers contained type I MHC exclusively. ST and ST&WS reduced the percentage of type I MHC fibers to 67% and 73%, respectively. Fibers co-expressing type I and IIa MHC accounted for 12% of all fibers in both ST groups vs. <1% in control. Fibers containing Emb/Neo MHC were observed rarely in the ST groups. Fibers containing slow (SSR), fast and slow (FSR&SSR) and fast (FSR) SR-ATPase could be identified. In controls, 98% of all fibers contained SSR exclusively. The number of SSR exclusive fibers was reduced to 67% in ST and 76% in ST&WS. Relatively few fibers (5% ST, 7% ST&WS) showed a complete transition to FSR compared to the number of fibers showing a complete transition to type II MHC (14% ST, 20% ST&WS). A relatively high percentage (8% ST and 10% ST&WS) of fibers showed dual expression of type I and II MHC and FSR&SSR isoforms. These results are consistent with a coordination of initiation of MHC and SR-ATPase protein transformation. The MHC transformation, however, appears to be more complete than the SR-ATPase. (Supported by NIH Grant NS16333 & NRSA (DE07212) from NIDR)

649.4

HISTOCHEMISTRY AND IMMUNOHISTOCHEMISTRY OF CAT TIBIALIS ANTERIOR MOTOR UNITS AFTER 6 MONTHS OF ELECTRICAL INACTIVITY. D.J. Pierotti*, R.R. Roy, and V.R. Edgerton. Brain Research Institute and Deptartment of Physiological Science, UCLA, LA, CA, 90024-1568.

The effects of chronic electrical inactivity on the myosin heavy chain (MHC) composition and succinic dehydrogenase (SDH), alpha glycerophosphate dehydrogenase (GPD), and myofibrillar ATPase activities of fibers in tibialis anterior (TA) motor units (MU) of adult cats were studied. Spinal cord isolation (SI) was used to produce inactivity in 10 cats. The cord was transected at T12-T13 and L7-S1 and a bilateral dorsal rhizotomy was performed between the two transection sites. Two 24-hr EMG recording sessions verified that the muscles in the lower limb were electrically silent. One MU from each hindlimb was isolated using ventral root isolation techniques, physiologically typed, and glycogen depleted (Bodine et al. *J Neurophysiol* 57 :1730, 1987). Optical density measurements from glycogen-stained frozen sections were used to classify fibers as depleted (unit) or non-depleted (non-unit). Myofibrillar ATPase activity was determined as described by Jiang et al. (*Muscle & Nerve* 13: 1037, 1990). SDH and GPD activities were determined as described by Martin et al. (*J Histochem Cytochem* 33 :1053, 1985). MHC composition was assessed using monoclonal antibodies specific for slow and fast MHCs (Donated by S.Schiaffino, Padova, Italy). A similar degree of variability in ATPase, SDH, and GPD activities was evident among fibers within a unit as well as among units as found in the normal TA. SDH activity decreased, GPD activity increased, and ATPase activity remained unchanged compared to control. No heterogeneity in the type of MHCs expressed was present among fibers of a MU or within a single fiber of a unit. The coordination of contraction time, force, and fatigability with metabolic properties was similar in SI and in control units. These data demonstrate that the coordination of expression of metabolic and contractile proteins, which in large part form the biochemical basis for physiological unit types, remains regulated at the MU level following chronic inactivity. (SUPPORTED BY NIH GRANT NS16333).

649.5

EMG/FORCE RATIO AS AN INDICATOR OF MUSCLE CELL DEATH FOLLOWING ECCENTRIC EXERCISE. J.N. Howell*, G. Chleboun, D. Karapondo and R. Conatser. Ohio University College of Osteopathic Medicine, Athens, OH 45701.

Following eccentric exercise to failure of the human elbow flexors under heavy load (90% of isometric max.; elbow angle = 90°), the integrated EMG amplitude during submaximal, isometric contractions (up to 20% of isometric max.) increased 2 to 3 fold (N=37). Associated with failure was a decrease in mean power frequency (MPF) of the EMG from about 60 Hz to less than 50 Hz. This decrease was less than the decrease in MPF associated with concentric exercise carried to failure, suggesting lesser metabolic fatigue. The increased EMG amplitude required to produce a given force after exercise indicates that at least some fibers, capable of generating action potentials, could generate little force, either because of fatigue or cellular injury. Maximum isometric force output of the eccentrically exercised muscles decreased by almost 50%. Recovery was slow, taking weeks in many subjects (50% recovery in 2 weeks; N=39). After concentric exercise, despite the greater downward shift in MPF, strength was far less compromised, recovering fully in 1-2 days. Thus the prolonged strength loss following eccentric exercise was related more to mechanical stress than to metabolic fatigue. Although strength returned slowly after eccentric exercise, the EMG amplitude required to produced submaximal contractions recovered in three days. It is likely that injured fibers, able to produce action potentials but little force after the exercise, subsequently lose their ability to produce action potentials and become inexcitable. The time course of recovery of the normal EMG/force ratio may indicate the time course of death of these cells, which must ultimately be replaced by the regeneration of new cells. The motor units involved must include those recruited during low level isometric contractions, <20% of isometric max. (Supported by the American Osteopathic Assn.)

649.6

FATIGABILITY OF CAT SOLEUS MOTOR UNITS ACTIVATED AT VARYING FORCE LEVELS. B.R. Botterman*, L.B. Graf, and K.E. Tansey. Dept. of Cell Bio. and Neurosci., Univ. of Texas Southwestern Med. Cent., Dallas, TX 75235.

The fatigue resistance of motor units in the soleus muscle has been appreciated for some time. Recently, however, it was shown that the relative fatigue resistance among these motor units can vary dramatically (*Cope et al., J. Neurophysiol. 66:1483, 1991*). The fatigue resistance of a motor unit was determined by noting the length of time (endurance time, E_t) it could maintain its tension output at 85% of maximum. Using computer feedback control, a unit's tension was clamped at the target level by altering the stimulation rate applied to its axon (maximum of 100 pps). E_t was found to be correlated with axonal conduction velocity (CV) but not with maximum tetanic tension (P_o).

In the present experiments, the same procedures were followed except that motor unit tension output was varied between 3 levels (50 - 70 - 90% of P_o) in ascending and descending order. The time at each level was held constant, although various step durations (10, 20 or 40 s) were selected at random for different units. A wide range of E_ts was found (~100x), regardless of the step duration used. In contrast to the earlier study, E_t was correlated with not only CV but also P_o. In addition, the relationship between stimulation frequency and tension output changed over time as a function of output level. The stimulation frequency necessary to maintain 50% and 70% of P_o remained fairly constant (~7 pps and ~10 pps respectively) or increased modestly throughout the endurance test, while the frequency necessary to maintain 90% of P_o increased steadily from ~30 pps to ~60 pps, after which it increased rapidly to 100 pps. Early in their endurance tests, some units showed a notable decrease in the stimulation rate required to clamp tension at 90% of P_o. This indicates that soleus motor units can undergo a form of potentiation which operates at relatively high tension output levels. Supported by NIH grant NS17863.

649.7

FATIGABILITY AND RELAXATION PROPERTIES OF THE HUMAN PARALYZED SOLEUS MUSCLE. R.K. Shields and C. Kukulka*. University of Iowa Hospitals and Clinics, Iowa City, Iowa 52242.

Spinalized animal models have been reported to show minimal changes in muscle fatigability as the result of reduced use. The fatigability of the human paralyzed soleus muscle was tested in both chronic (n=10) and acute (n=3) paralyzed individuals using a modified Burke fatigue protocol. In addition, the relaxation properties and electromyographic activity (emg) of the soleus muscle was determined during the fatigue protocol.

Plantarflexion torque was measured in both paralyzed groups from a seated position with the knee and ankle at 90 degree angles. A 20 Hz supramaximal stimulation was administered to the tibial nerve each second for 330 ms for a duration of four minutes.

Plantarflexion torque and the normalized maximum rate of relaxation (nMRR) were significantly reduced at two minutes of the fatigue protocol in the chronic group, but no significant changes were noted in the acute group. The emg (chronic) was significantly reduced at three minutes, but was minimally related to the changes in torque. The fatigability of the chronically paralyzed group is consistent with clinical reports of muscle fatigue during neuromuscular stimulation and contrary to the reports of unchanged muscle fatigability in long term spinalized animal models.

649.8

DIFFERENCES IN FATIGABILITY OF MOTOR UNITS OF THE SAME TYPE NOT RELATED TO DIFFERENCES IN ENERGY-METABOLISM. S. Sesodia*, R.J. Callister, P.M. Nemeth, R.M. Enoka, R.M.Reinking and D.G. Stuart. Dept. of Neurology, Wash. Univ. Med. Sch., St. Louis, MO 63110 and Dept. of Physiology, Univ. of Arizona, Tucson, AZ 85724

Earlier studies on muscle fatigue led to the recognition that resistance to fatigue of motor-units of different histochemical types was positively correlated to the levels of activity of energy-generating enzymes of the oxidative pathways. In this study we have examined the relationship between energy metabolism and fatigue in motor-units of the same histochemical type but of differing fatigabilities. Isolated single axons to rat EDL muscles were stimulated to fatigue fast-fatiguable (FF) motor-unit muscle fibers. After the fatigue test, the motor unit was continuously stimulated at 40 Hz for one hour to deplete its muscle fibers of their glycogen. Muscles were then rapidly dissected, snap frozen in liquid nitrogen and mounted on blocks for cross-sectioning. Series of thick (56 μm) and thin (14 μm) cross-sections were cut from the muscles with the thick sections being freeze-dried and then stored under vacuum at -70°C. Thin sections were stained for glycogen (Periodic acid-Schiff (PAS) reaction) and myosin adenosine triphosphatase. Glycogen depleted fibers as identified from the PAS-stained sections were dissected from the thick freeze-dried cross-sections. The fibers were divided into smaller pieces, weighed on quartz-fiber balance and assayed for activity of enzymes belonging to different metabolic pathways (i.e. glycolytic, oxidative and high energy phosphate). Our preliminary results show that while a difference in fatigue index (and hence fatigatiliby) exists, no correlatable difference exists in the activity of the enzymes of energy-generating pathways. However our data suggest that a relationship may exist between the ratio of certain energy-generating and energy-consuming enzymes and fatigability. We are presently examining this aspect in further detail.

649.9

IDENTIFICATION OF OPTIMAL INTERPULSE INTERVAL (IPI) PATTERNS FOR ACTIVATION OF FATIGUED HUMAN QUADRICEPS FEMORIS MUSCLE. S.A. Binder-Macleod* and Scott Baadte, School of Life and Health Sciences, Univ. of Delaware, Newark, DE, 19716.

This study attempted to identify the stimulation pattern during brief (10-pulse), subtetanic trains of pulses that produced the greatest force per pulse when the muscle was fatigueD by repetitive activation. Each train lasted ~600 ms; rest periods of ~600 ms separated each train. Subjects (N=8) participated in three experimental sessions. During the first session, each subject was stimulated with constant frequency trains (CFTs, all IPI durations = 70 ms) and five variable frequency trains (VFTs, first IPI duration = 5, 10, 15, 20 or 30 ms; the remaining 8 IPI durations = 70 ms). During the second experimental session, the second IPI duration was varied while the first IPI was kept at the interval that produced the greatest force during the first session. During the third session, the third IPI duration was varied. The sequencing of trains within each session was random, with each train repeated 30 times during each session (180 contractions). By the 120th contraction a stable level of fatigue was noted. The mean force produced during the last 10 contractions for each stimulation pattern was calculated to compare the response of the muscle to each stimulation pattern.

The results showed that one short IPI at the onset of the train produced 32% more force than the CFT, two short IPIs 64%, and three short IPIs 29%. Thus, two short IPIs produced marked force augmentation in a fatigued muscle compared to the CFTs and other VFTs ($F_{(2,14)}=9.411$, $P=0.003$). These results may have significant implications when electrical stimulation is used clinically to activate skeletal muscle repetitively. Such applications include stimulation of lower extremity muscles to assist spinal cord injured patients to ambulate and stimulation of the latissimus dorsi muscle to assist cardiac muscle function.

649.10

INNERVATION RATIO IS THE MAJOR DETERMINANT FOR THE WIDE RANGE OF MOTOR UNIT FORCE IN THE CAT MEDIAL GASTROCNEMIUS (MG) MUSCLE. V. Rafuse*, M.C. Pattullo and T. Gordon. Dept. of Pharmacology, Div. of Neuroscience, Univ. of Alberta, Edmonton, Alberta, Canada, T6G 2S2.

The extent to which motoneuron branching (innervation ratio; IR) determines the normal variation in motor unit (MU) force within a single muscle is a contentious issue. Determining the range of unit IRs in a large heterogenous muscle such as the cat MG muscle has been difficult due to 1) the wide range in muscle fiber cross-sectional areas (CSAs) between different MU types and 2) counting all muscle fibers by glycogen depletion is difficult due to the steep pinnation of muscle fibers. These problems for calculating the IR of MUs in the cat MG muscle have been overcome in recent experiments with long-term low-frequency electrical stimulation. Cat MG muscles were selectively stimulated (20Hz, 50% duty cycle) for 6 to 32 weeks via a MG nerve cuff electrode attached to a small portable stimulator fastened to the cat's back. In a final acute experiment the unit force distribution was determined by isolating and characterizing at least 14% of the MG MUs. The muscle fibers of a single MU were also depleted of glycogen by repetitive stimulation at a low frequency. The MG muscle was then removed and prepared for histochemical analysis. Following 19 $\pm$ 10 weeks of stimulation the normal 100- fold range in unit force was reduced to a 40- fold range. Concurrently, the range of CSAs for the whole muscle fiber population was similarly reduced from an 8 to 4- fold range. Normally, the range in muscle fiber CSAs within a single MU is 50% that of the whole muscle fiber population. Following stimulation the mean and range of muscle fiber CSAs within a single MU was the same as for the entire muscle. Since the mean muscle fiber CSA of all MUs became similar and IR is not changed by stimulation, these results provide strong evidence that the 40- fold range is IR of MG motoneurons. (Supported by MRC, AHFMR and NCE).

649.11

MOTOR UNIT HETEROGENEITY FOLLOWING FUNCTIONAL STIMULATION OF CAT AND HUMAN MUSCLES. M.C. Pattullo, V.F. Rafuse, D.J. Parry, J. Yang*, R.B. Stein and T. Gordon. Div. Neurosci., Univ. Alberta, Alta T6G 2S2 and Dept. Physiol., Univ. Ottawa, Ontario K1H 8M5, Canada

Although it is well recognized that whole muscle properties can be modulated by imposed activity, it is not known whether the predicted conversion to a homogeneous motor unit (MU) population occurs. In medial gastrocnemius (MG) in the cat and tibialis anterior (TA) in spinal injured patients, functional electrical stimulation (FES; 20Hz, 50% duty cycle for at least 2 hrs/day), caused a gradual increase in twitch conctraction time (CT) and fatigue resistance (Rafuse et al., Soc. Neurosci., 17: 256.4, 1991; Stein et al., J. Appl. Physiol. In press). We analyzed up to 14% of the total MU population in the cat MG at 6 weeks (short-term FES) and, in the rest, at 16-32 weeks after continuous FES (long-term FES). In both cat and human, increase in mean values of MU CT and fatigue indices corresponded with changes in whole muscle properties. However, the range of values was NOT greatly reduced and did not correspond to the range of the normal slow MU population. Immunohistochemical analysis of myosin heavy chain (MHC) isoforms in stimulated cat muscles showed that, at 6 weeks, muscles were heterogeneous in composition but long-term stimulated muscles were homogeneous in type I MHC composition with no evidence of hybrid fibers. The finding that all MUs become slower without reducing the range of CT in the MU population provides evidence that 1) factors other than MHC content which include Ca release and uptake rates, are rate limiting for CT and 2) that differences between fibers remain in stimulated muscles which account for the wide range of MU CTs, despite the change in gene expression of contractile proteins. (Supported by Canadian MRC and MDAC and AHFMR).

649.12

IMMUNOHISTOCHEMICAL ANALYSIS OF FIBER TYPES WITHIN PHYSIOLOGICALLY TYPED MOTOR UNITS OF RAT TIBIALIS ANTERIOR MUSCLE AFTER LONG-TERM CROSS-REINNERVATION. S. Fu, T. Gordon*, D.J. Parry and N. Tyreman. Dept. Pharmacology., Div. Neuroscience., Univ. of Alberta, Alta T6G 2S2, & Dept. Physiology. Univ. of Ottawa, Ont. K1H 8M5, Canada.

We have previously shown in the rat (Totosy de Zepetnek et al., Soc. Neurosci., 15: 65, 1989) and mouse (Parry & Wilkinson, Can. J. Physiol. Pharmacol. 68: 596-602, 1990) that the characteristic distribution of fiber types in the deep and superficial regions of Tibialis Anterior (TA) is also seen after self-reinnervation, under conditions in which nerves do not reinnervate their former muscle fibers. In this study, we have used antibodies to type I, IIa and IIb myosin heavy chains (MHCs) to investigate 1) the spatial distribution of fiber types 1 year after cross-reinnervation of rat TA by posterior tibial nerve and 2) the fiber type composition of characterized MUs. We found that the spatial distribution of fiber types was not statistically different from normal despite increased clumping of fiber types. Fibers which did not react with any of the 3 antibodies, presumably type IIx fibers, comprised up to 30% of the total, similar to the proportion of FI MUs in normal and self-reinnervated muscles (Totosy de Zepetnek et al., J. Neurophysiol., 67 #5, 1992). Within identified fast MUs, most fibers showed MHC expression which was congruent with the MU type (i.e. FF=IIB, FR= IIa). However, there was considerable variation in the intensity of staining and a significant proportion of fibers (>5%) in FF and FR MUs appeared to be type IIx. In FI MUs, 5-10% of fibers were IIb positive. These findings provide suggestive evidence for an intrinsic regulation of muscle fiber type which allows for the maintenance of the original spatial distribution and may account for the fiber heterogeneity within a single MU. (Supported by MDAC, MRC and AHFMR).

649.13

NEURAL REGULATION OF GLYCOGEN PHOSPHORYLASE EXPRESSION IN RAT SKELETAL MUSCLE C. C. Matthews and R. C. Carlsen*. Department of Human Physiology, School of Medicine. University of California, Davis 95616

Denervated skeletal muscle undergoes a series of metabolic changes, including a decrease in both the activity of glycogen phosphorylase and the content of muscle-specific glycogen phosphorylase (MGP) messenger RNA. We tested the hypothesis that the loss of neurotrophic substances following denervation is responsible for the decrease in MGP transcription. Several concentrations of vinblastine were used to block axonal transport in situ in the rat peroneal nerve. Nerve conduction velocities and tibialis anterior (TA) contractile properties were assessed at 7 days after initiation of the axonal transport block. MGP expression in the TA muscle was determined using standard Northern analysis procedures. The results show that axonal transport of acetylcholinesterase activity declined in nerves treated with 0.2% (w/v) and 0.4% vinblastine. There was also a dose-dependent decline in motor and sensory nerve conduction velocities one week after treatment with 0.2% or 0.4% vinblastine. The ability of the muscle to produce force likewise declined with the highest doses of vinblastine. The amount of MGP mRNA present 7 days after vinblastine treatment decreased substantially when the nerve was exposed to 0.4% vinblastine. A lesser decrease in MGP mRNA developed after treatment with 0.2% vinblastine. Lower concentrations of vinblastine produced no apparent change in the content of MGP mRNA. These results suggest that MGP transcription is under neurotrophic regulation, but by some means other than the release of an axonally transported diffusable substance.

649.14

IS MUSCLE LIKE PAINT? J.L.F. Weytjens* and J.A. Hoffer[1]. Dept. of Clinical Neurosciences, University of Calgary, Calgary, Alberta T2N 4N1, CANADA.

Hoffer et al. (Prog. Brain Res. 80: 75-85, 1989) found that the origin-to-insertion length of the cat medial gastrocnemius muscle (MG) and the length of its fibers do not vary in unison during locomotion: the maximum difference was 2 mm in stance and 5 mm in swing. In contrast, Elek et al. (J. Physiol. 429: 237-258, 1990), using the spindle-null method, estimated the length changes in the tendinous component of the muscle ("extramysial displacement") during simulated locomotion to be only 0.3 to 0.7 mm (stance). We have reassessed extramysial displacement during locomotion using direct measurements of muscle length and muscle fiber length in simulated locomotion with and without stimulation of the muscle.

In six chronically instrumented cats muscle force, muscle length, muscle fiber length, and the length of a small part of the ventral aponeurotic sheet were measured during treadmill locomotion. Locomotion was simulated in the same animals under anesthesia by matching muscle force and muscle fiber length (Weytjens and Hoffer, SN Abstr. 17: 648, 1991). Extramysial displacement was computed from the measurements made in the simulations, using simple assumptions about the structure of MG.

Tendon length and muscle force did not change in phase. After an initial increase in length more or less proportional to force, the rate of lengthening decreased abruptly. However, tendon length kept increasing (until after the last stimulus) even though muscle force declined. It shortened back to its original length only slowly, the last half of the shortening occurring in the absence of muscle force. The same pattern was observed in the measurements of aponeurotic sheet length. The mean maximum estimated change in tendon length was 2.53 mm.

These observations were most simply explained by assuming that muscle has paint-like, i.e., *thixotropic*, properties: that it "thins" when "stirred" by internal or external movement, and "thickens" when left "standing." On the basis of the same mechanism other hitherto unexplained muscle phenomena could also be explained: (1) the time course of force responses of passive muscle to stretch, (2) the shape of single motor unit force-velocity curves, and (3) movement-dependent motor unit force potentiation.

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MUSCLE II

650.1

Muscle unit architecture and motor pool organization of the pectoralis muscle in pigeons. A. Sokoloff*, J. Ryan, E. Valerie, D. Wilson and G. E. Goslow Jr. Biology, Brown University, Providence, RI 02912.

The pectoralis muscle of the pigeon *(Columba livia)* is composed of fast oxidative glycolytic (FOG) and fast glycolytic (FG) fibers thought to be differentially recruited during take-off and steady flight. To assess the neuromuscular organization of FOG and FG motor units, pectoralis muscle unit architecture and motor pool organization were studied with glycogen depletion and retrograde axonal tracing techniques. Individual motor units were identified in ventral root filaments by the presence of a stable EMG and force trace during a 5-10 fold increase in voltage and were stimulated for 1-3 hours. The pectoralis muscle was sectioned and stained for glycogen to allow muscle unit reconstruction. Three FOG units were 2.4, 2.5 and 3.5 cm in length, and consisted of 100, 250 and 300 fibers respectively. A single FG unit was 3.5 cm in length and consisted of 170 fibers. None of the muscle units extended more than 30% of the origin-to-insertion whole muscle length. Following application of a 30-40% solution of HRP to cut pectoralis nerves (N=3), retrogradely labeled neurons were located in cervical segments 10 to 12. Motoneuron soma area ranged from 3000-18000 sq μm (x = 9000 sq μm). A few exceptionally large neurons (15000-18000 sq μm) were observed in each bird. These results 1) demonstrate that individual muscle units occupy a small portion of the pigeon pectoralis and do not extend from muscle origin to muscle insertion and 2) suggest that force transmission in the pectoralis proceeds through myo-myous and/or endomysial connections between the fibers of in-series muscle units. The presence of large motoneurons in the pectoralis motor pool suggests a possible correspondence between motoneuron size and muscle unit area and/or fiber type. Supported by NSF grant DCB-87-18727.

650.2

MORPHOLOGY OF MOTONEURONS INNERVATING TWO REGIONS OF RAT MEDIAL GASTROCNEMIUS MUSCLE WITH DIFFERING PHYSIOLOGIAL AND HISTOCHEMICAL PROPERTIES. S. Vanden Noven*, P.F. Gardiner and R.A. Turcotte. *School of Physical & Occupational Therapy, McGill University, (H3G 1Y5) & Sciences de l'activité physique, Université de Montréal, Montréal, Québec, H3C 3J7, Canada.

We studied the medial gastrocnemius (MG) motoneuron pool/muscle complex in Sprague-Dawley rats in which the MG nerve enters the muscle as two branches. In situ experiments showed that the muscle region innervated by the proximal MG nerve branch generated 25% of total muscle tetanic force and demonstrated slower twitches and higher fatigue resistance than that innervated by the distal branch. Glycogen depletion experiments showed the proximal branch to innervate the axial core of the muscle which contains the slow twitch and most of the fast twitch, high oxidative fibers. In separate experiments, proximal and distal nerve branch motoneurons were labeled with horseradish peroxidase (HRP). 'Proximal-branch' alpha-motoneurons were smaller and accounted for up to 44% of the total MG pool. A systematic variation in rostral-caudal location of motoneurons from the two nerve branches was present within the MG pool. Thus, the unique organization within the MG motoneuron pool/muscle complex will permit comparative studies on the adaptive properties of different motor unit types within a given complex.

Supported by NSERC Canada (SVN & PFG) and McGill Graduate Faculty (RAT) grants.

650.3

FIBER TYPE DIVERSITY IN THE VIBRISSAL FACIAL MUSCLES OF RODENTS. L.E. Wineski*, S.A. Pitts and O.I. Weeks. Dept. Anatomy, Morehouse School of Medicine, Atlanta, GA 30310; Dept. Biological Sciences, Florida International University, Miami, FL 33199.

The golden hamster (Mesocricetus auratus), Norway rat (Rattus norvegicus), and guinea pig (Cavia porcellus) exhibit three types of exploratory behavior, as defined by the use of the mystacial vibrissae. The gross morphology of the superficial facial muscles that operate the vibrissae is very similar in the three species; however, the muscles may differ in their physiological characteristics. Preliminary enzyme histochemical tests indicate significant species differences. Myosin ATPase reactions show that the muscles that act to protract the vibrissae are composed predominantly of type IIa fibers in the whisking species (hamster, rat); those in the nonwhisking guinea pig are composed largely of types IIb and IIc fibers. The muscles involved in retraction are more heterogeneous in their composition: the hamster has predominantly type IIa fibers, the rat mainly types IIb and IM fibers, the guinea pig largely types IIc and IM fibers. NADH tetrazolium reductase reactions show that all vibrissal muscles in the hamster are composed entirely of highly oxidative fibers. Those in the rat and guinea pig contain large populations of intermediate-level fibers. In general, the vibrissal muscles are composed almost entirely of fast-twitch, fatigue-resistant fibers with minor populations of slow-twitch, fatigue-resistant fibers. However, there is great diversity in the subtype composition of the muscles. Supported by NIH S06-GM08248 and NIDR.

650.4

MUSCLE FIBER TYPE COMPOSITION AND SUCCINATE DEHYDROGENASE ACTIVITY OF SEXUALLY DIMORPHIC MUSCLES. G.C. Sieck*, C.F. Blanco, P. Popper and P.E. Micevych. Depts. of Physiology & Anesthesiology, Mayo Clinic, Rochester, MN 55905, and Dept. of Anatomy & Cell Biology, Laboratory of Neuroendocrinology, UCLA School of Medicine, Los Angeles, CA 90024.

The succinate dehydrogenase activity (SDH) of muscle fibers from the sexually dimorphic levator ani (LA) and bulbocavernosus (BC) muscles were determined using a quantitative histochemical technique from cryostat sections of fresh frozen tissues (Histochem. J. 20:230, 2988). The BC, LA and medial gastrocnemius (MG) muscles of male Long-Evans rats were excised and frozen in liquid nitrogen cooled isopentane. The MG muscle was included so that comparisons could be made with a non-sexually dimorphic muscle. All of the BC and LA muscle fibers were type IIb. Furthermore, the BC and LA muscle fiber populations were metabolically homogeneous. For type IIb fibers the mean SDH activity was greatest among fibers from the deep region of the MG followed in descending order by the superficial region of the MG, the BC, and the LA muscles. These results suggest that the BC and LA muscles are composed of highly fatigable fast-twitch muscle units. (Supported by grants HL 34817 and HL 37680.)

650.5

THE CATCH-LIKE PROPERTY OF TURTLE MUSCLE: EFFECT OF SHORTENING, LENGTHENING, AND FATIGUING ISOMETRIC CONTRACTIONS. R.J. Callister, D.H. Laidlaw, R.M. Reinking, and D.G. Stuart.*Department of Physiology, University of Arizona, Tucson AZ 85724.

We have shown previously in mammals (adult cat) that subtle changes in motor unit firing patterns can delay and reduce fatigue (Bevan *et al.*, J. Physiol. (Lond.) 449:85-108, 1992). To extend on these observations, we have recently changed our animal model from cat to turtle (*P. scripta*) as this species affords the unique opportunity, in vertebrates, to study segmental motor mechanisms in intact *(in vivo), in vitro* (slice) and cell-culture preparations. In this report, we examine the catch-like property in the whole external gastrocnemius muscle (EG; 27% SO, 39% FOG and 34 %Fg fibers) during shortening and lengthening contractions, and following fatiguing isometric contractions. Two different stimulation patterns, *constant-frequency* (10 pulses, 100 ms intervals) and *catch-inducing* (two additional 10 ms intervals inserted at the beginning of the constant-frequency pattern), were applied to the EG muscle nerve. The force-time integral attributable to each stimulus pattern was quantified. The catch-inducing pattern always produced greater force than the constant-frequency pattern. However, the magnitude of the increase depended on the type of contraction. A comparison of force enhancement, due to the catch-like property, prior to and during shortening (17% *vs.* 14%) and lengthening (20% *vs.* 16%) contractions did not reveal any significant differences. This contrasts with the marked difference (12% pre-fatigue *vs.* 37% post-fatigue) in force enhancement observed in muscles following a period of fatiguing (10 Hz trains, 10 p/train, 1 train/2 s for 4 mins) isometric contractions. It remains to be determined if a catch-inducing activation pattern becomes more important during dynamic contractions in fatiguing muscle, as is the case for isometric contractions. Supported by USPHS grants GM 08400, HL 07249, NS 25077, NS 07309, NS 20544, and NS20762.

650.6

SARCOMERE LENGTH-JOINT ANGLE RELATIONSHIPS OF SEVEN FROG HINDLIMB MUSCLES. R. L. Lieber* and C. G. Brown. Department of Orthopaedics and Biomedical Sciences Graduate Group, *U.C. San Diego Medical School and VAMC*, San Diego, CA 92161

The sarcomere length-joint angle relationship was measured in seven different muscle-joint complexes (n=49 muscles) of the frog hindlimb (*Rana pipiens*). Muscles studied included the cruralis, iliacus internus, gastrocnemius, gluteus magnus, gracilis major, semimembranosus, and the semitendinosus. Muscle-joint complexes were mounted in a jig and submerged in chilled Ringer's solution. Joints were rotated throughout their range of motion while sarcomere length was measured by laser diffraction. Sarcomere length change per degree of joint rotation (i.e., $dL_S/d\theta$) ranged from a low of 3.7 nm/° for the cruralis muscle acting at the knee to a high of 12.5 nm/° for the semitendinosus muscle acting at the hip. $dL_S/d\theta$ values for muscles acting at the hip joint were significantly greater than those for muscles acting at the knee ($p<0.005$). $dL_S/d\theta$ was also negatively correlated with fiber length, suggesting a balance between fiber length and moment arm in most muscle-joint systems. Many exceptions to this generalization were noted. These data suggest that various muscle-joint combinations are "designed" for differential contribution of muscle force production to the joint torque profile.

650.7

ELECTROPHYSIOLOGICAL PROPERTIES AND CALCIUM ACTIVATED POTASSIUM CHANNELS IN RAT CEREBROVASCULAR SMOOTH MUSCLE CELLS. Y.Wang* and D.A.Mathers. Department of Physiology, University of British Columbia, Vancouver B.C., Canada V6T 1W5.

Patch clamp methods were used to study the electrophysiological properties and the calcium activated potassium channels of smooth muscle cells (SMCs) from the cerebral arteries of adult Wistar rats. Procedures were developed for the enzymatic dissociation of these cells. Dissociated cells were maintained at 4°C for 1-3 days prior to use. Whole cell and inside-out patch clamp recordings were made at 21°C using a List EPC-5 amplifier.

During whole-cell, current clamp recordings from these cells, the resting membrane potential was found to be -41 mV. Application of strong depolarizing stimuli evoked only small regenerative responses and full action potentials were not observed. When measured at zero applied current, the slope membrane resistance was 3.2 gigaohm. The average membrane time constant was 78 ms and cell capacitance was 24 pF.

Isolated, inside-out membrane patches excised from these cells displayed calcium activated potassium channels with intermediate single channel conductance (92 pS) in symmetrical 140 mM KCl solution. These channels were activated by both increasing intracellular free calcium and depolarization of the patch membrane. Tetraethylammonium caused a reversible, dose-dependent reduction in the amplitude of current in the channels, when applied to the cytoplasmic membrane face (K_d=0.31 mM).

650.8

CYTOARCHITECTURE OF THE NEUROMUSCULAR JUNCTION IN DIABETIC MUSCLE. K. M. Klueber* and S. Stansel. Department of Anatomical Sciences and Neurobiology, University of Louisville, School of Medicine, Louisville, KY 40292.

During the pathogenesis of diabetes, a peripheral neuropathy occurs in the C57Bl/KsJ-dbm diabetic mouse. Prior work indicated that neuromuscular remodelling occurs in this mouse (Klueber and Stahr, Neurosci. Abts.15:51, 1989). The objective of the present study was to evaluate the cytoarchitecture of these neuromuscular junctions (NMJ) to provide an index of remodelling. The extensor digitorum longus muscles from young (8wk) and old (20wk) female diabetic and control mice were examined electron microscopically: In the diabetic muscles, thirty percent of the NMJ's examined at both time points exhibited various degrees of degeneration. Remodelling was also observed as indicated by the presence of sprouts (4%). Reinnervation was indicated by NMJ's exhibiting secondary synaptic clefts which extended beyond the axon (10%, 8wk; 5%, 20wk) while others exhibited only limited shallow secondary cleft formation (13%, 8wk; 9% 20wk). Both of these profiles are characteristic of reinnervation following nerve section and are hypothesized to be stages in the maturation of regenerating NMJ's (Hansen-Smith, Anat. Rec. 207:55, 1983). Thus regeneration and remodelling of NMJ's is more frequent in young rather than old diabetic muscle and occurs to a greater degree in diabetic than in normal muscle. (Funded by DK41553).

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS—CONDITIONING I

651.1

DISRUPTING CEREBELLAR DEVELOPMENT IMPAIRS EYEBLINK CONDITIONING IN THE INFANT RAT. M.E. Stanton[*1,2] & J.H. Freeman, Jr.[2] [1]Neurotox. Div., US EPA, RTP, NC, 27711; [2]Psychology Dept., UNC, Chapel Hill, NC 27514

There is a dramatic increase in rate of eyeblink conditioning (EBC) between Postnatal Day 17 (PND17) and PND24 in the rat (Stanton, Freeman & Skelton, Behav. Neurosci., in press), which may reflect postnatal development of the cerebellum. To examine this possibility, we exposed pups neonatally to the antimitotic agent, methylazoxymethanol (MAM), a treatment that impairs the development of cerebellar cortex, while leaving most other brain structures intact (Chen & Hillman, 1986, Exp. Neurol., 94, 103).

On PND0 and PND1, rat pups were injected (s.c.) with saline or 20 mg/kg MAM. On PND21 or PND22, they were trained on spatial delayed alternation, a task that is sensitive to early limbic and prefrontal cortical damage (Freeman & Stanton, in press; 1991, Behav. Neurosci, 105, 386-395). On PND23 or 24, they were trained on EBC (see Stanton et al., in press). Pups exposed to MAM were not impaired on delayed alternation (suggesting no functional impairment of hippocampus or prefrontal cortex). In contrast, pups that showed MAM-induced cerebellar hypoplasia were impaired on acquisition of EBC. These findings suggest a role for cerebellar development in the ontogeny of EBC.

651.2

EARLY CEREBELLECTOMY IMPAIRS EYEBLINK CONDITIONING IN THE INFANT RAT. J.H. Freeman[*1] & M.E. Stanton[1,2] [1]Psychology Dept., UNC, Chapel Hill, NC 27514; [2]Neurotox. Div., US EPA, RTP, NC, 27711

Initial studies have demonstrated that associative eyeblink conditioning (EBC) can be established in the infant rat (Stanton, Freeman, & Skelton, Behav. Neurosci., in press). One purpose of this research is to develop a preparation for the neurobiological analysis of the ontogeny of learning. As a first step toward this end, we sought to determine whether the cerebellum was critical for EBC acquisition in the infant rat. In the adult rat, lesions of the cerebellar deep nuclei abolish EBC (Skelton, Behav. Neurosci, 1988). However, it is not known whether early cerebellar damage would affect acquisition of EBC in the infant rat.

Pups received either sham surgery or cerebellar removal by aspiration on Postnatal Day 10 (PND10). On PND25, all pups were trained on EBC (see Stanton et al., in press). Animals with complete cerebellar removal were dramatically impaired when compared with animals in the sham-surgery group.

This experiment extends and confirms the structure-function homology between neuranatomical substrates of EBC in the rat and rabbit. Moreover, the results suggest a role for the cerebellum in the ontogeny of EBC.

651.3

EVIDENCE THAT THE PRINCIPAL ABDUCENS NUCLEUS UNDERLIES THE *IN VITRO* CONDITIONED EYE-BLINK RESPONSE. J. Keifer*, Dept. of Physiology, Northwestern Univ. Medical School, 303 E. Chicago Ave., Chicago, IL 60611.

Two distinct populations of abducens motor neurons contribute to the eye-blink reflex: the principal and the accessory abducens nuclei. Accessory abducens sends its axons to the retractor bulbi muscle and receives afferents from the trigeminal nucleus and premotor blink areas. Principal abducens projects to the lateral rectus and retractor bulbi muscles, and receives inputs from nerve VIII, the reticular formation, and a minor input from the trigeminal nucleus. Thus, the pathways that undergo modification during conditioning may be different depending on the relative contribution of the abducens nuclei to the conditioned response (CR). Previously, we reported that the *in vitro* turtle brainstem-cerebellum was a useful model to study the conditioned eye-blink reflex. Using the activity-dependent dye sulforhodamine, evidence has been obtained suggesting that the principal abducens nucleus, rather than the accessory abducens, has a predominant role in producing the conditioned response.

Paired electrical stimuli to the posterior nerve VIII (CS) and the trigeminal nerve (UCS) were applied to the *in vitro* preparation while recording activity in the abducens nerve as described previously (*Soc. Neurosci. Abs.* 16: 763, 1990). Once a conditioned response had been acquired, sulforhodamine was added to the bath and the preparation was given CS-only stimuli. Hence, during dye application, only CR's were recorded. The results show that during expression of the CR, labeled neurons were found predominantly in the principal abducens nucleus as compared to the accessory abducens nucleus. Labeled neurons were also observed in the red nucleus and lateral cerebellar nucleus, areas which never label during the UCR. Purkinje cells, the reticular formation, trigeminal nucleus, and the cochlear nucleus also labeled with dye.

The results suggest that the principal abducens nucleus is in the modifiable pathway leading to the CR. This is intriguing since activity in this nucleus, but not in the accessory abducens, is mediated by NMDA. Although not traditionally considered, the principal abducens and trigeminal nuclei, both of which receive CS-US convergence, are postulated to be potential sites of learning. (NSF BNS-9109572)

651.4

PERFORMANCE OF UNCONDITIONED NICTITATING MEMBRANE RESPONSES IN THE INTACT RABBIT IS AFFECTED BY MUSCIMOL INACTIVATION OF THE NUCLEUS INTERPOSITUS. V. Bracha*, M.L. Webster, J.R. Bloedel, Barrow Neurological Institute, Phoenix, AZ 85013.

The purpose of this study was to examine the specificity of involvement of the anterior nucleus interpositus (AIN) in the control of nictitating membrane reflexes. Animals were trained in the standard delay paradigm using 450 ms sound as the conditioned stimulus and 100 ms unilateral corneal air-puff as the unconditioned stimulus (ISI=350 ms, ITI=17-23 s). The trained animals were injected with muscimol in the AIN ipsilateral to the trained eye and tested in experiments in which trials consisting of paired conditioned and unconditioned stimuli were alternated with trials containing the unconditioned stimulus alone.

The muscimol microinjections (200 ng) completely abolished the conditioned responding. Explicit testing of the unconditioned reflex before and after drug administration revealed that activation of GABA-a receptors significantly decreases the amplitude of the unconditioned nictitating membrane responses. The analysis of behavior in animals which developed bilateral conditioned responses indicate that the drug effect is mostly restricted to the ipsilateral eye.

The results of the present study do not support the notion that the AIN is involved exclusively in mediating the classically conditioned nictitating membrane reflex. The behavioral effects of the muscimol injections in the AIN suggest that this cerebellar nucleus participates in control of both conditioned and unconditioned nictitating membrane responses in intact rabbits . NIH Grant R01 NS21958

651.5

LIDOCAINE INFUSION IN A CRITICAL REGION OF CEREBELLUM COMPLETELY PREVENTS LEARNING OF THE CONDITIONED EYEBLINK RESPONSE. A.F. Nordholm*, J.K. Thompson, S. Standley, G.Tocco, C. Dersarkissian & R.F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

New Zealand White rabbits were implanted with cannulae in the dorsal or ventral aspect of the anterior interpositus nucleus. Three days (and six days for some animals) of standard tone-airpuff training was given with continuous infusion (constant rate of 0.2 ul/min) of Lidocaine (2, 4, 8, 16%) or saline. All animals were then given three days of training with no infusion. The minimum dose (concentration) of Lidocaine necessary to abolish performance of the CR was then determined for every animal. Saline control animals learned to criterion during the three days of infusion training; Lidocaine animals with dorsal cannula locations and appropriate doses exhibited no CRs in the three (or six) days of infusion training and learned in the subsequent three days of no-infusion training as if naive, i.e. they exhibited no savings. Animals with ventral cannula locations and appropriate doses showed no CRs during Lidocaine infusion training but substantial savings in subsequent no-infusion training. Thus both acquisition and performance of the CR were completely abolished, depending on cannula location, in a dose dependent manner. These results strongly support the hypothesis that the essential memory trace for eyeblink conditioning is formed and stored in the cerebellum. (Supported by NSF and ONR grants to R.F.T.)

651.6

INACTIVATION OF CEREBELLUM WITH GABA AGONIST MUSCIMOL REVERSIBLY BLOCKS ACQUISITION AND RETENTION OF RABBIT'S CLASSICALLY CONDITIONED EYEBLINK RESPONSE D.J.Krupa*, J.K.Thompson, R.F.Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520

The GABA agonist muscimol was used to assess the cerebellum's role in eyeblink conditioning. In well trained rabbits, varying concentrations of muscimol were infused into the cerebellum through chronically implanted cannulae aimed at the ipsilateral interpositus nucleus. Conditioned responses (CRs) were completely blocked for up to 8 hours with no effect on unconditioned responses (URs). By 12 hours, CRs returned to pre infusion levels.

A group of naive rabbits (n=6) received 6 days of tone-airpuff conditioning. On the first 3 days, muscimol was infused into the cerebellum prior to training; no infusion was given on the last 3 days. A control group (n=5) received vehicle alone infusions on days 1-3 and no infusion on days 4-6.The muscimol group showed no learning on days 1-3 and no savings on day 4. These rabbits learned normally on subsequent days. Their rate of acquisition and average percent CRs on days 4-6 was identical to days 1-3 of controls. Controls were responding at a high percentage by day 3. Average UR amplitude on paired trials as well as airpuff alone trials was the same for both groups. Infusions of ^{3}H-muscimol following training and subsequent histology confirmed that the muscimol was localized to the dentate/interpositus nuclei and overlying cortical regions. Another group of rabbits (n=5) received infusions of muscimol into the red nucleus on days 1-3. These rabbits showed no CRs on those days but showed a significantly higher percent CRs on day 4 than the cerebellar group.

These results demonstrate that the cerebellum is required for acquisition and expression of eyeblink conditioning. The results also indicate that the cerebellum is a locus for storage of this memory. Supported by NSF, ONR, and McKnight grants to RFT.

651.7

Reversible Lesions of the Red Nucleus During Acquisition and Retention of a Classically Conditioned Behavior in Rabbits. Robert E. Clark* and David G. Lavond. Neuroscience Program, University of Southern California, Los Angeles, CA 90089-2520.

We have previously shown that temporary cooling of the interpositus nucleus prevents acquisition of a classically conditioned eyeblink. In the present study we assess the role of the red nucleus during conditioning. A cooling probe was implanted lateral to the red nucleus. Recording electrodes were implanted in the right red nucleus and the left interpositus nucleus. Animals were trained for five days with the cooling probe activated. No behavioral conditioned responses (CR) developed and multiple unit recordings related to learning did not develop in the red nucleus. However, a learning related model did develop in the interpositus. Animals were then given five days of normal training (cooling probe inactive) to assess retention. Substantial savings were evident as CRs appeared quickly and multiple unit models were present in both red nucleus and interpositus nucleus. These results support the idea that the red nucleus is a necessary efferent for the memory trace formed in the cerebellum. Supported by NSF: BNS8906612.

651.8

TEMPORAL DISCRIMINATION AND PAVLOVIAN EYELID RESPONSES: CEREBELLAR VERSUS PRE-CEREBELLAR MECHANISMS. S. P. Perrett* and M. D. Mauk. University of Texas Medical School, Houston, TX 77225.

We previously reported (Perrett et al, **Neurosci. Abstr.** 115.1, 1990) that lesions of the cerebellar cortex disrupt the adaptive timing of conditioned eyelid responses in the rabbit. The retention of inappropriately timed conditioned responses (CRs) after the lesion suggests a) the temporal discrimination necessary for adaptive timing of CRs occurs in the cerebellar cortex or b) occurs afferent and is conveyed to the cerebellar cortex. To distinguish these possibilities we tested the ability of individual animals to acquire differently timed CRs using stimulation of mossy fibers in the middle cerebellar peduncle (MCP) as the conditioned stimulus (CS).

Rabbits are conditioned by pairing a stimulus train in the MCP coterminating with periocular eyeshock as the unconditioned stimulus (US). Differently timed responses are produced in an individual animal by training at one interstimulus interval (ISI) until a criterion of 90% responding is reached, extinguishing this CR, then training at a different ISI. All animals with electrode placements in the mossy fiber bundle learned at least three differently timed responses at criterion levels before degeneration of the electrode. Peak amplitude for all CRs occurs approximately when the US is presented. The animals' ability to produce differently timed responses when a constant set of mossy fibers in the MCP are activated, along with the lesion data, suggests that a temporal discrimination mechanism exists in the cerebellar cortex. To test this hypothesis further we trained rabbits with single pulse stimulation as the CS to preclude temporal summation as a mechanism to recruit different subsets of mossy fibers. While the ISI range that supports conditioning is much smaller with a single pulse CS, rabbits learn differently timed responses at criterion levels. We hypothesize that temporal discrimination occurs via the activation of different subsets of granule cells, rather than different subsets of mossy fibers, throughout the ISI. This may result from the dynamic interaction of granule cells and inhibitory Golgi cells in the cerebellar cortex. If the granule cell synapses active in a time window around a climbing fiber response decreased in strength (e.g. LTD), nuclei cells would be disinhibited when this subset of granule cells is subsequently active and an appropriately timed CR could be elicited.

651.9

EFFECTS OF REWARDING ELECTRICAL STIMULATION OF LATERAL HYPOTHALAMUS ON CONDITIONED NICTITATING MEMBRANE RESPONSE IN RABBITS J. Arikoski*, T. Korhonen, M. Penttonen, and T. Ruusuvirta. Dept. of Psychol., Univ. of Jyvaskyla, P.O. Box 35, SF-40351, Finland

Possible facilitation or retardation effect of rewarding brain stimulation of lateral hypothalamus (ESB) was studied in classical conditioning of nictitating membrane responses in rabbits. A 250 ms train of ESB pulses were applied 250 ms after the unconditioned stimulus (UCS, corneal airpuff, 150 ms, 2.1 N/cm^2). The conditioned stimulus (CS, tone 1000 Hz, 400 ms) preceded the UCS 250 ms. In the control group ESB preceded the CS-UCS pair by 250 ms. A tone CS was classically conditioned to an air-puff UCS followed by stimulation of the lateral hypothalamus. The rabbits showed increased orienting and activity to brain stimulation. Preliminary observations showed also that the airpuff UCS can act as a CS eliciting conditioned responses.

651.10

SUBICULAR AND CINGULATE CORTICAL COMBINED LESIONS AND LIMBIC THALAMIC UNIT ACTIVITY DURING LEARNING IN RABBITS. E. Kang* and M. Gabriel, Dept. of Psychol. and Beckman Inst., Univ. of Illinois, Urbana, IL 61801.

Separate bilateral electrolytic lesions in the dorso-posterior subicular complex or in posterior cingulate cortex (area 29) enhanced the magnitude of training-induced unit activity (TIA) exhibited by anterior ventral (AV) thalamic neurons comprising circuitry involved in discriminative avoidance conditioning. Rabbits acquired a conditioned response (CR, stepping in an activity wheel) to prevent foot-shock 5 sec. after presentation of a .5-sec. tone conditional stimulus (CS+), and they learned to ignore a different tone (CS-) which did not predict shock (Gabriel et al., Exp Br Res, 1987, 67, 131-152). These findings indicated subicular and cingulate cortical modulation of limbic thalamic TIA. One account of these findings states that subicular and area 29 efferents in intact rabbits suppress TIA in the AV nucleus. Lesions in either area thus enhance AV thalamic TIA. Alternatively, efferents of the two cortical areas may enhance AV thalamic TIA and mutually inhibit one another. Lesions in one area would thus disinhibit the other thereby enhancing AV thalamic TIA. Combined lesions (1.0 DC mA. for 20 sec. each) at 3 sites along the subicular septo-temporal axis and at 9 sites from bregma to 9.0 mm posterior in area 29 (N=8) enhanced AV and medial dorsal (MD) thalamic TIA relative to TIA in sham lesion controls (N=9). As after single lesions, TIA was maximally enhanced in asymptotically trained rabbits, but discharge enhancement also occurred before training, unlike the single-lesion case. These results support the first and rule out the second of the previously stated accounts. The combined lesions increased CR incidence in the first conditioning session, as did the single subicular lesions. CR loss found in well-trained rabbits with single area 29 lesions was not found here in the rabbits with combined lesions. Also, an increased latency and decreased duration of shock-elicited locomotion occurred in the rabbits with combined lesions. (Supported by an NIH grant to MG).

651.11

CINGULATE CORTICAL AND LIMBIC THALAMIC NEURONAL RESPONSES TO UNEXPECTED CUE AND CONTEXTUAL STIMULI DURING EXTINCTION OF DISCRIMINATIVE AVOIDANCE BEHAVIOR IN RABBITS. A. Poremba*, Y. Kubota, E. Kang and M. Gabriel. Dept. of Psychol. and Beckman Institute, Univ of Illinois, Urbana IL 61801.

Hippocampal formation lesions enhanced the training-induced neuronal activity (TIA) in the anterior ventral (AV) thalamic nucleus during discriminative avoidance training, wherein rabbits performed a locomotory conditioned response (CR) in an activity wheel to avoid a footshock signaled by a tone (CS+) 5 sec. before the shock, and they learned to ignore a tone (CS-) of different auditory frequency than the CS+, which did not predict shock (Gabriel et al., Exp Br Res, 1987, 67, 131-152). Unexpected training experiences such as extinction (CSs presented without shock to a trained subject) did not effectively suppress CR performance in rabbits with lesions, suggesting the hypothesis that CR suppression by unexpected events is due to TIA suppression in AV and possibly other limbic thalamic nuclei. Here, CRs were suppressed but anterior and medial dorsal (MD) thalamic and cingulate cortical TIA was **not** suppressed during initial trials of extinction with an unexpected context (altered background odor and illumination) or tone. These results disconfirm the hypothesis. TIA in anterior cingulate cortical area 24b, and in the anterior dorsal, magnocellular AV and MD thalamic nuclei was enhanced by the unexpected context (but not by the unexpected tone) during the first 20 extinction trials, relative to TIA during extinction trials without novelty. The novel context-induced AV thalamic TIA enhancement corroborated preliminary results of a study (Kang et al., Soc Neurosci Abst., 1990, 16, 264) which also showed loss of the enhancement in rabbits with lesions of Ammon's horn. These results suggest that hippocampal efferents mediate the enhanced TIA. The cortical and thalamic sites exhibiting the context-related enhancement of TIA may be part of a circuit involved in the immediate suppression of CRs and/or mnemonic encoding of novel information, but this circuit does not appear to be engaged by unexpected CSs. (Supported by NIH).

651.12

SUBICULAR AND CINGULATE CORTICAL LESIONS AND ANTERIOR THALAMIC NEURONAL RESPONSES TO UNEXPECTED CUE AND CONTEXTUAL STIMULI IN RABBITS. M. Gabriel* and E. Kang. Dept. of Psychol. and Beckman Institute, Univ of Illinois, Urbana IL 61801.

Anterior and medial dorsal thalamic neurons are essential for discriminative avoidance conditioning, wherein rabbits learn to avoid footshock by stepping in response to a tone (CS+), and to ignore a different tone (CS-) which does not predict shock. CR acquisition, blocked by limbic thalamic lesions, is accompanied in intact rabbits by training-induced neuronal activity (TIA, increased tone-elicited discharges and discrimination, i.e., greater discharges to CS+ than to CS-). Standard CSs presented to intact trained rabbits following the introduction of unexpected contextual stimuli (alteration of odor and illumination cues) increased anterior ventral (AV) thalamic TIA and suppressed CR incidence, an effect blocked by hippocampal lesions. Presentation of an unexpected tone stimulus in the standard training context also suppressed behavior but did not increase TIA (see abstract by Poremba et al, this volume, and Kang et al., Soc Neurosci Abst. 16, 1990, 264). These results suggested that hippocampal efferents in the presence of altered contextual stimuli induce: a) AV thalamic TIA enhancement; b) CR suppression. Ammon's horn efferents gain access to AV nucleus via subicular and cingulate cortical (area 29) synaptic relays. If true, then subicular/area 29 lesions should, as Ammon's horn lesions, diminish AV thalamic discharges elicited in the presence of unexpected contextual stimuli. Instead, AV thalamic novel-context-specific discharges were enhanced, relative to controls (N=11), in rabbits with the combined lesions (N=11). These results suggest that Ammon's horn neurons do not drive directly the AV thalamic discharges. Computations of interactive Ammon's horn and subicular circuitry may permissively allow the thalamic discharges (driven by as yet unknown afferents) to occur. Ammon's horn lesions may unbalance the permissive interactions, with the result that subicular/area 29 influences suppress the thalamic discharges. (Supported by an NIH grant to MG).

651.13

LESIONS OF THE CEREBELLAR FASTIGIAL NUCLEI FAIL TO AFFECT APPETITIVE PAVLOVIAN CONDITIONING OF JAW MOVEMENT (JM). C.M. Gibbs* and K.L. Watson. WJB Dorn Veterans' Hospital and University of South Carolina, Columbia, SC 29201.

We recently reported [Gibbs et al: Soc Neurosci Abstr 17(1991)870] that bilateral lesions of the cerebellar interpositus n. have no effect upon the acquisition of conditioned JM responses in rabbits with repeated pairings of tone (CS) and an intraoral pulse of water (US), though such lesions do disrupt circuitry associated with climbing fiber responses to intraoral stimulation. Since the vermal cortex receives gustatory information [Somana & Walberg: Neurosci Lett 11(1979)41-47], the present studies sought to determine whether bilateral lesions of its deep nuclear target, the fastigial n. (FA), influence JM conditioning.

To date, 4 animals with lesions of the rostral FA and 8 sham-operated animals have been subjected to 4 daily sessions of appetitive Pavlovian training (30 pairings/day of a 2-s, 1216-Hz tone with a 1-ml pulse of 0.5M sucrose solution). Evaluation of a variety of dependent measures indicated that lesioned and sham animals exhibited orderly and statistically indistinguishable JM CR acquisition functions, reaching levels of 84-90% CRs during training days 3-4. Moreover, FA lesions did not affect JM URs to unsignalled sucrose presentations. These data thus provide further evidence suggesting an extracerebellar substrate for appetitive JM conditioning. (Supported by VA Institutional Research funds and NSF Grant BNS 88-20379.)

651.14

CHANGES IN STIMULATION THRESHOLDS FOR DLPN-ELICITED EYEBLINKS REFLECT CR-BEHAVIOR IN THE RABBIT. J. Tracy*, D. J. Krupa, N. L. Bernasconi, J. S. Grethe & R. F. Thompson, Neuroscience Program, University of Southern California, Los Angeles, CA 90089-2520.

Classical conditioning of the rabbit eyeblink response using paired presentations of dorso-lateral pontine nucleus (DLPN) stimulation as a CS, and corneal airpuff as an US, results in rapid acquisition. In order to better localize the plasticity associated with pontine/airpuff conditioning we measured the stimulation thresholds required to elicit eyeblinks, in both the DLPN and the interpositus nucleus (IPA), before and after training. New Zealand White rabbits (n=6) received paired presentations of DLPN stimulation (350 ms., 200 Hz.) and airpuff to the eye (100 ms., 3 psi). All CS stimulation intensities were calibrated to be well below the threshold required for eliciting any kind of motor activity. A second group of rabbits (n=6) received an equivalent number of unpaired presentations prior to training. After training all rabbits were extinguished with CS-alone presentations.

Paired training reliably lowers the DLPN stimulation threshold required for eliciting eyeblinks to well below the training intensity. Subsequent extinction brings the threshold back up to a level not statistically different from pre-training levels. Unpaired presentations of DLPN stimulation and airpuff do not change thresholds. Thresholds for eyelid movement from IPA stimulation do not change following paired, unpaired, or extinction training. Interpositus lesions following paired training abolish CR's elicited with pontine stimulation. These results strongly suggest that changes in neural activity due to conditioning are occurring efferent to the pons and provide further evidence of the critical role of the cerebellum in eyelid conditioning. Support:NSF(BNS-8117115) and ONR(N00014-88-K-0112) to RFT.

651.15

UNCONDITIONED STIMULUS (US) EFFECTS DURING AN INTRACEREBELLAR STIMULATION PARADIGM. R.A. Swain* & R.F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

Intracerebellar stimulation of lobule HVI white matter as US produces robust classical conditioning when paired with either tone or intracerebellar stimulation CS. CS alone presentations produce extinction and reinstatement of paired trials produces rapid reacquisition. In addition, explicitly but not randomly unpaired presentations of the conditioning stimuli profoundly retard learning in subsequent acquisition trials (Swain, et al., 1992).

In the current experiment, we examined the nature of US elicitation of movement and any nonassociative effects of US preexposure on learning. Previous lesion results have underscored the essential role of the interpositus (IP) in the generation of movement in this paradigm (Swain et al., 1989; 1991). However, as HVI stimulation necessarily activates Purkinje cell, mossy fiber and climbing fiber afferents to IP, we sought to determine the relative contribution of these fibers to the IP's generation of movement. 108 US trials of varying stimulus durations (50-300 msec) were presented to 7 rabbits. Analyses indicated that IP is differentially sensitive to climbing and/or mossy fiber input. UR onset occurs within 100 msec of US onset eliminating the possibility that movement arises from rebound excitation of IP during Purkinje cell hyperpolarization. Maximum UR amplitude, however, always occurs 50-100 msec from US offset.

The nonassociative impact of US preexposure on learning was examined in these same rabbits by presenting up to 10 days of paired CS-US trials. Comparison with non-preexposed controls (N=9) indicated that US alone trials impair learning as measured by total number of trials to criterion and also by variability of CR performance from session to session.

Supported by NSF BNS8718300, ONR N0001488K0112, & McKnight to R.F. Thompson.

651.16

INTRACEREBELLAR ELECTRICAL STIMULATION AS BOTH CS AND US IN CLASSICAL CONDITIONING OF DISCRETE MOTOR RESPONSES. P.G. Shinkman*, R.A. Swain, and R.F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

In earlier experiments we showed that electrical stimulation of cerebellar white matter elicits discrete motor responses of the facial and neck musculature; pairing a tone CS with the intracerebellar electrical US leads to the development of robust conditioned responses. The present experiment was designed to test for associative learning in a preparation in which both CS and US are delivered directly to the cerebellum. Chronic stimulating electrodes were implanted in rabbit cerebellum, providing an electrical CS activating cortical parallel fibers and thence Purkinje cells, and an electrical US activating underlying white matter and eliciting unconditioned responses. Paired CS-US presentations led reliably to the development of conditioned responses; also, increased local excitability was observed in cerebellar cortex. Pseudorandom unpaired presentation of CS and US did not produce any CRs, indicating that true associative learning, and not sensitization, is responsible for the observed results. Subsequent pilot data suggest that similar conditioning may occur when intracerebellar electrical CS and US are delivered via the same intracortical electrode, at different intensities. This preparation provides a model for the study of plastic neuronal interactions within cerebellar networks critically involved in associative learning. (Supported by NSF BNS-8718300, ONR N00014-88K-0112, and the McKnight Foundation, to R.F. Thompson, and by the BDRC, University of North Carolina.)

651.17

MOTOR CORTEX LESIONS DO NOT AFFECT ACQUISITION OR RETENTION OF THE CLASSICALLY CONDITIONED NICTITATING MEMBRANE RESPONSE IN RABBITS.

D. Ivkovich* and R.F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520

Rabbits were classically conditioned using the delay paradigm with a tone conditioned stimulus (CS; 350 ms, 1KHz, 85 dB) and an airpuff unconditioned stimulus (US; 100 ms, 3 psi, coterminating with the CS). Seven rabbits received bilateral motor cortex lesions prior to receiving 5 days of acquisition training. Another 7 rabbits received 5 days of acquisition followed by the lesion and 5 days of retention training. Each training session consisted of 12 blocks of 9 trials (1 CS-alone followed by 8 paired CS-US trials). Bilateral motor cortex lesions did not affect the acquisition or retention of the classically conditioned nictitating membrane response. The percentage of conditioned responses did not differ between lesioned and unlesioned animals during acquisition. Animals lesioned following acquisition showed no conditioned or unconditioned response deficits during subsequent training on any measure (percentage, amplitude, onset or peak latency, and amplitude-time area). Reflexive eyeblinks to 4 different US intensity levels (1, 2, 3, & 4 psi) measured over the course of training were unaffected by motor cortex lesions.

(Supported by NSF BNS-8718300, ONR N0001488K0112, & McKnight to R.F. Thompson)

651.18

INTRA-OLIVARY INFUSIONS OF PICROTOXIN PREVENT "BLOCKING" OF RABBIT CONDITIONED EYEBLINK RESPONSE. J. J. Kim*, D. J. Krupa and R. F. Thompson. Neurosciences Program, USC, Los Angeles, CA 90089-2520.

This experiment investigated the effect of a GABA antagonist picrotoxin (PTX) on the phenomenon of "blocking" using the conditioned eyeblink response in the rabbit. Kamin's two-stage paradigm was employed. The blocking group received 7 days of tone-airpuff conditioning followed by 5 days of tone-light-airpuff compound conditioning. Half of the blocking animals received intra-olivary infusions of PTX (1 nM) during the compound conditioning phase, while the other half received artificial cerebrospinal fluid (CSF). The control group received 5 days of tone-light-airpuff compound conditioning only. Preliminary results indicate that the CSF animals that received tone-airpuff conditioning prior to the compound conditioning did not show any conditioned eyeblink responses to the light. Subsequent light-airpuff training in this group indicates that there was no *savings* to the light. Animals that received PTX, however, showed reliable conditioned responses to the light as well as a marked *savings*. These results support the view that the inferior olive provides the unconditioned stimulus information to the cerebellum for classical eyeblink conditioning.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS—CONDITIONING II

652.1

SEXUALLY DIMORPHIC EFFECTS OF DEXAMETHASONE ON ACTIVE AVOIDANCE LEARNING AND CENTRAL MUSCARINIC ACETYLCHOLINE RECEPTORS IN RATS. Ş.Pöğün*, L.Kanıt, S.Demirgören, F.Z.Kutay, B.Okur, H.Özkan, H.Semerci, M.Altınışık. Depts.of Physiol. and Biochem., Ege Univ. School of Med.,35100 İzmir, Turkey

Glucocorticoids have prominent roles in the modulation of behavior and the proposed psychoneuroendocrine interactions are sexually dimorphic. The present study was undertaken to study the effects of dexamethasone (DEX) on active avoidance learning and 3H-QNB binding in 8 brain regions. Male and female Sprague Dawley rats (4 months) were injected with either 1 mg DEX in sesame oil or only the vehicle at 8:00 and were given active avoidance learning trials at 10:00. Following 5 days of learning trials, the rats were decapitated, brains removed and dissected. Blood corticosterone levels were assayed. Receptor binding was determined in the cerebellum(cb), hypothalamus(hyp), corpus striatum(c.st), hippocampus(hip)and frontal(frn), parietal(par), temporal(tem) and occipital(occ) cortices. ANOVA revealed significant differences between the groups with regard to corticosterone levels($p<0.01$), learning performance at day 5 ($p<0.05$) and 3H-QNB binding in the cb, hip, frn and par cortices ($p<0.05$). There was a significant correlation between learning performance at day 5 and corticosterone levels ($p<0.001$), 3H-QNB binding in the hip, c.st, tem and occ cortices ($p<0.05$). Correlations were also observed between corticosterone levels and receptor binding in the hip, c.st and tem cortices ($p<0.05$). Our results show that DEX effects active avoidance learning in rats through the muscarinic cholinergic system and more prominently in males.

652.2

INVOLVEMENT OF THE LATERAL AMYGDALA AND PERIRHINAL CORTEX IN FEAR POTENTIATED STARTLE TO ACOUSTIC AND VISUAL CONDITIONED STIMULI S. Campeau* and M. Davis. Dept. of Psychology and Psychiatry, Yale Univ. Sch. of Med, 34 Park St., New Haven, Ct 06508.

Prior studies in our laboratory have indicated that post-training lesions of the lateral/basolateral complex of the amygdala or perirhinal cortex block fear-potentiated startle using a visual conditioned stimulus (CS). The goal of the present studies was to test the generality of the involvement of these nuclei in fear-potentiated startle using an acoustic CS. Anatomical data show that the medial geniculate nucleus of the thalamus, the source of all auditory information to the forebrain, projects subcortically to the lateral nucleus of the amygdala, and cortically to the auditory and perirhinal cortex. The auditory cortex projects to the lateral nucleus of the amygdala, via a relay in the perirhinal cortex.

Rats received 10 pairings of a 70-dB, 3.7-s noise centered at 2 kHz, mixed with 10 pairings of a 3.7-s fluorescent light, each coterminating with a 0.5-s, 0.6-mA footshock, presented at a variable intertrial interval of 2.5 min, on each of 2 consecutive days. To preoperatively match rats into groups with similar fear-potentiated startle to the noise and light CSs, a startle test consisting of 30 startle stimuli alone, or 5 startle stimuli 3.2 s after the onset of each noise or light CS was used. Twenty-four hr later, rats were given either electrolytic (n = 6), neuro-toxic NMDA (n = 8) or sham (n = 5) lesions of the lateral amygdala, or electrolytic (n = 8) or sham (n = 6) lesions of the perirhinal cortex. Ten days later, a startle test, similar to the pre-lesion test, revealed that specific and complete lesions of the lateral nucleus of the amygdala (electrolytic and neurotoxic) or anterior perirhinal cortex reliably blocked potentiated startle to the acoustic and visual CSs.

These post-training lesion results indicate that the lateral nucleus of the amygdala and the perirhinal cortex are relays along a pathway normally mediating the expression of visual as well as acoustic aversive memories. Experiments in progress are testing the effects of pre-training lesions of the lateral amygdala and perirhinal cortex on fear-potentiated startle.

652.3

A PRIMARY ACOUSTIC STARTLE CIRCUIT : LESION AND ANATOMICAL TRACING STUDIES. Y. Lee*& M. Davis. Dept of Psychology & Psychiatry, Yale Univ. Med. Sch., 34 Park St., New Haven, CT 06508.

The delineation of neural circuits that mediate behavior provides a foundation for determining where behavioral plasticity might occur, and how these changes might alter behavior. The acoustic startle reflex in the rat has proven to be extremely sensitive to a range of experimental manipulations and is currently being used as a model system to analyze behavioral plasticity in vertebrates.

Using a variety of techniques, our laboratory has proposed a primary acoustic startle circuit that consists of the auditory nerve, posteroventral cochlear nucleus (PVCN), an area including the ventral nucleus of the lateral lemniscus (VLL), paralemniscal zone (PLZ), and the rostroperiolivary nucleus (RPO), an area just dorsomedial to the lateral superior olive (LSO) in the nucleus reticularis pontis caudalis (RPC), and motor neurons in the spinal cord. (Davis et al 1982; Cassella & Davis 1986). While lesion and stimulation studies generally support this circuitry, some important details that are crucial for future electrophysiological studies remained to be found. For example, because the original studies used relatively large lesions in the vicinity of the VLL, it is not known which structure in this area is critical in relaying information from PVCN to RPC. The present studies attempted to identify more precisely the locus of this relay in the startle circuit.

Discrete, bilateral electrolytic lesions of the RPO region completely eliminated startle, while lesions of areas just lateral (VLL) and medial to the RPO (PLZ), showed a partial blockade of startle depending on the size, and the location of the lesions. Retrograde (Fluro-Gold) and anterograde (Fluro-Ruby) tracers deposited into the RPO clearly labeled cell bodies in the contralateral PVCN, and terminals in both ipsilateral and contralateral RPC, respectively.

To investigate the functional significance of these connections more definitively, currently we are using ibotenic acid to destroy cell bodies of each of these structures (VLL, PLZ, & RPO) without damaging fibers of passages. To be sure that the PVCN projects to the part of the RPO critical for startle, we are injecting retrograde tracerS into the RPO in combination with ibotenic acid lesions of the adjacent areas such as the VLL and the LSO, which are known to receive dense projections from PVCN (Lee & Davis unpublished observation; Thompson et al 1991).

652.4

VISUAL CORTEX ABLATIONS FAIL TO PREVENT EXTINCTION OF FEAR-POTENTIATED STARTLE USING A VISUAL CONDITIONED STIMULUS. W.A. Falls*& M. Davis. Depts. of Psychology & Psychiatry, Yale Univ. Med Sch., 34 Park St., New Haven, Ct. 06508.

Paired presentation of a neutral stimulus, such as a light, with an aversive stimulus, such as shock, leads to the acquisition of a variety of behavioral effects that are used to define a state of conditioned fear. If the same light is then presented in the absence of shock, it loses its ability to produce these behavioral effects. This reduction in conditioned fear is called experimental extinction and is thought to involve the acquisition of a new memory that may mask or inhibit the expression of the original conditioned fear. Several reports indicate that sensory cortex is not critically involved in the acquisition or expression of conditioned fear. However, it has been suggested that sensory cortex may be a component of a neural circuit that is responsible for inhibiting subcortically mediated conditioned fear (LeDoux et al., *J. Cog. Neurosci.*, 1989, 1, pp 238-243; Teich et al., *Brain Research*, 1989, 480, pp 210-218). Following the observation that sensory cortex ablations prevent extinction of conditioned fear, the present experiments sought to investigate the generality of this finding by examining whether visual cortex ablations would prevent the acquisition and/or expression of extinction of conditioned fear as assessed by fear-potentiated startle using a visual conditioned stimulus.

Rats underwent training in which a light was paired with footshock. Three to 5 days after training, the rats' intial level of fear-potentiated startle was assessed by measuring the amplitude of noise-elicited startle in the presence or absence of the light. On each of the next two days, the rats were given 30 light-alone extinction trials. Following this, fear potentiated startle was assessed again.

Visual cortex ablations *performed prior to fear conditioning* did not prevent the subsequent acquisition of extinction of fear-potentiated startle to a visual conditioned stimulus. Visual cortex ablations *performed following extinction* did not reverse pre-operatively established extinction as would be expected if visual cortex is a component of a neural circuit that is responsible for inhibiting subcortically mediated conditioned fear. Together these results indicate that sensory cortex is not required for the acquisition or expression of extinction of conditioned fear in all behavioral systems.

652.5

ELECTROLYTIC LESIONS OF THE AMYGDALA BLOCK ACQUISITION AND EXPRESSION OF CONDITIONED FEAR EVEN WITH EXTENSIVE TRANING, BUT DO NOT PREVENT REACQUISITION, AS ASSESSED BY FEAR-POTENTIATED STARTLE. M. Kim* & M. Davis. Dept. of Psychiatry, Yale University School of Medicine, 34 Park St., New Haven, CT 06508

The amygdala is well-known to be important for aversive conditioning. Lesions of the amygdala block the expression of fear-potentiated startle in which the amplitude of the acoustic startle reflex is elevated when elicited in the presence of a cue previously paired with footshock. However, it has not been determined how extensive training would alter this lesion effect.

In Exp.1, rats received 2 training trials (light CS paired with 0.6 mA footshock US) and 6 fear-potentiated startle test trials per day for 30 days, and either sham or electrolytic lesions aimed at the amygdala central nucleus (ACe) on day 32. The rats showed asymptotic performance by day 12 (after 22 training trials). ACe lesions totally abolished fear-potentiated startle, but did not prevent reacquisition of potentiated startle when the rats were retrained over the next 7 days. In Exp.2, rats were first sham- or ACe-lesioned, and then given 24 days of training. Shock intensity was always 0.6 mA for controls, but increased to 1 mA on day 15 for ACe-lesioned rats. The lesioned rats showed no acquisition at either shock intensity. In summary, ACe lesions given after extensive training abolished expression, but did not prevent reacquisition, of fear-potentiated startle. In contrast, ACe lesions given before training totally blocked acquisition.

These results suggest that the amygdala is normally involved in the expression of fear-potentiated startle regardless of the degree of learning. Also, the amygdala is necessary for the acquisition of fear-potentiated startle and other brain structures do not support this form of learning when training occurs without the amygdala. However, when learning occurs with an intact amygdala, other brain structures can reacquire conditioned fear after amygdala damage, suggesting that the amygdala may induce some functional change in those brain structures during original acquisition.

652.6

ROLE FOR THE AMYGDALA CENTRAL NUCLEUS IN ATTENTION. Michela Gallagher* and Peter Holland. Dept. Psychology, Univ. N. Carolina, Chapel Hill, NC 27599 and Dept. Experimental Psychology, Duke University, Durham, NC 27706.

Conditioning episodes can modify attentional processing of conditioned stimuli (CSs). Some of these changes can be characterized as incremental and others as decremental. Elsewhere we reported that neurotoxic lesions of the amygdala central nucleus (CN) in rats disrupt normal conditioning-dependent enhancement of orienting responses (OR) but do not affect the ability of a CS to elicit ORs prior to conditioning. This work examined whether CN damage alters selected CS processing functions.

Our results indicate that CN lesioned rats fail to exhibit conditioning that depends on increments in CS processing. This deficiency was evident in failures to show the enhanced associability of a CS that is normally observed when either an inconsistent predictive relation is arranged between that CS and another cue, or the reinforcement value of the unconditioned stimulus (US) is reduced in an unblocking procedure. In contrast to these impairments, CN damage did not affect the reduction in associability of a CS produced by CS preexposure (latent inhibition), blocking, or consistent reinforcement procedures. Thus the CN appears to be part of a neural system that regulates broadly-based increments in processing or attending to signals for biologically significant events, but is not critical for tuning out redundant or uninformative cues. Supported by NIMH grant 35554 and a NIMH RSDA to MG (KO2-MH00406).

652.7

POSTTRAINING INJECTIONS OF LIDOCAINE INTO THE LATERAL/ BASOLATERAL COMPLEX OF THE AMYGDALA IMPAIR RETENTION OF INHIBITORY AVOIDANCE. M.B. Parent* & J.L. McGaugh. Center for Neurobio. of Learning & Memory and Dept. of Psychobio., U. of Calif., Irvine, CA 92717

The central nucleus (ACe) and the lateral/basolateral complex (LBL) are two areas within the amygdala that have been extensively implicated in learning and memory. The present experiment examined the role of the ACe and LBL in the retention of inhibitory avoidance by reversibly inactivating these regions after training. Male Sprague Dawley rats (175 - 200 g) were implanted bilaterally with cannulae aimed at the ACe (AP - 0.23; ML ± 0.4; DV - 0.5 cm) or the LBL (AP - 0.31; ML ± 0.51; DV -0.5 cm). One week after recovery, the rats were trained in a one-trial inhibitory avoidance task (0.45 mA footshock; 1 sec) followed immediately by injections of lidocaine hydrochloride (Sigma; 0.01 mg; 40 mg/ml; 0.25 µL/1 min) or phosphate buffer administered via the cannulae. On a retention test 48 hr later, the latency to enter the dark/shock compartment (maximum 600 sec) was recorded and used as an index of retention. Animals that received buffer in the ACe did not differ from those that received buffer in the LBL. However, in comparison with buffer controls and ACe-lidocaine animals, LBL-lidocaine animals had significantly shorter entrance latencies. This retrograde impairment produced by posttraining reversible inactivation of the LBL suggests that the LBL may be involved in regulating the consolidation of aversively-based memory. This interpretation is in agreement with the finding of Liang (1991), who reported that pre-retention test injections of lidocaine into the basolateral amygdala impair retention only when retention is tested 2 days after learning, but not 21 days later.

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652.8

ACQUISITION OF NEGATIVE PATTERNING IN HIPPOCAMPAL LESIONED RATS. D.M. Skinner*, A. Bechara and D. van der Kooy. Dept. of Anatomy and Cell Biology, University of Toronto, Toronto, Ontario, M5S 1A8.

Hippocampal lesions do not impair acquisition of simple, Pavlovian associations, but more complex forms of learning do seem to be disrupted. We have previously shown that performance on complex, conditional tasks is not affected by hippocampal lesions when visceral cues (drug states and flavors) are used. We suggested that visceral cues were unique in that they can (unlike visual, auditory, and somatosensory cues) enter into configural associations independent of hippocampal processing. As a further test of this hypothesis we have designed a negative patterning task using visceral cues. In a negative patterning task each of two cues is followed by an unconditioned stimulus when presented singly, but the unconditioned stimulus is withheld when the two elements are presented together. We used a novel 0.1% saccharin solution and an injection of 5 mg/kg morphine sulfate as cues. Each of these cues alone signals an injection of 100 mg/kg lithium chloride. However, when presented together they signal the absence of lithium chloride. The acquisition performance of rats that had undergone aspiration lesions of the hippocampus was no different than control animals. Substituting contextual cues (visual and textural cues from a distinctive environment) for the morphine drug state made the task slightly more difficult, as evidenced by the need for a longer training period, but again lesioned and control animals performed at a similar level. The data provide further support for the idea that visceral cues can enter into configural associations in the absence of the hippocampus, whereas the same task employing visual and auditory cues does appear to depend on the hippocampus.

652.9

OPPOSITE ROLES OF VENTRAL AND DORSOLATERAL PERIAQUEDUCTAL GRAY IN CONDITIONAL FEAR-RELATED DEFENSIVE BEHAVIOR. J. Landeira-Fernandez*, M. S. Fanselow, & B. M. De Oca. Dept of Psychology, UCLA, L.A., CA 90024.

Lesions of the ventral portion of the periaqueductal gray (vPAG) reduce defensive freezing to conditional fear stimuli. The function of the more dorsal portions of this structure lateral to the aqueduct (dlPAG) on conditional freezing is unclear. In order to investigate the role of these regions of the PAG in mediating freezing, rats were given electrolytic lesions of either the vPAG or dlPAG. There were two types of controls (sham & superior colliculus lesioned). The rats were given electric footshock immediately after placement in a chamber for 3 consecutive days. This procedure does not provide the animal with ample time to appreciate the contextual cues prior to shock and therefore does not normally condition defensive behavior. However, rats with dlPAG lesions were unique in acquiring conditional freezing using this procedure. Following this treatment, all the rats received a shock after being given a 3 min period to explore the chamber. All rats, except those with vPAG lesions, evidenced a high level of conditional freezing . During several extinction tests, dlPAG lesioned animals showed enhanced, and vPAG lesioned animals showed reduced, freezing. Superior colliculus lesioned animals never differed from shams. These data suggest that the vPAG mediates conditional freezing while the dlPAG inhibits acquisition of this defensive behavior.

652.10

DORSOLATERAL PERIAQUEDUCTAL GRAY LESIONS AND BENZODIAZEPINE AGONISTS AND ANTAGONISTS DISSOCIATE ASSOCIATIVE AND NONASSOCIATIVE FEAR CONDITIONING. J. P. DeCola* & M. S. Fanselow, Dept. of Psychology, UCLA, Los Angeles, CA 90024.

Previously we demonstrated that administration of the NMDA antagonist APV, blocks associative fear conditioning but does not block the sensitization of conditional fear. The present experiments further dissociate associative and nonassociative fear conditioning. Sensitization of fear can be demonstrated behaviorally as an enhanced response to a conditional stimulus (CS) when conditioning is preceeded by exposure to unconditional stimuli (US). In our paradigm, the sensitization pretreatment is done by exposing rats to a series of footshocks in a distinct context the day prior to a single pairing of a novel contextual CS, and a single shock US. The sensitized animals exhibit enhanced fear to the novel context as compared to a nonsensitized group. Lesions of the dorsolateral periaqueductal gray (dlPAG) prior to the sensitization treatment resulted in an enhanced sensitization of fear to the novel context. Paradoxically, both the sensitization treatment and dlPAG lesions produced a reduction in unconditioned responding (activity) to the shock. Administration of the benzodiazepine (BZD) agonist tetrazepam (20 mg/kg, i.p.) prior to the sensitization treatment in the first context did not affect the sensitization of fear observed in the second novel context. However, it did block the acquisition of conditional fear to the first context where the pretreatment shocks were delivered. The BZD antagonist RO15-1788 (7mg/kg, i.p.) had no effect on conditional fear but tended to enhance the sensitization effect.

652.11

THE DORSAL HIPPOCAMPUS AND CONTEXTUAL FEAR CONDITIONING. S. L. Young*, M. S. Fanselow & D. L. Bohenek. Department of Psychology, University of California, Los Angeles, CA 90024.

Previously, we reported that electrolytic lesions of the dorsal hippocampus, made shortly after Pavlovian fear conditioning, eliminated the fear response to contextual stimuli associated with shock. The present studies sought to extend that finding to pre-conditioning elimination of cell bodies in that structure. We made excitotoxic lesions, which spare fibers of passage, prior to conditioning. Conditioning to contextual cues immediately after the first conditioning trial was not impaired in the lesioned animals. However, as training progressed, hippocampal animals showed markedly attenuated conditional responding. In a shock-free retention test given 24 hours later, hippocampal lesioned animals showed severe impairments in responding to contextual fear cues. This pattern of results closely parallels the effects of competitive NMDA antagonists on contextual fear conditioning. In conclusion, hippocampal lesions made either before or after training reduce contextual fear and this effect appears to depend on neurons intrinsic to the hippocampal formation.

652.12

BEHAVIORAL AND NEUROLOGICAL DIFFERENCES IN BXSB-Yaa AND BXSB-Yaa+ MICE L.M. Schrott*, G.F. Sherman, N.S. Waters G.D. Rosen, A.M. Galaburda and V.H. Denenberg Biobehavioral Science Graduate Degree Program, University of Connecticut, Storrs, CT 06269; and Beth Israel Hospital and Harvard Medical School Boston, MA 02215.

BXSB mice are autoimmune and have approximately a 50% incidence of cortical ectopias. Because of associations between immune disorders, ectopias and developmental learning disorders in humans, we have been investigating behavior in these mice. The autoimmunity in BXSB mice is not hormonally mediated and is more severe in males because of a Y-chromosome autoimmune accelerator gene. To determine the effect of this gene on behavior, BXSB mice with the autoimmune accelerator gene (BXSB-Yaa) were compared to BXSB mice lacking the gene (BXSB-Yaa+). The mice were tested on a behavioral battery, blood samples were obtained for immune analyses and their brains were examined for ectopias. Significant difference between the two genotypes were found for activity and learning measures. BXSB-Yaa were more active in a swimming rotation task, and performed better in discrimination learning and avoidance conditioning. BXSB-Yaa+ had better performance in water escape, the Lashley maze, and the Morris maze. Ectopias, in interaction with strain, sex, and paw preference, significantly affected learning measures. There was no difference in the incidence of ectopias between the two strains, although there was a significant sex difference with males having more ectopias (60%; and 40% for females).

Strain differences in males might be expected since they differed genetically. Strain differences between the two female groups are indicative of genetic drift. Therefore, it is unlikely that the males differ only with respect to the Yaa gene. The reason for the sex difference in ectopia incidence is not known, but is not due to the autoimmune accelerator gene, since a higher incidence was also seen in the non-accelerated strain. This work was supported in part by NIH grant HD 20806.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS—CONDITIONING III

653.1

EVIDENCE OF INTERACTION OF NEURAL PATHWAY SYSTEMS DURING ASSOCIATIVE LEARNING: UNCONDITIONED STIMULUS CAN MODIFY EVOKED SLOW POTENTIAL CHARACTERISTICS OF THE ALPHA RESPONSE. T.Korhonen* and M.Penttonen, Dept.of Psychol.,Univ.of Jyvaskyla,40100 Jyvaskyla, Finland.

General facilitation effect of the unconditioned stimulus (UCS) on the conditioned response has been reported in many studies. Here, more specific effect is shown: the time amplitude characteristics of evoked slow potential response in the conditioned stimulus (CS) period changed as a result of paired training similar to the waveform of the unconditioned response (UCR). A tone CS was given to one ear of the cat eliciting orienting head movements to the direction of the tone accompanied with evoked neural response in recorded brain sites. The brain stimulation UCS to the lateral hypothalamus elicited, in turn, a typical head movement and unconditioned evoked slow potential response pattern. Compared to the unpaired presentation of the CS and UCS a significant correlation (between .46 and .95) was found to emerge as a result of paired conditioning.Three alternative explanations were examined: first, the observed similarity between the evoked neural UCR and developing conditioned response during the interstimulus interval (ISI) might be results of a general, increased facilitation of the CS pathway, second, any strong stimulus such as an UCS might disclose some typical "maximum orienting response", specific to the CS pathway system measured, and third, the UCS may actually modify the CS-pathway system; it might leave a temporal "memory trace" on the time-amplitude course of the CS pathway system. The results presented here supported the "memory trace"-hypothesis.

653.2

FUNCTIONAL NEUROIMAGING OF CONDITIONED INHIBITION EFFECTS ON THE AUDITORY SYSTEM OF THE RAT: FLUORODEOXYGLUCOSE MAPPING AND STRUCTURAL MODELING. A.R. McIntosh* & F. Gonzalez-Lima, Dept of Psych and Inst of Neurosci, Univ of Texas, Austin, TX, 78712, USA.

[^{14}C(U)]2-fluoro-2-deoxyglucose (FDG) was used for functional neuroimaging of the auditory system processing an acoustic stimulus trained as either a conditioned excitor (A^+) or inhibitor (X-). Structural modeling (McIntosh & Gonzalez-Lima, *Brain Res.* 547, p. 295, 1991) used the mapping data to demonstrate interactions between auditory structures related to conditioning. Rats were trained in two phases. In the A^+ phase, a tone was paired with footshock for Group 1 and a light was paired with footshock for Group 2. In the X- phase, reinforced trials of A^+ were intermixed with nonreinforced trials of the tone-light compound (AX-). For Group 1, the tone was the excitor, for Group 2 the tone was the inhibitor. After conditioning, both groups were injected with FDG and presented with the same tone. Group 1 showed greater FDG uptake in the dorsal cochlear n. (DCN) and the external n. of the inferior colliculus. Structural models of the auditory system showed that effects through the direct lemniscal path were similar for both groups, but effects of lemniscal-adjunct paths were in opposite directions for the two groups. Extra-auditory influences on DCN were stronger for Group 2 suggesting that differentiation of the significance of the tone was a function of alterations in the interactions between parallel auditory pathways. These data indicate that auditory pathways can code both the physical parameters of stimuli and the behavioral significance acquired through learning. (Supported by NIMH grant RO1 43353).

653.3

SELECTIVE ACTIVATION OF THE CEREBRAL CORTEX OF RATS DURING CONDITIONED AND UNCONDITIONED STRESS - REVEALED BY THE IMMEDIATE EARLY GENE *C-FOS*. C. H. M. Beck* and H. C. Fibiger. Department of Psychology, University of Alberta, Edmonton, AB, T6G 2E9 and Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, Vancouver, BC, V6T 1Z3.

Male hooded rats were individually shocked acutely (1 session), shocked chronically (3 sessions), or were placed in the shock apparatus without being shocked. With the multiple cues of the shock box serving as contextual cues, the rats were assigned to groups (n=7) as unconditioned (shocked on the final session), conditioned (shocked previously but not on the final session), or control animals. Two hours after the beginning of the final session, the animals were terminated, perfused, and their brains removed. Following sectioning and immunohistochemistry, Fos-positive nuclei were counted on coronal sections of the cerebral cortex. Exposure to conditioned cues, compared to being placed in the box without shock, resulted in increased Fos counts in several allocortical (piriform, infralimbic, cingulate, retrosplenial, lateral orbital, perirhinal, and entorhinal) and isocortical structures (secondary occipital, frontal Area 2, forelimb, and hindlimb). Suprisingly, the unconditioned effect of acute shock appeared only in the retrosplenial cortex. The unconditioned effect of chronic shock was observed only in the insular, perirhinal, and hindlimb cortices. The relative unconditioned effects were small in part because of the large effect of simply exposing the rat to the apparatus.

653.4

FUNCTIONAL NEUROIMAGING OF DIFFERENTIAL CONDITIONING EFFECTS ON THE RAT AUDITORY SYSTEM: A FLUORODEOXYGLUCOSE WITHIN-SUBJECTS MAPPING STUDY. F. Gonzalez-Lima[1]* & J. Agudo[2]. [1]Dept Psych and Inst Neurosci, Univ of Texas, Austin, TX 78712; [2]Dept Cell Biol, Univ of Valladolid, Spain.

Autoradiography with [^{14}C(U)]2-fluoro-2-deoxyglucose (FDG) was used to functionally map the effects of a differential conditioning paradigm consisting of two tones of high and low frequencies, with one tone paired with water reward. Control rats were presented tones and water randomly or explicitly unpaired. This paradigm allowed direct comparison of the effects of the two tones within each auditory structure in the same subject, taking advantage of the separate tonotopic spatial maps for the two tones visualized with FDG. Differentially enhanced activity to the tone paired with the reinforcer was demonstrated within the cochlear nuclei, inferior colliculus, medial geniculate nucleus and auditory cortex. These effects were not simply a generalized increase in activity in the conditioned rats, but involved a frequency-dependent effect specific to the tone used to signal reward. Cortical tonotopic effects as revealed by FDG have never been obtained with the 2-DG method, suggesting that FDG is better suited for experiments where the goal is to map functional effects rather than calculation of glucose utilization. The FDG results provide a direct image within each auditory structure of the specific modification of the reinforced tone representation induced by the associative process, as opposed to the pure sensory effects of the tone. This supports previous studies showing that the auditory system is involved in associative learning of sounds signaling positive or negative reinforcers (Gonzalez-Lima & Agudo, Neuroreport 1, 161-4, 1990), and is not merely a feature detector system. (Supported by R01 MH43353).

653.5

CONDITIONAL CONTROL OF FLUID CONSUMPTION BY DISCRIMINATIVE CUES IS INDEPENDENT OF THEIR PAVLOVIAN ASSOCIATIONS. G.M. Martin, D.M. Skinner, A. Pridgar, and D. van der Kooy*. Dept. of Psychology, Memorial University, St. John's, Newfoundland, A1B 3X9. Dept of Anatomy and Cell Biology, University of Toronto, Toronto, Ontario, M5S 1A8.

Conditional associations involve discriminative cues that predict whether or not a target stimulus will be followed by a motivational event (the US). An important question in conditional associations is how much of the discriminative control is due to simple, Pavlovian associations between the discriminative cue and the US and how much is due to a more complex type of association among all three elements. In the first experiment, two groups of rats were trained using distinct contexts as cues (visual and textural stimuli from boxes). In the Pavlovian group a distinct, novel context was paired with an injection of lithium chloride (LiCl) while a second novel context was paired with injections of saline. In the Conditional group one novel context predicted that access to a saccharin solution would be followed by an injection of LiCl and the alternate context predicted that saccharin would be followed by saline injections. After conditioning, the Pavlovian group showed larger aversions to the context previously associated with LiCl than did the Conditional group. In contrast, the Conditional group showed greater suppression of consumption of saccharin and other novel fluids in the context associated with LiCl than did the Pavlovian group. These results suggest that the Conditional group learned something separate and different from the Pavlovian group. It might be possible to dissociate Pavlovian and conditional associations in the same animals. We predict that if the Conditional group were given unreinforced exposure to the distinctive environments this would result in the extinction of the mild place aversions seen in this group (presumably measuring the weak Pavlovian association) without abolishing the ability of the contexts to control saccharin consumption. Indeed, results from experiments using drug states or novel fluids as cues to predict when saccharin will be followed by LiCl suggest that extinction of the Pavlovian properties of the discriminative cue do not abolish the conditional control over fluid consumption. Thus, conditional suppression of saccharin consumption by various discriminative cues seems to result from complex, non-Pavlovian associations.

653.6

DOUBLE DISSOCIATION OF PASSIVE AVOIDANCE AND MILK MAZE DEFICITS WITH DISCRETE LESIONS OF THE SUBSTANTIA INNOMINATA OR GLOBUS PALLIDUS. R.C. Meyer* and G.D. Coover. Department of Psychology, Northern Illinois University, DeKalb, IL 60115.

We previously reported (Soc. Neurosci. Abstr., 17:480) that discrete electrolytic lesions along the rostrocaudal extent of the substantia innominata (SI) in the rat produced differential deficits in drinking passive avoidance (dPA), step-through PA (sPA), and milk maze (MM) performance. The present study examined the effects of bilateral lesions at three sites in the SI (placed relative to bregma): central (SI: A-1.4 mm, L±3.0, V-8.4), lateral (lSI: A-1.4, L±3.7, V-8.4), or rostral (rSI: A- 0.7, L±2.5, V-8.2). Also, lesions were placed in the globus pallidus: central (GP: A-1.4, L±3.2, V-7.2), or rostral (rGP: A-0.7, L±2.7, V-7.0).

Significant deficits in dPA were produced by SI and also lSI lesions, but not rSI, GP, or rGP lesions. The control group (CON) required 23 ±2 (M ±SE) footshocks of incrementing intensity to avoid drinking for 5 min. Groups SI and lSI required 38 ±2 and 35 ±3, and rSI, GP, and rGP required 24 ±2, 27 ±2, and 28 ±2 footshocks, respectively. Parallel results were found for sPA. In contrast to PA behavior, MM performance was poor in Groups rSI and GP, and markedly impaired in Group rGP. Particularly notable is that Groups SI and lSI, compared to Group rGP, were significantly worse in PA and better in MM performance: a double dissociation.

653.7

AMYGDALA CENTRAL NUCLEUS LESIONS ATTENUATE THE BRADYCARDIA, TACHYCARDIA AND BEHAVIORAL FREEZING ELICITED BY ACOUSTIC STIMULI IN RATS. B.J. Young* and R.N. Leaton. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

In a preliminary experiment phasic heart-rate (HR) responses of rats to a 120-dB startle stimulus were characterized by decelerations which habituated across trials, and accelerations which developed across trials in a manner that paralleled the development of freezing behavior. A 92-dB stimulus evoked little freezing or tachycardia yet evoked decelerations of similar magnitude to the 120-dB stimulus. Since lesions of the amygdala central nucleus (ACE) disrupt both conditioned freezing and HR responses, we studied the effects of ACE lesions in this startle-HR paradigm. Bilateral electrolytic lesions were made in one group of rats and another group served as sham-operated controls. Subgroups received 10 presentations of either an 87- or 120-dB white noise stimulus for 10 trials (60-s interstimulus interval) on four consecutive days. As predicted, ACE lesions impaired the development of both HR accelerations and freezing to the 120-dB stimulus. Surprisingly, the lesions also impaired HR decelerations to both the 87- and 120-dB stimuli. This latter finding conflicts with previous data which suggest no involvement of ACE in the unconditioned HR orienting response. In continued testing in a new context five presentations of a 1000-Hz, 85-dB tone were given to more directly contrast the present results with previous data. The HR orienting response remained impaired in the ACE animals. Thus, the ACE appears to play a role in mediating HR orienting responses as well as the development of fear-related tachycardia and conditioned freezing.

653.8

ACOUSTIC STARTLE STIMULATION SUPPRESSES IMMUNOLOGICAL FUNCTIONING AS INDEXED BY A CONTACT SENSITIVITY RESPONSE. R. Brown and J. Cranney* Univ. of NSW Australia, 2033.

The present studies investigated the immunological effects of novelty, startle stimulation and context conditioning in adult male Wistar rats. Behavioural indices of fear responding (freezing, grooming, defecation and exploratory behaviours) were investigated alongside immunological measures. Experiment 1 demonstrated that startle stimulation and exposure to a novel environment induced suppression of cell-mediated immune functioning, as indexed by the contact (skin) sensitivity response to a chemical allergen, specifically: diminished response magnitude, deceleration of the recruitment of that response and alteration in the nature of peak responding. Startle stimulation induced greater immunosuppression than just placing animals into a novel (startle chamber) environment alone. Experiment 2 demonstrated that the immunosuppressive effects of startle stimulation could become conditioned behaviourally in the rat. This suppression was more transient and of a smaller magnitude than that observed in rats exposed to unconditioned aversive stimulation, and manifested as the diminution of contact sensitivity response amplitude; freezing behavior correlated with immunosuppression.

653.9

EXCITOTOXIC LESIONS OF THE CENTRAL NUCLEUS OF THE AMYGDALA BUT NOT OF THE PERIAQUEDUCTAL GRAY BLOCK INTEGRATED FEAR RESPONDING AS INDEXED BY BOTH FREEZING RESPONSES AND AUGMENTATION OF STARTLE. M. Kiernan* and J. Cranney. UNIV. OF NSW, Australia, 2033.

Two experiments examined the effects of ibotenic acid lesions of the central nucleus of the amygdala (CeA) and the dorsal (dPAG) and ventral (vPAG) periaqueductal gray on the relationship between freezing responses and the sensitization of the acoustic startle response in adult male Wistar rats. CeA lesions attenuated both the non shock related sensitization of the startle and the sensitization of startle by shock, and attenuated post shock freezing and freezing elicited by shock associated cues. vPAG lesions attenuated freezing but failed to attenuate sensitization of startle, eliciting a tonic augmentation instead. dPAG lesions had no significant effect on either startle or freezing responses. These results support the hypothesis that the CeA constitutes part of the final common pathway of an integrated defensive response.

653.10

ABOLITION OF LATENT INHIBITION BY SYSTEMIC AMPHETAMINE TREATMENT. M. B. Noel*, A. Gratton & J. Rochford. Douglas Hospital Research Center, Dept. Psychiatry, McGill University, Verdun, Quebec, H4H 1R3, CANADA.

Preexposure to a to-be-conditioned stimulus (CS) retards conditioning when the CS is subsequently paired with an unconditioned stimulus (US). This phenomenon, known as latent inhibition (LI), can be abolished by systemic pretreatment with low doses of amphetamine. The majority of studies demonstrating dopaminergic involvement in LI have used relatively simple behavioral measures (e.g., conditioned licking) and have employed but a single test trial to assess LI. The present study was conducted to extend the generality of these results by using a more complex behavioral measure (lever pressing) and by employing multiple test trials. Male, Long Evans rats (300-325g) were deprived to 85% of their free feeding weights and trained (VI 30) to press a lever for sweetened milk reinforcement. Following acquisition, rats were preexposed to either 40 or 0 presentations of a tone CS. On the next day, all animals were administered 2 tone-shock (0.6 mA, 0.5 sec) pairings. Half of the animals were administered 2.0 mg/kg amphetamine (i.p.) 15 min prior to both the exposure and the conditioning day, the other half received vehicle. On the test day all animals were exposed (drug free) to three tone alone presentations, and the suppression of ongoing lever press responding was measured. LI was demonstrated by the finding that rats given 40 CS preexposures without drug were less suppressed on all three test trials than those not preexposed to the CS. Amphetamine treatment abolished LI; there were no significant differences in the level of suppression between amphetamine-treated rats preexposed to the tone and those not preexposed. These results confirm and extend the generality of previous evidence demonstrating that amphetamine-induced enhancement of dopaminergic transmission inhibits the ability to ignore irrelevant stimuli.

653.11

DIFFERENTIAL DISTRIBUTION OF PROTEIN KINASE C (PKCα,β and -γ) ISOENZYME IMMUNOREACTIVITY IN THE BRAIN OF THE DOMESTIC CHICK. J. J. Bolhuis[1], E.A. van der Zee[2], G. Horn[1] and P.G.M. Luiten[2]. [1]Univ. of Cambridge, Dept. Zoology, Cambridge CB2 3EJ, England and [2]Univ. of Groningen, Dept. Animal Physiology, Haren, The Netherlands (SPON: European Neuroscience Association).

PKC is involved in neural plasticity, and phosphorylation of a PKC substrate (MARCKS) in part of the chick hyperstriatum (IMHV) has been shown to correlate significantly with the strength of learning in filial imprinting (McCabe *et al*, Soc. Neurosci. Abs. 17, 140). The distribution of PKCγ and PKCα,βI,βII in the brain of day-old dark-reared chicks was determined immunocytochemically. Frozen coronal sections (20 µm) were incubated with MC5, or with 36G9, monoclonal mouse anti-PKCα,β or anti-PKCγ, respectively. PKCγ-stained cells were distributed widely in the telencephalon, incl. all hyperstriatal structures (incl. IMHV), the Hippocampus (H), Neostriatum (N), Ectostriatum (E) and Archistriatum (A). There was less dense staining in the Septum (S), and the least cellular staining was in the Paleostriatal complex (P). The distribution of PKCα,β-stained cells was more limited, with staining in A, H and S but not in the hyperstriatum. However, there was PKCαβ staining of fibres in part of IMHV (but little elsewhere in hyperstriatum ventrale), in N, P and in Lobus parolfactorius. These results raise the possibility that in IMHV, some axonal inputs contain αβ-PKC, whereas some postsynaptic cells contain the γ-form of PKC. (supported by the AFRC (UK)).

653.12

PASSIVE SHOCK AVOIDANCE (PSA) INDUCES CHANGES IN PKC-, MAP2- AND MUSCARINIC ACETYLCHOLINE RECEPTOR-IMMUNOREACTIVITY IN SINGLE CORTICAL NEURONS. E.A. Van der Zee*, B.R.K Douma, A.D. Strosberg, B. Bohus and P.G.M. Luiten. Lab. Animal Phys., Univ. of Groningen, Haren, The Netherlands.

Cortical activity is enhanced during PSA. In experiment I, changes in immunoreactivity (ir) for mAChRs, PKCγ and MAP2 after PSA were examined. 18 Young adult male Wistar rats were divided into 3 groups: naive controls (n=6), nonshocked controls, introduced to the testapparatus (n=6), and shocked animals (n=6). 24 Hr after the last trial, the animals were perfused. Cryosections were labeled for mAChRs (M35), PKCγ (36G9),and MAP2. In addition,fluorescencedouble-labeling was performed for mAChRs/MAP2and mAChRs/PKCγ. Cholinoceptiveneurons often express PKCγ,and mAChRs appeared to be highly colocalized with MAP2. Notably in the shocked group, the ir for all 3 markers was increased in individual neurons. These neurons were found in radial cortical columns, often in a lateralized fashion (either in left or right hemisphere). In experiment II, we examined the contribution of ACh to the enhanced ir. Of 6 rats, the nucleus basalis magnocellularis was unilaterally lesioned. Cortical columns with enhanced ir were still observed, both on the lesioned and nonlesioned side. However, clear deficits in PSA was observed when the columns overlapped with ACh depletion. The results reveal that 1) enhanced ir for mAChRs, MAP2 and PKCγ is found in single cortical neurons, 2) neuronal activation induced by PSA caused the changes in ir, 3) ACh is not required for this induction, but 4) learning deficits arise when the behaviorally activated neurons lack cholinergic innervation.

653.13

ROLE OF NITRIC OXIDE IN THE INDUCTION AND MAINTENANCE OF LONG LASTING POTENTIATION IN THE CHICK BRAIN SLICE. P.M. Bradley, B.D. Burns and A.C.Webb. Spon: Brain Research Association. Division of Neurobiology, Medical School, Newcastle upon Tyne, England.

The intermediate medial hyperstriatum ventrale (IMHV) is an area of the chick forebrain known to be involved in learning. We have shown that in a slice preparation it is possible to induce long lasting potentiation of a locally evoked field response within the IMHV by administration of tetanising stimulation at 5Hz. The present study was designed to investigate whether the transneuronal messenger nitric oxide (NO) was involved in the induction and/or maintenance of this potentiation.

A stable field response was evoked in the IMHV in a 500µm thick coronal forebrain slice by electrical stimulation at 0.1Hz. A single burst of 300 stimuli at 5Hz was then administered in an attempt to induce potentiation. The extent and duration of potentiation achieved during superfusion of the slice with Krebs' solution was compared to that obtained during superfusion of 1µm of the NO scavenger bovine haemoglobin.

In both situations the potentiating stimuli were followed by a rapid rise in the amplitude of the response. Under Krebs' this potentiation was maintained for at least 60 min whereas in the presence of haemoglobin no long term effects were seen and response amplitude returned to baseline values in a period with a mean duration of 20.2 ± 7.5 min (n=10).

The results presented here would suggest that nitric oxide has a role in the maintenance (but not the induction) of potentiation in the IMHV of the chick brain. Since our previous work has shown a predominantly postsynaptic locus for the changes associated with potentiation it may be that in the chick brain NO is acting as an intracellular rather than a transneuronal second messenger.

This work was supported by the SERC.

654.1

MIDAZOLAM IMPAIRS RETENTION OF BEHAVIORAL CONTRAST. J.A. Salinas*, H. Dickinson-Anson, & J.L. McGaugh. Center for the Neurobio. of Learning & Memory and Dept. of Psychobio., U. of Calif., Irvine, CA 92717.

Crespi (1942) showed that rats trained to run an alley for a large food reward display sharply increased latencies when shifted to a small reward. This effect is referred to as behavioral contrast and is usually interpreted as an aversive emotional reaction to a reduction in reward magnitude. Benzodiazepines have been shown to attenuate the behavioral effects of reward reduction, but the emphasis has been on their anxiolytic, not memory-impairing effects. To examine benzodiazepine effects on memory of a reward decrease we trained male Sprague-Dawley rats (175-200g) that were food deprived and maintained at 80% of body weight to run a straight alley (six trials per day) for either ten 45 mg food pellets or one 45 mg food pellet until asymptote was reached. Twenty minutes prior to the next training session, half the animals were injected (i.p.) with 1 mg/kg midazolam (MDZ) or saline. During the training session one half of the high reward group in each injection condition was shifted to one pellet. The reward conditions were maintained for two additional days of training. No further injections were given. Shifted animals receiving saline displayed a characteristic sharp increase in latency on the second day of shift training. This increase was not visible in the shift/MDZ animals on that day but it did appear on the third day. This study suggests that MDZ injected immediately prior to a reduction in reward magnitude impairs the retention of the aversive consequences of such a decrease.

Supported by NSF fellowship RCD-9054728 (JS) and PHS MH12526 (NIMH and NIDA) and ONR N00014-90-J-1626 (JLM).

654.2

INTRA-AMYGDALA INJECTIONS OF FLUMAZENIL DO NOT BLOCK THE AMNESTIC EFFECTS OF SYSTEMIC DIAZEPAM H. Dickinson-Anson*, C. Tomaz & J.L. McGaugh. Center for Neurobio. of Learning & Memory and Dept. of Psychobio., U. of Calif., Irvine, CA 92717

It is well known that benzodiazepines (BZD) induce anterograde amnesia in humans and laboratory animals. Recent findings suggest that the memory impairing effects of BZD's involve the amygdaloid complex (AC). Bilateral intra-AC injections of the BZD antagonist flumazenil (FZ) and agonist midazolam enhance and impair, respectively, retention performance of rats trained in aversively motivated tasks. We recently reported that excitotoxic lesions of the AC block diazepam (DZP)-induced retention impairment. Furthermore, bilateral lesions of the basolateral nucleus (BL) attenuated systemic DZP-induced retention deficits. Lesions of the central (CE) or lateral (LAT) nuclei of the AC had no effect. These data indicate the BL nucleus is necessary for systemic DZP to influence memory processes. To investigate this implication male rats (250-300g) were implanted with bilateral cannula in either the CE or BL nuclei of the AC. One week later, they were trained on a one-trial inhibitory avoidance (IA) task and tested 48-h later (step-through latency). Twenty min prior to training the rats received either FZ (1.5 or 2.5 μg) or vehicle (VEH) in the CE or BL nuclei followed by either DZP (2.5 mg/kg) or VEH ip. Pre-training administration of VEH ip plus FZ in either amygdaloid nuclei produced enhanced retention performance. In contrast, a robust retention impairment was observed in animals that received DZP ip and intra-AC injections of VEH. The DZP-induced retention deficits were not attenuated by administration of FZ in the AC. These results suggest that although endogenous BZD activity within the AC can influence memory processes, systemic DZP may influence memory by acting on other brain systems. The present results are consistent with our previous findings suggesting that the BL nucleus must be intact in order for DZP to initiate its memory modulating effects in other brain systems. [Supported by PHS MH12526 (NIMH & NIDA) & ONR N000-14-90-J-1626 (to JLM)]

654.3

THE ACQUISITION AND RETENTION DEFICITS OF DIAZEPAM IN A MODIFIED STEP-DOWN PASSIVE AVOIDANCE PARADIGM DO NOT TOLERATE FOLLOWING CHRONIC DRUG ADMINISTRATION IN RATS. A.D. Kastello and J.V. Cassella*. Behavioral Biology, Neurogen Corporation, Branford, CT 06405.

Avoidance paradigms are useful in studying learning and/or memory processes following various physiological or pharmacological manipulations. The present procedure used a modified step-down passive avoidance task allowing for assessment of acquisition and retention processes.In normal animals the shock-response retention function was non-monotonic. Pharmacological studies were conducted (drugs given IV prior to acquisition trials). Scopolamine (0.2-0.8mg/kg) impaired acquisition only while MK-801(0.025-0.5mg/kg) produced both acquisition and retention deficits. Acute dosing with the BZ agonists diazepam(0.12-0.5mg/kg) or triazolam (0.015-0.125mg/kg) impaired both acquisition and retention. Both the BZ partial agonist, RO 16-6028 (0.125-2.0 mg/kg), and the Type-I selective BZ agonist, alpidem (1.0-4.0 mg/kg) produced retention deficits only. A 0.5mg/kg(IV) dose of DZ produced marked sedation and significant acquisition and retention deficits. However, following chronic treatment with DZ (5 mg/kg,IP; b.i.d.; 7 days), a 0.5mg/kg(IV) dose of DZ produced the same avoidance deficits without sedation. Thus, this paradigm demonstrates that the learning and memory impairments of diazepam do not tolerate and suggests that the drug's sedative and amnestic effects are mediated by different mechanisms.

654.4

INTRASEPTAL INFUSIONS OF CHLORDIAZEPOXIDE IMPAIR PLACE LEARNING IN THE MORRIS WATER MAZE: REVERSAL BY FLUMAZENIL BUT NOT TETRAHYDROAMINOACRIDINE (THA). R. K. McNamara* & R. W. Skelton. Dept. Psychology, Univ. Victoria, P.O. Box 3050, Victoria, British Columbia, Canada, V8W 3P5.

Peripherally administered chlordiazepoxide (CDP) impairs place learning in the Morris water maze (MWM; McNaughton & Morris, 1987, Behav. Brain. Res., 24: 39). The present study sought to determine if this impairment is mediated by the septohippocampal cholinergic system.

Rats were implanted with a cannula aimed at the medial septum. CDP (10, 30 or 60 nmol) was dissolved in a 1 μl volume of aCSF and infused over a three minute period; controls were infused with aCSF. Rats were trained in the MWM (1 d, 20 trials followed by probe; 1 min ITI: 15 s on platform, 45 s in warming cage). Intraseptal infusions of CDP at concentrations of either 30 or 60 nmol, but not 10 nmol, impaired place learning; rats took longer swim paths to locate the submerged platform and did not show a bias for the correct quadrant during the probe trial. None of the CDP concentrations impaired performance on a visible platform task. On the following day, all rats were able to acquire a new platform position in the absence if intraseptal infusions, suggesting that the impairment found during initial acquisition was not due to septal damage. In a new group rats, systemic flumazenil (10 mg/kg) or THA (1 or 3 mg/kg) was coadministered with intraseptal CDP (60 nmol). Flumazenil, but neither dose of THA, reversed the place learning deficit produced by CDP. Together, these results suggest that the amnesic effects of CDP in the MWM are mediated by septal benzodiazepine/GABA-A receptors, independent of the septohippocampal cholinergic system.

654.5

INTRASEPTAL DIAZEPAM-BINDING INHIBITOR (DBI) AND FLUMAZENIL ENHANCE RETENTION OF A SINGLE SESSION SPATIAL WATER MAZE TASK. R.W. Stackman* and T.J. Walsh. Department of Psychology, Rutgers University, New Brunswick, NJ 08903.

Cholinergic cells in the medial septum (MS) innervate the hippocampus (HPC) and modulate spatial memory processes. GABA/BDZ receptors located on these cells are capable of functionally regulating the cholinergic activity in the HPC. Intraseptal injection of BDZ agonists decrease the activity of these neurons; while BDZ antagonists and inverse agonists increase their activity (Walsh et al., submitted). Systemic and intraseptal, but not intraamygdala, injection of the BDZ chlordiazepoxide impairs working memory in rats (Stackman & Walsh, 1992). The MS contains a high density of endogenous BDZ agonist-like molecules that are released during stressful events (Wolfman et al. 1991). DBI, an endogenous neuropeptide, induces a negative modulatory action at BDZ receptors, and behavioral effects characteristic of an inverse agonist. The involvement of DBI and BDZ receptors in spatial memory formation was evaluated in this experiment. Adult male Sprague-Dawley rats were implanted with single guide cannulae in the MS. The rats were trained in the Morris water maze for a single session of 16 trials, during which the platform's position remained constant. Immediately after the 16th training trial, rats were injected into the MS with either DBI (4 nmoles), flumazenil (10 nmoles), or artificial CSF. Retention of memory for platform location was assessed 24 hr. after training, by a 60 sec probe trial. During the first 15, and 30 sec of the probe trial, DBI- and flumazenil-treated rats spent a significantly greater amount of time in the training quadrant than the CSF-treated controls. These data indicate that intraseptal infusion of DBI and flumazenil enhanced retention, possibly via an antagonism of endogenous BDZ-like molecules released during the training event. These data support an endogenous BDZ mechanism in the MS that is capable of modulating the processing and retention of spatial information. Supported by NSF grant BNS 9109163 to TJW.

654.6

INTERACTIONS BETWEEN THE EFFECTS OF BASAL FOREBRAIN LESIONS AND CHRONIC TREATMENT WITH MDL 26,479 ON LEARNING AND MARKERS OF CHOLINERGIC TRANSMISSION. L.A. Holley[1], J.A. Miller[2], P. Chmielewski[2] and M. Sarter[1]*. [1]Dept. Psychology, Ohio State Univ., Columbus, OH 43210, and [2]Marion Merrell Dow Research Institute, Cincinnati, OH 45215.

Behavioral and biochemical data have suggested that the triazole MDL 26,479 acts as a benzodiazepine receptor selective inverse agonist. It has been assumed that the beneficial behavioral effects of such compounds are likely to depend on the activation of cortical cholinergic afferents. The interactions between the effects of MDL 26,479 and basal forebrain lesions were tested. Lesioned animals were impaired in learning a conditional visual discrimination task and showed a marked decrease in cortical hemicholinium binding. Chronic treatment with MDL 26,479 (5 mg/kg; i.p.) produced a modest facilitation of learning in lesioned animals but did not significantly affect hemicholinium binding. Performance of all animals significantly correlated with frontal cortex hemicholinium binding. It is assumed that the relatively limited potency of MDL 26,479 to facilitate learning was based on the fact that the lesion destroyed about 95% of all basal forebrain cholinergic cell bodies and therefore significantly confined the drug's potential to activate cortical cholinergic transmission via the basal forebrain GABA-cholinergic link.

654.7

DISSOCIATION BETWEEN THE EFFECTS OF BENZODIAZEPINE RECEPTOR AGONISTS ON BEHAVIORAL VIGILANCE AND RESPONSIVITY. P. Dudchenko*, B. Paul and M. Sarter. Dept. Psychology and Neurosci. Program, Ohio State Univ., Columbus, OH 43210.

The effects of the benzodiazepine receptor (BZR) full agonists chlordiazepoxide (CDP) and midazolam, and the partial agonist ß-carboline ZK 91 296 on the performance of rats in a simple reaction time paradigm were examined. This task required the animals to respond to rarely and unpredictably occurring brief (50 msec) visual stimulus. The dose-dependent effects of midazolam on signal sensitivity and general responsivity occurred in parallel. In contrast, the effects of CDP on signal sensitivity were largely independent from effects on response bias. The partial agonist ZK 91 296 in general had little effect on performance. Extension of the stimulus presentation time attenuated the effect of CDP on signal sensitivity. These results support the hypothesis that BZR agonist-induced disruption of attentional abilities is not necessarily confounded by effects on general responsivity or sedation, and thus may represent a discrete pharmacological property of these compounds. We are currently examining the hypothesis that the CDP-induced impairment in behavioral vigilance depends on the integrity of the basal forebrain GABA-cholinergic link.

654.8

NEUROANATOMICAL DISSOCIATION OF THE ANXIOLYTIC AND AMNESIC ACTIONS OF CHLORDIAZEPOXIDE. W. B. Gallagher*, R. K. McNamara & R. W. Skelton. Dept. Psychology, Univ. Victoria, P.O. BOX 3050, Victoria, British Columbia, Canada, V8W 3P5.

Both clinical and experimental evidence have found systemic chlordiazepoxide (CDP) to reduce anxiety and impair new learning. Whether these two actions are causally related is unknown. The present study sought to determine if different neuroanatomical regions mediate the anxiolytic effects of CDP on exploration (thigmotaxis) in an open field and the amnesic effects of CDP on place learning in the Morris water maze.

Firstly, rats received systemic (i.p.) injections of either CDP (5 mg/kg) or saline and tested for anxiety in the open field and amnesia in the water maze (1 d, 20 trials followed by probe; 1 min ITI, 15 s on platform, 45 s in warming cage). Systemic CDP suppressed thigmotaxia (reduced anxiety) and impaired place learning (produced amnesia). New rats were implanted with a cannula aimed at either the medial septum, or bilateral cannulae aimed at amygdalea or frontal cortex. CDP (60 nmol) was dissolved in a 1 µl volume of aCSF and infused over a three minute period. Controls were infused with aCSF. Intra-amygdala infusions of CDP suppressed thigmotaxia but had little effect on place learning and intra-septal infusions of CDP had little effect on thigmotaxia but impaired place learning. Infusions of CDP into the frontal cortex had little effect on either thigmotaxia or place learning. These results suggest that the anxiolytic and amnesic actions of systemic CDP are mediated by the amygdala and medial septum, respectively. (Research Supported by B.C. Health Care Research Foundation and NSERC, Canada)

654.9

REVERSAL OF CHLORDIAZEPOXIDE-INDUCED IMPAIRMENT IN DISCRIMINATION PERFORMANCE BY PICROTOXIN. S. O. Cole* and J. V. Martin. Departments of Psychology and Biology, Rutgers University, Camden, NJ 08102.

The effects of chlordiazepoxide (CDP) alone and in combination with picrotoxin (PTX) on the performance of a previously-learned go-no go successive discrimination were studied in male Sprague-Dawley rats. CDP 10 mg/kg impaired discrimination in five successive drug sessions, with animals demonstrating recovery in a single post-drug session. The impairment in discrimination performance was due to an increase in responding during the no go periods of the task (errors of commission). The γ-aminobutyric acid (GABA) antagonist PTX (0.5, 1.0 mg/kg) reversed the impairment in discrimination performance and reduced the number of incorrect responses in a generally dose-dependent manner when co-administered with CDP.

Although the co-administration of CDP and PTX also produced a significant reduction in responding during go periods of the task (which further reduced the overall response rate), this effect worked against an improvement in discrimination performance rather than contributing to it. When administered alone, PTX produced no significant change in discrimination performance.

These findings suggest that the impairment in discrimination performance by CDP is mediated by actions on the central $GABA_A$ receptor-benzodiazepine receptor-chloride channel complex.

654.10

MEMORY DEFICITS RESULTING FROM DIENCEPHALIC DAMAGE IN MICE ARE REVERSED BY AN I.P. ADMINISTRATION OF METHYL β-CARBOLINE-3-CARBOXYLATE. D.J. Beracochea (*1), A. Krazem (2) and R. Jaffard (1). (1)Lab. Neurosciences Cognitives et Comportementales, CNRS URA 339, Univ. Bordeaux 1, Av. des Facultés,33405 Talence, France and (2) Université de Tizi-Ouzou, Algeria.

Diencephalic damage was induced either by a chronic (12-months) alcohol consumption (AC)(see **Bontempi et al** poster) or by experimental ibotenic acid lesions of the mamillary bodies (MB). Previous studies showed that both AC and MB lesions decreased anxiety in the open-field and in the elevated-plus maze, and induced memory impairments in spatial alternation tasks. We hypothetized that hypoanxiety might be in part responsible for these deficits. Accordingly, increasing anxiety in experimental subjects should attenuate the memory impairments. Three doses of β-CCM (0.25, 0.5, and 1.0 $mg.Kg^{-1}$) were used, according to their previously determined anxiogenic properties. Results showed that the administration of β-CCM improved alternation rates in all groups, as compared to saline-treated subjects.However, the alternation deficits were reversed by a lower dose in the AC group ($0.5mg.Kg^{-1}$) than in the MB group ($1.0 mg.Kg^{-1}$). Since AC induced weaker lesions of the MB than a direct administration of ibotenic acid, one can conclude that part of the facilitative effect of β-CCM might be a function of the severity of damage to the mamillary system.

LEARNING AND MEMORY: PHARMACOLOGY—OTHER II

655.1

STATE-DEPENDENT EFFECTS IN TASTE AVERSION LEARNING. Bernard M. Rabin* and Walter A. Hunt. Behavioral Sciences Dept., AFRRI, Bethesda, MD 20889 and Dept. of Psychology, Univ. of MD, Baltimore, MD 21228.

State-dependent learning can occur when the experimental conditions under which a response is acquired differ from those under which its recall is tested. As a result, the learned response may not be performed. Attempts to prevent conditioned taste aversion (CTA) learning may be confounded by the failure to maintain identical conditions on both acquisition and test days. Studies were run using histamine to block a radiation-induced CTA; AMPT to block an amphetamine-induced CTA; and diazepam to block a lithium chloride-induced CTA. In each experiment, the greatest disruption of CTA learning occurred when the drug was administered prior to the conditioned stimulus on the conditioning day, but not on the test day. Pretreating the animals with the drug on both conditioning and test days significantly reduced the effectiveness of the drugs in preventing the acquisition of a CTA.

655.2

COMPARISON OF AVERSIVELY MOTIVATED TASK (PASSIVE AVOIDANCE TASK) AND APPETITIVE WATER-FINDING TASK ON ACQUISITION OF LEARNING IN MICE. M. Hiramatsu[1]*, K. Maezawa[1], K. Ichihara[1], T. Nabeshima[2] and T. Kameyama[1]. [1]Dept. of Chem. Pharmacol., Meijo Univ., Nagoya 468, Japan and [2]Dept. of Neuropsychopharmacol. & Hospital Pharmacy, Nagoya Univ. Sch. of Med., Nagoya 466, Japan.

We examined the development of a latent learning behavior using 'water-finding task' in mice. If animals without deprivation have had previous experience of exploring an open field containing a water-tube, they find the water source more quickly than non-experienced mice when they are deprived water. In this method, mice are not required to perform a special behavior in the pre-exposure stage. We compared this learning behavior with that in one-trial passive avoidance task in mice. The mice were deprived of water for about 24 hr before the test trial. The latencies to entering the alcove (entering latency), drinking water (drinking latency) and starting to drink water after first entering the alcove (finding latency) were measured for each mouse. In water-finding task, finding and drinking latencies were time dependently prolonged: these latencies 5 and 6 days after training were significantly longer compared with those of 1-day group. Up to, at least, 14 days after training, step-down latency in the passive avoidance task did not alter. Cycloheximide treatment immediately after training significantly prolonged finding and drinking latencies but shorten step-down latency. These results indicate that the finding and drinking latencies seem to be indices of learning and memory. Extinction rate of memory from non-reinforced learning is faster than that from negatively reinforced learning. This result also indicates that the process of memory (motivation, acquisition and/or retention) may be different in these two types of behavioral tasks using appetitive and aversive stimuli.

655.3

BEHAVIOURAL, BIOCHEMICAL AND HISTOLOGICAL EFFECTS OF TRIMETHYLTIN-INDUCED BRAIN DAMAGE IN THE RAT.

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The nature and extent of the behavioural, biochemical and histological changes induced by the organotin compound trimethyltin (TMT) were assessed. Male Sprague-Dawley rats were treated with a single injection of TMT (6.0, 7.0 or 8.0 mg/kg i.p.) and behavioural experiments were conducted 21 - 28 days afterwards. The behavioural consequences of TMT administration were dose-dependent and included (a) hyperactivity in the open-field test, (b) increased locomotor activity and (c) deficits in passive avoidance behaviour, T-maze alternation and Morris water maze performance. Post-mortem histological analysis revealed damage to hippocampal pyramidal cells, an effect which was particularly apparent at 8.0 mg/kg. After a single injection of TMT, HPLC and fluorimetric assays showed that 5-HT and GABA levels in the hippocampus and amygdala were decreased. Furthermore, using quantitative autoradiography, M_1 and M_2 receptor binding sites were found to be markedly diminished in the hippocampus. These results suggest that the toxic interaction of TMT with the hippocampus and other limbic brain regions may be responsible for its detrimental effect on learning and memory.

655.4

NEUROCHEMICAL CHANGES IN BRAIN OF RATS WITH TRIMETHYLTIN-INDUCED HIPPOCAMPAL LESION AND EFFECT OF NEURAL GRAFTS.

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Trimethyltin(TMT) is reported to produce learning impairment, hyperactivity and tail mutilation and to induce a specific loss of pyramidal cells in hippocampal CA3 and CA4 subfields. We found fetal septal grafts, rich in cholinergic neurons, ameliorated learning deficits in TMT-treated rats.

We here examined whether TMT and neural grafting can affect brain levels of somatostatin and neuropeptide Y and the activity of choline acetyltransferase (ChAT). Both peptides are known to exist abundantly in hippocampus and limbic structures including entorhinal cortex, afferent pathway into hippocampus. Male Sprague-Dawley rats were gavaged with TMT-hydroxide (9mg/kg) at 6 wks of age and were grafted with fetal septal cells (ED 15) or CA3 cells (Day 1) a week later. Morris water maze task was performed 6 wks after transplants, and then the rats were subjected to neurochemical evaluation. Both peptides were found to markedly increase after TMT treatment in entorhinal cortex, but not in hippocampus. Transplants successfully ameliorated place-navigation deficits in TMT-treated rats, nevertheless failed to alter hippocampal levels of both peptides. In TMT rats a significant elvation of ChAT activities was noted in CA3 but not in CA1.

655.5

MEMORY EFFECTS OF NEUROSTEROIDS INJECTED INTO THE NUCLEUS BASALIS MAGNOCELLULARIS OF THE RAT.

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Since the discovery of marked cell loss and various pathological alterations in the nucleus basalis magnocellularis (NBM) in patients suffering from senile dementia of the Alzheimer type, this structure has been the subject of much attention. The NBM has been reported to be the main source of cortical cholinergic innervation. Various afferents of the NBM have been identified, and particularly a GABAergic afferent originating in the nucleus accumbens, that forms contacts with NBM cholinergic cells. GABA-A and Benzodiazepines (BZD) binding sites have been detected in the NBM. Functionally, local injections of GABAergic agonists into the NBM have been shown to block or reduce the activity of NBM cholinergic neurons. Behavioral studies using local injections of GABAergic agonists or antagonists into the NBM have been shown that this structure was involved in memory processes.

Recently it has been demonstrated an interaction between endogenous steroids and the central GABA receptors. Steroids-GABA receptor interactions may have a role in the regulation of the NBM cortical projections. In order to test this hypothesis, we investigated the effects of local injection of Pregnenolone sulfate (a GABA-like antagonist) and Tetra-Hydro-Progesterone (a GABA-like agonist) into the NBM on performance in a new two-trial memory task.

Our results show that local injection of Pregnenolone sulfate into the NBM improves memory performances in the Y maze recognition task. Conversely the injection of Tetra-Hydro-Progesterone decreases memory performance in this task.

In conclusion, endogenous steroids can modulate memory processes and NBM-related dysfunctions. However, it remains to be elucidated if this effect results from an interaction at the GABA receptor level.

655.6

SELECTIVE DECREASE IN DECLARATIVE MEMORY PERFORMANCE IN NORMAL HUMAN SUBJECTS TREATED WITH DEXAMETHASONE

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A range of evidence supports the role of hippocampus in declarative memory performance. Hippocampal glucocorticoid (GC) receptors are an important neural target for GCs and brief physiologic GC exposure produces site-selective hippocampal GC receptor down-regulation. Four days of double-blind, placebo-controlled treatment with physiologic doses of dexamethasone (DEX) was given to normal subjects, hypothesizing an effect on declarative memory performance, but not performance on other cognitive measures. Subjects received either oral placebo (N = 9) or DEX (N = 10) on study days 1-4, in doses of 0.5, 1, 1, and 1 mg. Cognitive testing, using paragraph recall (immediate and 30 min. delay), serial addition, auditory trails/vigilance, and the Benton line orientation task, was performed on days 1 (baseline), 2, 5, and 12 (7 day recovery condition).

DEX, and not placebo, treatment resulted in a selective decrease in declarative memory performance, followed by post-treatment recovery. In DEX-treated subjects, repeated measures ANOVA found an overall effect of study day on delayed recall ($F[3,27] = 6.06$, $p < 0.01$), with a trend toward an effect on immediate recall ($F[3,27] = 2.37$, $p < 0.09$). The greatest decrease from baseline delayed recall performance was observed at day 5 ($F[1,9] = 7.80$, $p < 0.05$). No other cognitive measure showed a significant effect of study day. These results demonstrate a selective effect of DEX on declarative memory performance.

655.7

ACUTE ETHANOL DISRUPTS PATTERN CONDITIONING BUT NOT CONTEXT CONDITIONING IN ADOLESCENT AND ADULT ANIMALS.

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Effects of alcohol administration on conditioning to a visual stimulus and an olfactory context was examined in both adult (60-70 day old) and periadolescent (35-38 day old) rats. Conditioning occurred in a patterned chamber divided into two compartments , one with vertical black and white alternating stripes (CS-) and one with horizontal stripes (CS+); both contained an orange contextual odor. Paired animals were given 6 training trials consisting of a 20 sec placement in the CS- chamber followed by a 20 sec exposure to the CS+ chamber, during which two 3 sec 1 mA footshocks were given. Unpaired animals received the same regimen of footshock exposure in a black chamber 10 min prior to exposure to the patterned chamber and the orange odor. Ethanol (2.0 g/kg) or saline was intragastrically administered 10 min prior to conditioning. Animals were given one of two 5 min preference tests after conditioning: either between the CS+ and CS- or between a novel lemon odor and the orange odor. Ethanol did not disrupt conditioning to the odor context; at both ages paired animals pretreated with saline or alcohol exhibited an equivalent aversion to the orange odor. However, conditioning to the CS+ was significantly impaired by alcohol. While paired animals at both ages given alcohol did not acquire an aversion to the CS+, paired saline animals did learn the CS-US association. Thus it appears that a modest dose of alcohol impairs conditioning to a visual CS+ but not to the more general olfactory context in which the CS+ occurred.

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655.8

PHARMACOLOGICAL MODULATION OF ACUTE-ETHANOL-INDUCED MEMORY BLACKOUTS IN RATS.

D.H. Epstein and L.A. Pohorecky*. Center of Alcohol Studies, Rutgers University, Piscataway, NJ 08855.

This study explored the neurochemical mechanisms of ethanol-induced amnesia. Behavioral interactions of ethanol (ET) with drugs whose actions are neurotransmitter-specific were evaluated in a paradigm that assessed the clinically relevant type of memory (episodic) under clinically relevant conditions (intoxication during acquisition). Male Long-Evans rats were trained in a Y-maze active-avoidance task whose episodic-memory component took the form of repeated reversals, each of which had to be remembered over a delay of up to 180 minutes. ET (20% w/v in saline, 2 g/kg IP) was shown to produce delay-dependent deficits in memory consolidation, which were not dependent on deficits in acquisition or procedural memory. This ET-induced amnesia was partly prevented by coadministration of the antiGABAergic drug Ro 5-3663 (5 mg/kg IP), mimicked and enhanced by $GABA_A$-receptor activation with chlordiazepoxide (1, 3, or 10 mg/kg IP), and also mimicked and enhanced (but to a lesser degree) by general GABAergic augmentation with aminooxyacetic acid (15 mg/kg IP). This finding suggests that $GABA_A$ receptors have a greater role than $GABA_B$ receptors in ET-induced amnesia. Activation of $5\text{-}HT_{1A}$ receptors with 8-OH-DPAT (.16, .32, or .64 mg/kg IP) unexpectedly produced the most severe and behaviorally specific amnesia of any of the treatments, but the interaction of 8-OH-DPAT with ET, and the two drugs' differing interactions with a $5\text{-}HT_{1A}$ antagonist ([-]propranolol, 8 g/kg IP), ruled out $5\text{-}HT_{1A}$ activation as the mechanism of ET-induced amnesia. The significant but not complete prevention of blackouts by GABAergic antagonism suggests the involvement of other transmitter systems.

655.9

STAGES OF MEMORY FORMATION IN 2 AREAS OF CHICK BRAIN. M.R. Rosenzweig*, D.W. Lee, S.S. Shrawder, & E.L. Bennett. Dept. of Psychology, Univ. of California, Berkeley, CA. 94720, USA.

Two-day-old chicks were given bilateral i.c. injections into the intermediate medial hyperstriatum ventrale (IMHV), trained on a 1-trial peck avoidance task using a target bead dipped in methyl anthranilate (MeA) then tested from 10 s to 24 h posttraining. After strong training (100% MeA), memory formation is impaired by 5 min posttraining by glutamate (GLUT, a short-term memory inhibitor), by 20 min by ouabain or scopolamine (OUAB or SCOP, intermediate-term memory inhibitors), and by 90 min by anisomycin (ANI, a long-term memory inhibitor). Memory for weak training (10% MeA) is also impaired by GLUT and ANI at the same doses and times as for strong training. Neither ITM inhibitor performed as predicted: OUAB impaired weak memory only at a dose that resulted in severe side effects, and SCOP inhibited weak memory at 24 h only. In contrast, injections of OUAB into lobus parolfactorius (LPO) resulted in significant memory impairment at the same doses and times as for strong training. Supported by NSF grant BNS-88-10528 & NIDA grants DA04795 and DA05396.

655.10

SEASONAL MODULATION OF LEARNING AND MEMORY IN CHICKS. D.W.Lee*, G.G. Murphy, E.L. Bennett, & M.R. Rosenzweig. Dept. of Psychology, Univ. of California, Berkeley, CA. 94720, USA.

Two-day-old chicks were trained on a 1-trial peck avoidance task using a target bead dipped in either 5, 10, or 100% methyl anthranilate (MeA). Groups were tested at times from 10 s to 24 h posttraining. Runs were conducted during 2 summers (Jun-Aug) and winters (Nov-Jan). At every test time, groups of chicks trained with 100% MeA showed the highest avoidance, followed by 10%, then 5% MeA; retention is dependent upon strength of training.

Winter chicks did not differ from summer chicks during training but showed less retention at test. They were also heavier and more active, but neither measure correlated with test performance. Performance for each strength of training was positively correlated to daylength.

In summer, test performance averaged over all 3 training strengths showed retention deficits ("dips") at 1', 15', and 60' test times. In winter, dips were present at 1-5', 30', and 90' test times - thus winter not only impairs but also slows down memory formation on this task.

Supported by NSF grant BNS-88-10528 & NIDA grants DA04795 and DA05396.

655.11

MOLECULAR MECHANISMS FOR MEMORY FORMATION IN THE 2-DAY OLD CHICK: THE ROLE OF PKC AND OTHER KINASES. P.A. Serrano*, M.L. Leissring, E.L. Bennett, and M.R. Rosenzweig. Department of Psychology, University of California, Berkeley, CA. 94720.

The three stage model of memory formation proposed by Gibbs and Ng (1977) and extended by results from our laboratory was used to determine the role of protein kinase (PK) activity during memory formation. Groups of chicks were trained on a 1-trial peck-avoidance task 5 min after amnestic agents were injected bilaterally into the intermediate medial hyperstriatum ventrale (IMHV). The time of appearance of amnesia induced by these PK inhibitors indicates the stage of memory formation disrupted by each agent. Our results show that intermediate-term memory (ITM) formation can be disrupted by agents affecting calcium or CAM kinase activity (e.g., W-9, W-13, HA-1004, TFP, A-3). Long-term memory formation can be disrupted by inhibiting PKC, PKA or PKG by agents such as H-7, H-8, H-9, ML-9 and HA-156; these agents had no effect on ITM. W-9, TFP, H-7 and HA-156 produce amnesia when injected into the left IMHV but not when injected into the right IMHV.
Supported by NSF grant BNS-88-10528

655.12

IMPAIRMENT AND ENHANCEMENT OF SPATIAL DISCRIMINATION LEARNING INDUCED BY THE INTRA-HIPPOCAMPAL INJECTION OF POLYMYXINE B : A PROTEIN KINASE C INHIBITOR. X. Nogues, J. Micheau* and R. Jaffard. Lab. Neurosciences comportementales et cognitives, URA CNRS 339, Univ. Bordeaux I, Avenues des Facultés 33405 Talence Cedex France.

Electrophysiological and morphological data suggest that protein kinase C (PKC) is implicated in several forms of neuronal plasticity. Thus it has been proposed that activation of PKC and phosphorylation of particular substrates are critical events in the neuronal plasticity leading to an organic support of memory traces. In accordance with this statement, we have previously shown that spatial learning induced a decrease in cytosolic PKC activity in the hippocampus (NOGUES et al., 1990, Soc. Neurosci. Abst., Vol. 16, 316-14). In order to investigate the functional aspects of these modifications, we have compared the mnemonic effects of intrahippocampal injections of either polymyxine B (PMB, 10 mM) or 2% lidocaïne (0.2 µl bilaterally) on a mixed reference working memory task in a radial maze. When injected 15 minutes before each daily session, PMB was shown to slow down acquisition rate of reference memory. However in contrast with all other groups PMB-treated animals exhibited no forgetting on a retention test performed 16 days following acquisition. The beneficial influence of PMB treatment on forgetting might be explained by a post-acquisition rebound effect on PKC produced by the cessation of the PMB treatment. An alternative hypothesis (i.e. the induction of a direct extrahippocampal long-term storage) is currently under investigation.

655.13

IN VIVO AND IN VITRO EFFECT OF COGNITION ENHANCING DRUGS ON RAT BRAIN PROTEIN KINASE C ACTIVITY. L. Lucchi, S. Govoni, F. Battaini^, M. Trabucchi*^ Inst. Pharmacol. Sci, Univ. Milano, ^Dept.Exptl. Med. and Biochem. Sci., II[nd] Univ. of Roma, Italy.

Associative behaviour produces brain Protein Kinase C (PKC) translocation suggesting a role for this enzyme in learning and memory. The present data demonstrate that some cognition enhancing drugs promote PKC activation in rat brain following "in vivo" administration. In particular, the acute treatment with oxiracetam (OXI), a nootropic drug that stimulates cholinergic transmission and glutamate release, produced in rat cortex an increase of PKC activity in particulate fraction (+40%) paralleled by a decrease of enzymatic activity in the soluble fraction (-28%). The time course curve showed that the peak effect was reached 1 h after treatment, whereas at 5 hrs PKC activity was down-regulated. In order to understand the mechanisms of interaction between OXI and PKC "in vitro" studies were carried out. OXI was able to induce PKC translocation in cortical slices after 15 minutes of incubation at 200 nM concentration (+30% in particulate and -25% in soluble PKC activity). A bell shaped dose-curve was observed in this condition as well as in "in vivo" experiments: the peak effect was reached at 15' (OXI 200 nM). The results obtained may be related to a direct cortical effect of oxiracetam in stimulating PKC translocation. Another cognition enhancing drug was tested in a similar experimental protocol: 2 α-Glycerylphosphorylcholine (GPC), which stimulates acetylcholine synthesis and release. PKC activity was measured in soluble and particulate fractions from rat cortices after acute GPC treatment. The drug elicited an increase of particulate PKC (+50%) and a decrease of soluble fraction activity (-30%). The peak effect was reached 1 h after drug administration and at 5 hs enzyme down-regulation occurred. OXI and GPC did not produce significant effects on hippocampal PKC activity after acute treatment in "in vivo" as well as "in vitro" conditions. Experiments from literature indicate that specific cognition trainings preferentially increased selected PKC isoforms in rat cortex but not in hippocampus. These results are in line with the hypothesis that PKC translocation in brain areas important for cognition may contribute to learning and memory processes.

656.1

THE EFFECTS OF COCAINE ALONE AND IN COMBINATION WITH SEROTONERGIC ANTAGONISTS ON SCHEDULE-CONTROLLED BEHAVIOR OF SQUIRREL MONKEYS. S.L. Serdikoff *, C.A. Sannerud, C.W. Schindler & S.R. Goldberg. NIDA-Addiction Research Center, Baltimore, MD 21224.

Recent findings have established that there is a serotonergic (5HT) component involved in the behavioral effects of psychomotor stimulants. Additionally, it has been shown that cocaine (COC) potently inhibits 5HT reuptake as well as depresses the spontaneous activity of the dorsal raphe. In the present study, the effects of acute pretreatments of cocaine alone, cocaine together with the $5HT_2$ antagonist ketanserin (KET), and cocaine together with the $5HT_3$ antagonist MDL 72222 (MDL) were evaluated in 4 squirrel monkeys responding under a multiple FI 5 min - FR 30 schedule of food reinforcement. Control responding was characterized by lower overall response rates during the FI component as compared to the FR component (0.32 & 1.3 r/sec, respectively). During both components, COC (0.032 - 3.2 mg/kg, i.m.) produced an inverted U-shaped dose-response function for response rate with the higher doses also resulting in an increase in latency to respond. KET (0.1-10 mg/kg, i.m.) produced differential antagonism of COC's (.032-3.2 mg/kg, i.m.) effects on response rates during the two components; KET appears to have antagonized the rate-decreasing effects of high cocaine doses during the FI component and antagonized the rate-increasing effects of low cocaine doses during the FR component. During both components, however, response latencies following 1.0 mg/kg COC were shortened by concomitant KET administration. To date, MDL (5.6-20 mg/kg, i.m.) in combination with COC produces equivocal effects on responding under this multiple FI-FR schedule.

656.2

TOLERANCE TO THE REINFORCING PROPERTIES OF COCAINE: DOSE-EFFECT DETERMINATION USING A MULTI-DOSE SINGLE-SESSION METHOD. M. W. Emmett-Oglesby, R. Depoortere, C. Pickering, M. Hooper and J. D. Lane*. Dept. of Pharmacology, TCOM, 3500 Camp Bowie Blvd, Fort Worth, TX 76107

We tested the hypothesis that a chronic regimen of cocaine infusion would produce tolerance to the reinforcing properties of cocaine. We also assessed if a multi-dose single-session method, in which different doses of cocaine are tested in a single session, could be applied to demonstrate this tolerance phenomenom. The multi-dose method consists of switching among infusion pumps that drive syringes containing different concentrations of cocaine. Rats were first trained daily to self-administer 15 injections of cocaine (0.25 mg/0.1 ml for each injection) under a FR 2 schedule. They were then subjected to a regimen of chronic cocaine infusion (10 days) for induction of tolerance (5 mg in 20 infusions every 8 hours). At the end of the 10th day, a dose-effect relationship was assessed for self-administration of cocaine (0.125, 0.25 and 0.5 mg/0.1ml) using the muti-dose method. The chronic cocaine regimen produced tolerance, as indicated by a significantly faster intake of cocaine across the three doses tested. This study provides further evidence that tolerance occurs to the reinforcing properties of cocaine. It also demonstrates that a multi-dose method can be used to generate dose-effect data for self-administration of cocaine. Supported by grants DA RO1-4137 (M. E-O) and TX-ATP 3711 & 9768031 (J. L.).

656.3

DIFFERENT BEHAVIORAL EFFECTS OF COCAINE IN WINNERS AND LOSERS OF COMPETITION TESTING. M.C. Wilson, S.M. Chowdhury and J.C. Matthews* Dept Phcol. Sch. of Pharmacy, Univ. of Mississippi, University, MS 38677.

The objective of the study was to identify winners(W) and losers(L) in a fixed pair food competition(COMP) paradigm and to determine differential sensitivity to the effects of cocaine (C) in these pair housed rats. No significant differences were observed in cocaine induced locomotor activity(A) or in stereotypy(S) between W and L. A and S were significantly increased by 10 and 20 mg/kg of C in both W and L. In C based conditioned place preference, W and L did not demonstrate a significant change in time spent in the initially preferred chamber paired with saline. W spent significantly more time in the initially nonpreferred chamber after being paired with C(10 mg/kg). A similar but nonsignificant effect was seen in L. There were no significant differences in D_1 and D_2 receptor density(N. accumbens, caudate-putamen, and hypothalamus) or in plasma testosterone and cortisol levels between W and L. These data suggest that long term COMP testing and/or paired housing does not result in differential sensitivity to C.(Supported in part by the Res. Inst. Phar. Sci.)

656.4

THE AVERSIVENESS OF COCAINE WITHDRAWAL IS REVEALED IN A PLACE CONDITIONING PARADIGM. J.L.Parker* and D. van der Kooy. Dept. Anatomy, University of Toronto, M5S 1A8 Toronto, CANADA.

Opiate abstinence after prolonged opiate exposure results in an withdrawal syndrome consisting of somatic withdrawal symptoms and aversive motivational effects. Abstinence from stimulants, such as cocaine, produces somatic withdrawal symptoms that are much less dramatic. We now report that place conditioning paradigms can reveal the aversiveness of cocaine withdrawal following chronic exposure. Rats received 35mg/kg i.p. injections once daily for 10 days. Following this pre-exposure, 5 different groups of animals were injected with cocaine (35mg/kg) and placed in a visually and textually distinct environment 6, 11, 16, 20, and 24 hours later. Each animal was conditioned for one hour, and each animal received a total of four pairings. Place preferences were tested in a drug free state 10 days after the last injection and conditioning trial. The group trained at 20 hours after cocaine injection showed a significant aversion for the paired environment. No other group showed significant aversions. In a separate experiment, we asked if the 10 day pre-exposure phase was necessary for producing aversions for an environment paired with withdrawal from cocaine. Two groups of previously naive rats received four place conditioning trials at 11 or 20 hours following a 35mg/kg cocaine injection. No conditioned place aversions were seen in these two groups without cocaine pre-exposure. We conclude that in cocaine pre-exposed rats, the aversive motivational effects of withdrawal peak at 20 hours after injection and are of short duration.

656.5

EFFECT OF INTERSTIMULUS INTERVAL (ISI) ON COCAINE PLACE PREFERENCE CONDITIONING. W.H. Bridger* and E. Yadin. Department of Psychiatry, The Medical College of Pennsylvania/EPPI, Philadelphia, PA 19129.

The behavioral paradigm of place preference was used to examine the effect of the interstimulus interval (ISI) on conditioning, with the chamber cues acting as the conditioned stimulus (CS) and intravenous cocaine as the unconditioned stimulus (US). Rats fitted with jugular catheters were first allowed to spend 5 min in an apparatus consisting of two distinctly different chambers and a neutral middle section. Three free choice sessions were run and a side preference was determined. During the next eight 5 min conditioning sessions, a connector was attached to the catheter and animals were placed in one of the chambers, a guillotine door blocking its exit to the rest of the apparatus. Upon being placed in the chamber, animals received a 1 mg/kg dose of cocaine hydrochloride intravenously with a short ISI (1 sec) against their original preference. During alternating sessions they received the cocaine with a long ISI (60 sec in one group, 120 sec in another) in their originally preferred chamber. They were then given a 5 min free choice session. The percent of time spent in each chamber was recorded.

After conditioning with cocaine as the US with a 120 sec ISI (but not with a 60 sec ISI), rats showed preference to the chamber associated with the short ISI, a preference that was against their original choice. These results may have implications for the degree of addiction seen with various routes of cocaine administration in humans.

Supported by funds from the Department of Psychiatry

656.6

UNCONTROLLABLE STRESS INCREASES VULNERABILITY TO SELF-ADMINISTER COCAINE IN RATS. N.E. Goeders* and G.F. Guerin. Dept. of Pharmacology and Psychiatry, LSU Med. Center, Shreveport, LA 71130-3932.

Adult male Wistar rats were screened and assigned to groups of three based on similar responses to a novel environment and the locomotor stimulating effects of cocaine. Each group of rats was trained to respond on a discrete-trial 30 sec (100 trials/session), fixed-ratio 10 schedule of food reinforcement (45 mg). A 20 sec limited hold was in effect during each trial. When stable baselines of responding were established for all three rats in each group, a random-ratio schedule of shock presentation (0.6 mA) was added to the food schedule for the first rat in each group. This rat therefore had some degree of control over footshock delivery. Footshock for the second rat in each group was yoked to the first rat so that each time the first rat received response-contingent electric shock, the second rat would receive a simultaneous but non-contingent footshock. Approximately 50 shocks were delivered each session. The third rat was never shocked. When stable baselines of responding were obtained, the second component of the multiple schedule was initiated. During the second hour of the session, intravenous cocaine was available (0.2 ml delivered over 5.6 sec) when the rats pressed a second lever in the experimental chamber. The animals were initially tested with 0.031 mg/kg/infusion cocaine, and the cocaine dose was gradually increased (0.0625, 0.125, 0.25 mg/kg/infusion) every three to five days until self-administration was observed. In every case, the second rat (with no control over footshock presentation) self-administered cocaine sooner, at a lower dose and at a higher rate than either the rat with control over footshock delivery or the rat that was never shocked in each group. These data demonstrate that lack of control over environmental stress increases vulnerability to cocaine self-administration. This research was supported by USPHS grant DA06013.

656.7

PAVLOVIAN CONDITIONING OF HYPERTHERMIA BY COCAINE.

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The present study examined unconditioned and conditioned thermal responses to repeated infusions of cocaine. Rats implanted with an i.v. catheter and a biotelemetry device which measured core body temperature, were housed in cages enclosed in sound-attenuating chambers, and subjected to 16-hr sessions. Each session consisted of two 30-min trials. Subjects were presented with a light/noise cue (CS) for 30 min during the first trial: 15 min after the start of the CS, an i.v. infusion of 2 mg/kg of cocaine was administered to 'Paired' subjects. 'Unpaired' subjects received an identical cocaine infusion in the absence of the CS, 3.5 hrs later. 'Paired' animals did not receive any programmed events during this second trial. Initial infusions of cocaine produced little change in body temperature. However, with repeated exposure, cocaine was found to produce a mild hyperthermia in both groups. An anticipatory hyperthermia also became evident in the 'Paired' group following 20 conditioning sessions. This conditioned hyperthermia did not appear to summate with the thermal response to cocaine. Thus, in contrast to Pavlovian conditioning of thermal responses by ethanol and morphine, the cocaine-induced thermal conditioned response did not appear to mediate either tolerance or sensitization. Since previous data suggest that conditioned thermal responses alter drug self-administration, the present findings raise the possibility that conditioned responses may also play a role in cocaine self-administration. (Supported by NIDA grant DA03608.)

656.8

TASTE REACTIVITY RESPONSES ELICITED BY COCAINE, PHENCYCLIDINE AND METHAMPHETAMINE PAIRED SUCROSE SOLUTION.

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The nature of flavor-drug associations produced by a range of doses of the reinforcing agents cocaine (5 - 40 mg/kg, sc), phencyclidine (.5 - 20 mg/kg, sc) and methamphetamine (2 - 10 mg/kg, ip) were assessed by the taste reactivity (TR) test and the conditioned taste avoidance (CTA) test. Even at the highest doses tested, none of the agents produced aversive TR responses. At doses that produced equivalent strength CTA, lithium did establish aversive TR responses. These results provide evidence that aversive TR responses are only produced by non-reinforcing drugs.

656.9

ELECTROPHYSIOLOGICAL RECORDINGS OF NUCLEUS ACCUMBENS NEURONAL ACTIVITY DURING A MODIFIED FR-3 SCHEDULE FOR COCAINE SELF-ADMINISTRATION.

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We have previously demonstrated that neurons within the core region of the nucleus accumbens (NAc) exhibit anticipatory responses during cocaine self-administration bar pressing behavior. The purpose of the current study was to examine further correlations between neuronal activity in NAc and goal-directed behavior by using a modified fixed ratio 3 (FR-3) schedule to determine if the sequential bar pressing associated with different cues and consequences would evoke different neuronal responses in NAc. A bundle of eight 62μm diameter teflon coated stainless steel microwires were chronically implanted in the NAc for extracellular single unit recording. After recovery from surgery, rats were trained to self-administer cocaine with the modified FR-3 schedule. A retractable bar was mounted on one side of the conditioning chamber. The first bar press resulted in bar retraction; the second bar press turned on the light and retracted the bar; the third bar press turned off the light and delivered cocaine (1 mg/kg in 0.1 ml Ringer's solution, i.v.). A two second delay was imposed between each bar press during which the bar was kept in a retracted position. The anticipatory unit responses, observed as an increase in a firing rate before bar pressing, were not significantly different between each bar pressing, whether it led to cocaine delivery or not. In another experiment, in which a FR-1 schedule was associated with or without cocaine injection, the anticipatory response also remained essentially the same during extinction. These initial results suggest that the anticipatory neuronal responses expressed in the NAc are likely related more to a general goal-directed motoric behavior towards an object with significance rather than a reward-specific phase of the behavior. *Supported by DA02338, and MH44339.*

656.10

COCAINE-INDUCED CONDITIONED PLACE PREFERENCE IN RHESUS MONKEYS.

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Although numerous studies in rats have used a conditioned place preference (CPP) paradigm to assess the reinforcing actions of drugs, no studies have yet to demonstrate whether such a procedure can be employed in primate species. The aim of the present study was to develop a CPP paradigm in rhesus monkeys and evaluate the ability of cocaine to produce a CPP. A U-shaped test apparatus was designed comprising of a central compartment and two end compartments with colored side and floor panels. The experiment was run in three phases (Pretest, Drug Conditioning, and Posttest). In Pre- and Posttests, monkeys were able to explore the entire test apparatus for 30 min, whereas during conditioning, monkeys were restricted to one or the other end compartments for 30 min. Conditioning lasted 8 days. On odd-numbered training days monkeys were injected with cocaine (IM) and placed in the originally less-preferred compartment and on even-numbered days they were injected with saline and placed in the opposite, initially more-preferred compartment. The effect of 0, 50, and 100 μg/kg cocaine (N=8) and 400 and 800 μg/kg cocaine (N=4) on the acquisition of a CPP has been studied. Compared to their pretest, after being conditioned in one of the compartments with 0, 50, 100, 400 and 800 μg/kg cocaine, monkeys spent on average 5±24, 45±20, 91±25, 113±40, and 80±40 more 5-sec blocks in the cocaine-paired compartment, respectively. Moreover, since the increased time in the cocaine-paired compartment was also associated with decreased time in the saline-paired compartment a reversal in place preference was observed. These data demonstrate that a CPP procedure can be used in rhesus monkeys to investigate the reinforcing properties of drugs.

656.11

THE RUNWAY BEHAVIOR OF COCAINE-REINFORCED RATS RESEMBLES THAT OF SUBJECTS RUNNING FOR FOOD+SHOCK.

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Rats traversing as straight-alley for positive reinforcement typically exhibit faster running times, or *goal latencies* (GL), as training proceeds. However, when reinforced with i.v. cocaine, we observed that subjects took progressively longer to enter the goal box over trials. Closer observation of the data revealed that the increasing GLs were the result of a unique "retreat behavior" (i.e., stopping and returning to the start box). We hypothesized that such behavior reflected an inherent "conflict" that stemmed from the drug's well documented reinforcing and anxiogenic properties. To test this idea, the runway behavior of animals experiencing other concurrent positive and negative stimuli (i.e., food and mild footshock) was examined. Hungry animals were trained to traverse a runway for food reinforcement coupled with footshock. These subjects showed higher GLs and *retreat frequencies* than a control group which received only food in the goal box. The nature and pattern of the retreat behavior in the food+shock group strongly resembled that of cocaine-reinforced rats.

656.12

EVIDENCE FOR CONDITIONAL NEURONAL ACTIVATION FOLLOWING EXPOSURE TO A COCAINE-PAIRED ENVIRONMENT: ROLE OF FOREBRAIN LIMBIC STRUCTURES

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The classical conditioning of cocaine's behavioral effects with specific environmental stimuli is an important aspect of its actions. This property of cocaine is of major significance with respect to this drug's abuse potential, as intense craving can be evoked by stimuli previously associated with drug taking. To better understand the neurobiology of this phenomenon the expression of the putative metabolic marker *c*-Fos and locomotor behaviour were examined in rats that were exposed to an environment in which they had previously received cocaine.

Compared to saline treated controls, acute administration of cocaine produced an increase in locomotor behaviour that was accompanied by an increase in *c*-Fos expression within specific limbic regions (cingulate cortex, claustrum, lateral septum, paraventricular nucleus of the thalamus, lateral habenula and amygdala) as well as the basal ganglia (dorsomedial striatum and nucleus accumbens). Exposure of rats to the cocaine-paired environment also produced an increase in locomotion, as compared to pseudoconditioned and control subjects. In addition to this behavioural effect, conditioned subjects exhibited a significant increase in *c*-Fos expression within the cingulate cortex, claustrum, lateral septum, paraventricular nucleus of the thalamus, lateral habenula and the amygdala, suggesting an increased neuronal activation within these regions. In contrast to the dramatic effects observed within these limbic structures, no conditional activation was observed within the nucleus accumbens or dorsal striatum. The present findings suggest that specific limbic regions exhibit increased neuronal activation during the presentation of cocaine-paired cues and may be involved in the formation of associations between cocaine's stimulant actions and the environment in which the drug administration occurred.

656.13

THE 5HT1A RECEPTOR: RELATIONSHIP TO IMPULSIVE AND AGGRESSIVE BEHAVIOR IN COCAINE ABUSE.

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The neurotransmitter serotonin is linked to aggressive behavior in animals and humans. In order to examine the role of 5HT in aggressive and impulsive behavior in cocaine abuse, the 5HT1a agonist buspirone (0.4mg/Kg orally) was administered as a neuroendocrine challenge agent to 7 cocaine dependent male human subjects, and 9 healthy male controls. The ACTH, growth hormone and prolactin levels after buspirone administration were then compared between the healthy controls and the cocaine dependent patients. Hormone levels were also correlated with measures of aggression including the Buss-Durkee Hostility Inventory. Buspirone levels were obtained on all subjects to control for differences in metabolism between groups. Results of this analysis will be presented, and the implications for treatment of aggressive behavior in cocaine dependent patients will be discussed.

656.14

EFFECTS OF INTRA-ACCUMBENS AND INTRA-PREFRONTAL CORTEX COCAINE INFUSIONS ON SCHEDULE-INDUCED POLYDIPSIA.

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Schedule-induced polydipsia (SIP), the excessive drinking induced by exposure to intermittent schedules of food-delivery, has been functionally linked to forebrain dopamine projections and in particular the mesolimbic dopamine system. This study compared the effects of cocaine microinfusions into the nucleus accumbens (NACC), the medial prefrontal cortex (MPFC), and IP cocaine injections on schedule-induced drinking, locomotor activity and panel presses to gain access to the food.

SIP was induced in male Wistar rats reduced to 85% of their free-feeding weight and exposed to a fixed-time 60 second schedule of food-presentation. When performance reached stable levels subjects were bilaterally infused with either vehicle, 12.5, 25, 50, or 100 ug cocaine HCl via chronically implanted guide cannula aimed to give access to either the NACC (n=12) or MPFC (n=12). The sequence of infusions was according to a Latin square design and infusions were administered immediately before the daily 30 min sessions. Following the series of intra-cranial infusions all subjects received IP injections of either saline, 2.5, 5, 10, or 20 mg/kg cocaine HCl.

The 3 routes of drug administration produced different profiles of behavioural effects. For example, both IP and NACC cocaine dose-dependently decreased SIP and increased locomotor activity suggesting a reduction in SIP through response competition. However, MPFC cocaine also dose-dependently decreased SIP but did not significantly affect locomotor activity. In addition, IP cocaine increased low rates of panel pressing without affecting high rates whereas NACC and MPFC cocaine had no effect on low rates but decreased high rates of responding.

656.15

A PHARMACOLOGICAL INVESTIGATION OF GBR 12909-INDUCED BEHAVIORAL SENSITIZATION.

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The present study was designed to investigate the mechanisms underlying GBR 12909-induced behavioral sensitization. GBR 12909, a psychomotor stimulant which selectively inhibits dopamine reuptake, was repeatedly administered to male Sprague-Dawley rats. Specifically, chronic treatment consisted of intraperitoneal injections of 20 mg/kg GBR 12909 or vehicle, administered every other day over a 12-day interval. Following cessation of chronic treatment, the animals were challenged with vehicle, GBR 12909 (6 mg/kg), and the following drugs, which were tested in separate experiments: apomorphine (0.1 mg/kg), nomifensine (0.75 mg/kg, 1.5 mg/kg), d-amphetamine (0.25 mg/kg, 0.75 mg/kg), bupropion (20 mg/kg), and scopolamine (0.1 mg/kg, 0.5 mg/kg). The dependent variable was locomotor activity, measured in photocell testing cages.

Results show that animals chronically treated with GBR 12909 display a potentiated locomotor response to the 6 mg/kg GBR 12909 challenge, compared with their vehicle-pretreated counterparts. In addition, GBR 12909-sensitized rats display a hypersensitivity to other drugs which act at the dopamine uptake site: nomifensine, d-amphetamine, and bupropion. However, cross-sensitization to apomorphine, a direct dopamine receptor agonist, or to scopolamine, a cholinergic muscarinic antagonist, was not observed. These results are discussed with regard to possible presynaptic changes on dopamine neurons whch may underlie behavioral sensitization to cocaine-like drugs.

656.16

COMPARISONS BETWEEN DOPAMINE UPTAKE BLOCKERS IN RATS TRAINED TO DISCRIMINATE COCAINE OR BUPROPION.

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Bupropion (Wellbutrin) is a novel, non-tricyclic antidepressant; it is a weak inhibitor of dopamine uptake, and of several ligands to the dopamine transporter. Although its neurochemical and behavioral profile *in vivo* resembles that of a psychomotor stimulant, bupropion does not reliably produce stimulant effects in humans. This experiment examines and compares the discriminative stimulus effects of bupropion and cocaine in rats. One group of rats was trained to press one lever when injected IP with cocaine (10.0 mg/kg), and another lever when injected with saline; a second group of rats was trained similarly, but with bupropion (17.0 mg/kg) instead of cocaine. In substitution tests, full dose-response curves were obtained for several monoamine uptake inhibitors. In both cocaine- and bupropion-trained rats all nine dopamine uptake blockers tested to date fully substituted for the training compound. Across a 20-fold range, ED_{50} values in both conditions were highly correlated ($r=0.81$; $p<0.05$). Serotonin and norepinephrine uptake blockers failed to substitute even partially for either training compound. The results demonstrate a surprising similarity between cocaine and bupropion in terms of discriminative stimulus effects, and given the limited abuse of bupropion, suggest that this compound deserves further study as a potential therapeutic agent in cocaine addiction.

656.17

INJECTIONS OF COCAINE PRIOR TO MEMORY REACTIVATION FACILITATE LATER LEARNING OF AN AVOIDANCE RESPONSE IN RATS.

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Two noncontingent footshocks (700 μA, 1 sec) were administered to rats in a dark compartment of a two-compartment, one-way avoidance, chamber. Twenty-four hr later, cocaine or saline was administered 5 min prior to a 30 sec re-exposure to selected stimuli present during the initial conditioning trials. On Day 3 the rats were trained to move from the chamber's dark compartment to the light compartment in order to avoid a footshock. Intermediate (5.0 or 7.5 mg/kg IP), but not low (3.3 mg/kg IP) or high (11.25 or 16.88 mg/kg IP), doses of cocaine given on Day 2 enhanced acquisition on Day 3 of the avoidance response. These results suggest that cocaine administered prior to the reintroduction of cues associated with a conditioning episode can modulate memory processes, and that the dose-response function for this effect is U-shaped.

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656.18

COCAINE POTENTIATION OF LATERAL HYPOTHALAMIC BRAIN STIMULATION REWARD: A DOSE RESPONSE AND REPEATED TREATMENT ANALYSIS.

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The curve-shift rate-frequency paradigm was used to assess the ability of cocaine to potentiate the rewarding impact of lateral hypothalamic brain stimulation. In Experiment 1 animals (n=7) received daily saline or cocaine (0.5, 1, 2, 4, 8, 16, and 32 mg/kg i.p.) in ascending dose order. Cocaine produced dose orderly parallel leftward shifts of the rate-frequency functions; cocaine lowered the "dose" of brain stimulation required to produce normal responding. The highest dose shifted the rate-frequency curve by 0.4 log units thereby reducing self-stimulation thresholds by approximately 60%. The aim of Experiment 2 was to determine whether repeated testing (as in Experiment 1) might progressively alter the effectiveness of cocaine. A new group of animals (n=6) was treated 5 times with cocaine (16 mg/kg i.p.) at 48-h intervals. The magnitude of the reward-potentiating effect of cocaine did not change from treatment to treatment; there was neither tolerance nor sensitization to cocaine's reward-potentiating action.

657.1

DIFFERENT GENOTYPE-DEPENDENT FACTORS MODULATE SENSITIVITY TO THE BEHAVIORAL EFFECTS OF AMPHETAMINE. S. Cabib*, A. Badiani, S. Puglisi-Allegra, Inst Psicobiologia e Psicofarmacologia (CNR), via Reno 1,Roma I-00198, Italy

The existence of robust strain differences in the effects of chronic amphetamine treatment suggests that genetic factors influence behavioral sensitization processes. However, this phenomenon is produced by different experimental paradigms either involving or not involving classical conditioning and also by chronic or repeated exposure to environmental stress. Thus the question arises as to wether similar strain differences are revealed by the different situations. DBA/2 are less sensible to the stimulatory effects of amphetamine on locomotion than C57BL/6, although they are more susceptible to behavioral sensitization induced by repeated amphetamine 7 days after the end of the treatment. On the other hand, no sensitization was observed in either strains of mice tested 24 hrs after the end of the treatment and C57BL/6 showed a rapid and robust sensitization when repeated psychostimulant injections were paired with the test environment. Following ten days of daily restraint sensitization developed to the behavioral effects of amphetamine in DBA/2 but not in C57BL/6 mice tested 24 hrs after the last stressful experience. Results obtained in B6D2F1 hybrids suggested that the response to repeated stress of the C57BL/6 strain is inherited through a dominant mode of inheritance. Finally, 7 days after the last stressful experience no sign of behavioral sensitization could be detected in this strain of mice.

These results indicate that behavioral sensitivity to amphetamine depends on interaction between genotype-dependent factors and organism's experience. Moreover, sensitivity to the behavioral effects of amphetamine does not predicts susceptibility to either stress- or amphetamine-induced sensitization. Finally, each treatment capable of inducing sensitization produces different strain-dependent responses to amphetamine.

657.2

DISCRIMINATIVE STIMULUS EFFECTS OF N- AND RING-SUBSTITUTED AMPHETAMINE ANALOGS IN METHAMPHETAMINE-TRAINED RATS. T.D. Steele*, G.A. Ricaurte, J.M. Witkin, and J.L. Katz, Department of Neurology, Johns Hopkins School of Medicine, and NIDA Addiction Research Center, Baltimore, MD, 21224.

Aromatic- and side-chain N-substitution is known to alter the behavioral and neurotoxic effects of substituted amphetamine analogs. The present studies evaluated the effects of ring-substituted and N-methylated amphetamine analogs in rats trained to discriminate the dopaminergic/serotonergic neurotoxin methamphetamine (MA) from saline. Of the compounds tested, only N,N-dimethylamphetamine (N,N-DMA) completely substituted for 1 mg/kg MA. Consistent with the reported potency difference for producing dopamine depletion (Ricaurte *et al.*, 1989), N,N-DMA was approximately seven-fold less potent than the parent compound in evoking MA-appropriate responding. The ring-substituted analogs *p*-methoxyamphetamine, *p*-methoxymethamphetamine, and 3,4-methylenedioxymethamphetamine (MDMA), which are selective serotonergic neurotoxins, only partially substituted for MA. The maximum degree of MA-appropriate-responding for the three compounds ranged from 35-50%. All test compounds produced a dose-related decrease in response rate. Thus, N-methylation appears to cause parallel decreases in behavioral potency as well as dopaminergic neurotoxic activity of MA. In contrast, aromatic substitution can alter or attenuate MA-like discriminative cues, in addition to conferring selective serotonergic neurotoxic activity. Further evaluation of structural analogs may aid in dissociating the behavioral and neurotoxic effects of compounds in this series. (Supported by USPHS DA 06275)

657.3

INDIVIDUAL DIFFERENCES IN FEEDING CAN PREDICT INDIVIDUAL DIFFERENCES IN THE LOCOMOTOR RESPONSE TO AMPHETAMINE. [1]T. L. Sills* & F. J. Vaccarino[1,2]. Departments of [1]Psychology and [2]Psychiatry , University of Toronto, Toronto, Ont., M5S 1A1.

Previously we demonstrated individual differences in the feeding response to a low dose of amphetamine (AMP); AMP stimulated intake in low baseline feeders and inhibited intake in high baseline feeders. Individual differences in the locomotor response to AMP, as a function of baseline activity, have also been reported. Intrinsic variation in nucleus accumbens dopamine activity has been suggested to underlie individual differences in both feeding and locomotion. In light of the feeding/locomotor parallels, the present experiment examined whether individual differences in baseline feeding would be predictive of individual differences in the locomotor response to AMP.

Thirty-four male Wistar rats (Charles River, Quebec) were divided into low and high feeders based on a median split of their sugar intake in response to 0.9% saline administration (ip). The locomotor response of each group to both a novel environment and a 1.75 mg/kg dose of AMP (ip) was subsequently determined.

Results indicate that low feeders were significantly less active in response to a novel environment than high feeders. Similarly, low feeders exhibited significantly less locomotion in response to AMP than high feeders. These results are consistent with previous observations of individual differences in the locomotor response to AMP. Further, these results are consistent with the notion that individual differences in both the feeding and the locomotor response to AMP reflect intrinsic variation in a common substrate.

This research was supported by a NSERC grant to FJV.

657.4

THE EFFECTS OF CROWDING ON LOCOMOTOR ACTIVITY FOLLOWING CHRONIC METHAMPHETAMINE ADMINISTRATION. M. A. Blackshear* and B. Peoples, Department Of Biological Sciences, Tennessee State University, Nashville, TN 37209-1561

This study compares the effects of chronic methamphetamine administration on locomotor activity in isolated and crowded mice, and examines the effects of crowding on methamphetamine-induced "reverse tolerance". Swiss ICR male mice (26-30g) were used as experimental animals. The animals were either singly housed with 562cm^2 floor space/mouse or crowded with only 56cm^2 floor space/mouse throughout the duration of the study. Methamphetamine was administered at a dose of 4 mg/kg. For the acute studies, the mice received a single injection of methamphetamine, while in the chronic studies, methamphetamine was administered daily for 7 days. Locomotor activity was monitored immediately after drug administration on day 1 (acute studies) and again (on day 9) at 48 hours after the last dose following a challenge dose of 1 mg/kg of methamphetamine. Control animals were housed in a similar manner and received physiological saline. Predictably, methamphetamine induced locomotor activity was higher on day 9 than on day 1 in mice that were singly housed (5,883 ± 778 vs 7,744 ± 525, respectively for days 1 and 9. In both instances, locomotor activity was higher in the drug treated animals than in the controls. In contrast, methamphetamine-induced changes in locomotor activity in crowded mice were essentially the same on day 1 and day 9 (5998 ± 974 vs 5532 ± 821 for day 1 and day 9, respectively) and suggests the development of tolerance, rather than reverse tolerance. Considering that reverse tolerance is thought to be a form of receptor sensitivity that is mediated by dopamine release, these findings suggest that the effects of crowding may precipitate changes in dopamine receptor sensitivity.

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657.5

INVOLVEMENT OF SITUATIONAL VARIABLES ON THE EXPRESSION OF BEHAVIORAL SENSITIZATION TO AMPHETAMINE. S.H. Ahmed, L. Stinus, M. Le Moal and M. Cador*, Unité INSERM 259, rue Camille Saint Saëns 33077 Bordeaux Cedex, France.

Behavioral sensitization is a progressive and long lasting enhancement to the psychostimulant effects of amphetamine (AMPH). In a conditioning paradigm, it has been shown that the expression of this phenomenon is under situational control. However, the two sets of contextual stimuli used in this paradigm for an association with the presence or absence of the unconditioned effects of AMPH usually do not possess equivalent predictability value. In the present study, this possible bias has been circumvented by using the following protocol: two very distinct situational contexts both different from the home cage have been used. One was paired with AMPH injection (1 mg/kg, s.c., for 6 days, 2 days apart), the other with vehicle injection. Tests for sensitization were conducted by injecting AMPH (0.5 mg/kg, s.c.) in one of these two situational contexts and the locomotor activity was recorded. The main results are: 1) the paired group (rats tested in the situational context paired with AMPH) showed behavioral sensitization compared to the control group (rats that have received vehicle in the two contexts). The unpaired group (rats tested in the situational context paired with vehicle) failed to exhibit behavioral sensitization 2) when comparing the unpaired group with the control group, a great variability between rats appeared, some rats showed a response similar to the control group whereas other animals showed a response *below* the control group. In this latter subgroup, the influence of the situation appears to be more than an inhibition of the conditioned component of the behavioral response to AMPH but a reduction of its unconditioned effects. This variability can be explained either by a difference in the sensitization process or a difference in the capacity to index context. Our preliminary results indicate that this variability could reflect a difference in the capacity of contextual indexation between animals.

657.6

DIFFERENTIAL EFFECTS OF NUCLEUS ACCUMBENS LESIONS ON THE CONDITIONED PLACE PREFERENCE INDUCED BY MORPHINE OR AMPHETAMINE. M.C. Olmstead* and K.B.J. Franklin, Dept. Psychology, McGill University, Montreal, Canada, H3A 1B1

It has been suggested that the mesolimbic dopamine system, originating in the ventral tegmental area and projecting to the nucleus accumbens (NAS) is involved in the reinforcing effects of both psychomotor stimulants and opiates. The post synaptic elements of this system have also been implicated in drug reinforcement. The present study further examined the role of the NAS cell bodies on morphine and amphetamine reinforcement.

Excitotoxin lesions were produced by bilateral microinjections of kainic acid (0.5 ug in 1 ul) into the NAS. Following recovery, different groups or rats were tested for the development of a CPP to morphine (2 mg/kg X 3 pairings) or amphetamine (1.5 mg/kg X 3 pairings). In both experiments, sham lesioned animals developed a CPP for the drug paired environment. Lesioned animals also developed a CPP for the morphine paired environment. Lesioned animals conditioned with amphetamine, however, did not show a significant preference for the drug paired versus the saline paired environment. These results support the suggestion that the reinforcing effects of psychomotor stimulants and opioids do not involve identical neural substrates.

657.7

3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) INHIBITS GLUTAMATE-EVOKED FIRING OF NUCLEUS ACCUMBENS CELLS. S.R. White* and K.C. Paros. Dept. of VCAPP, Washington State Univ., Pullman, WA 99164.

MDMA is a recreationally used amphetamine derivative that has been reported to release serotonin (5HT) and, less potently, dopamine (DA) from nerve terminals in the forebrain (Schmidt et al., Biochem. Pharmacol. 36, 1987, 747). The nucleus accumbens (NAc), a major component of the brain reward pathway, is innervated by both 5HT- and DA-containing nerve terminals. This study examined the effects of local application of MDMA on excitability of NAc neurons *in situ*. Extracellular single unit recording was combined with microiontophoretic drug application in urethane-anesthetized male rats. NAc cells were driven at a slow, stable firing rate by cycled pulses of glutamate. Microiontophoretic application of MDMA (10-40 nA, 60 sec) produced a slowly developing, dose-dependent inhibition of glutamate-evoked firing that was not mimicked by application of equivalent currents applied to a pH control solution. Application of 5HT and of DA produced a dose-dependent inhibition of glutamate-evoked firing that was similar to the effect of MDMA. The nonselective DA antagonist haloperidol partially attenuated but did not block the MDMA effect. These results suggest that MDMA-induced inhibition of NAc neuronal excitability may be mediated by both DA and 5HT receptors. (Support by Washington State Alcohol and Drug Abuse Program.)

657.8

DIZOCILPINE (MK-801) POTENTIATES LOW-DOSE FACILITATION OF BRAIN STIMULATION REWARD BY BOTH AMPHETAMINE AND MORPHINE.

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It has been reported that the non-competitive NMDA antagonist dizocilpine (MK-801) can block both the behavioral sensitization observed after repeated amphetamine, and the tolerance and dependence observed after repeated morphine. The purpose of the present study was to evaluate the effects of blockade of the NMDA receptor on the reward-facilitating effects of amphetamine and morphine using brain stimulation reward (BSR). In rats (n=8) with stimulating electrodes aimed at the medial forebrain bundle (MFB), acute administration of a low dose of amphetamine (0.25 mg/kg, ip) caused a leftward shift in the function that relates stimulation frequency to response rate, causing a 14% decrease in BSR threshold. Dizocilpine, at a dose (0.05 mg/kg, ip) that elicited a minimal (9%) decrease in threshold, potentiated the threshold-lowering effects of amphetamine: the combination produced a 29% decrease in threshold. Furthermore, after tolerance had developed to its sedative side effects, a small (12%) decrease in threshold could be observed after administration of morphine (2.5 mg/kg, ip); this threshold-lowering effect was also potentiated (to 23%) by dizocilpine. These effects do not appear to be due to sensitization to amphetamine, morphine, or dizocilpine. Thus, the stimulant effects of the NMDA antagonist dizocilpine summate with those of the indirect dopamine agonists amphetamine and morphine, implying that disruption of normal glutamatergic tone can have significant effects on the rewarding impact of habit-forming drugs.

657.9

THE NONCOMPETITIVE NMDA ANTAGONIST MK-801 FAILS TO BLOCK THE REWARDING OR LOCOMOTOR-ACTIVATING EFFECTS OF AMPHETAMINE IN RATS. D.C. Hoffman* and H. Donovan. Behavioral Biology, Neurogen Corp., Branford CT 06405.

The noncompetitive NMDA receptor antagonist MK-801 prevents the development of sensitization to the locomotor-activating effects of amphetamine (Karler et al., 1989; Wolf and Khansa, 1991). In the present study, the possibility that the NMDA receptor might also play a role in the rewarding effects of amphetamine (as measured in the conditioned place preference paradigm) was investigated. Male Sprague-Dawley rats received amphetamine (2.0 mg/kg IP) paired with one side of a two-compartment box and saline paired with the other side. During these pairings, locomotor activity was measured. On the test day, the amount of time drug-free rats spent in each compartment was determined. Rats trained with amphetamine alone showed a significant increase in time spent on the drug-paired side from pre- to post-conditioning, indicating a place preference. When rats were injected with MK-801 (0.03, 0.1, 0.3 mg/kg SC) prior to amphetamine, no significant effects on amphetamine place conditioning were observed. Rats treated with MK-801 alone showed no consistent place conditioning effects, although a place preference was observed at the intermediate dose. On conditioning days, MK-801 produced a dose-dependent enhancement of amphetamine-induced locomotor activity, however, MK-801 alone caused a similar increase in activity. These data suggest that the NMDA receptor is probably not involved in either the rewarding or locomotor-activating effects of amphetamine.

657.10

THE NON-COMPETITIVE NMDA ANTAGONIST, MK801, BLOCKS THE DEVELOPMENT OF SENSITIZATION TO THE LOCOMOTOR ACTIVITY EFFECTS OF APOMORPHINE.

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Several studies have shown that the NMDA antagonist, MK801, blocks the development of sensitization to amphetamine-induced activity. Here we show that this effect cannot be explained by interference by MK801 with the release of dopamine (DA) by amphetamine in that MK801 also blocks the development of sensitization to the direct mixed DA receptor agonist apomorphine (APO). During preexposure, rats were pretreated with either 0.25 mg/kg MK801, i.p., or saline prior to injections of 2.0 mg/kg APO HCl, i.p. or saline, on five occasions every three days. Group PAIRED received APO in the activity boxes and saline in the home-cage, group UNPAIRED, saline in the activity boxes and APO in the the home-cage, and group CONTROL, saline in both places. All animals were tested for conditioned activity following injections of saline and for sensitization following 0.5 mg/kg APO.

MK801 blocked the development of sensitization to APO seen over the five preexposure days and conditioning when animals were tested in the activity boxes with saline only. On the test for sensitization, the saline-pretreated PAIRED group that had received repeated injections of APO in the activity boxes showed marked sensitization compared to the UNPAIRED and CONTROL groups which did not differ from each other. There was no evidence for sensitization in the MK801 pretreated groups.

657.11

AMPHETAMINE SENSITIZATION AND NMDA RECEPTORS: NEUROCHEMICAL AND ELECTROPHYSIOLOGICAL CORRELATES IN THE MESOACCUMBENS DOPAMINE SYSTEM. R.J. Brooderson*, F.J. White and M.E. Wolf. Departments of Psychiatry and Pharmacology, Wayne State University School of Medicine, Detroit, MI 48207.

The repeated intermittent administration of d-amphetamine (AMPH) results in a progressive augmentation of its locomotor stimulant effects, a phenomenon known as behavioral sensitization. We have shown previously that the development of AMPH sensitization requires stimulation of N-methyl-D-aspartate (NMDA) receptors since sensitization does not occur when AMPH is coadministered with the noncompetitive NMDA antagonist MK-801. The purpose of the present experiments was to further characterize the roles of dopamine (DA) and NMDA receptors in AMPH sensitization. Rats were treated for 5 days with either saline, AMPH (5 mg/kg, i.p.), or MK-801 (0.25 mg/kg, i.p.) followed 30 min later by AMPH. The following were performed after either 3 or 10 days off: 1) electrophysiological studies of postsynaptic D1 and D2 receptor sensitivity in the nucleus accumbens (NAc), 2) electrophysiological studies of DA autoreceptor sensitivity in the ventral tegmental area (VTA), and 3) microdialysis studies of AMPH-stimulated DA levels in the NAc. AMPH-treated rats were behaviorally sensitized at both 3 and 10 days off. At 3 days off, autoreceptor sensitivity in the VTA and AMPH-stimulated DA release in the NAc were similar in the saline and AMPH groups. However, supersensitivity of both D1 and D2 receptors in the NAc was observed in the AMPH group. MK-801 coadministration prevented the development of D1 receptor supersensitivity. Studies at 10 days off are in progress. Supported by DA-07735 (MEW), DA-04093 (FJW), MH-40832 (FJW), NARSAD (MEW), the PMA Foundation (MEW) and Institutional NRSA GM-08164 (RJB).

657.12

BEHAVIORAL SENSITIZATION TO MK-801: NEUROCHEMICAL AND ELECTROPHYSIOLOGICAL CORRELATES IN THE MESOACCUMBENS DOPAMINE SYSTEM. M.E. Wolf*, F.J. White, R.J. Brooderson and M.R. Khansa. Departments of Psychiatry and Pharmacology, Wayne State University School of Medicine, Detroit, MI 48207.

We have shown previously that repeated administration of MK-801, a noncompetitive N-methyl-D-aspartate (NMDA) antagonist, results in sensitization to its locomotor stimulant effects (Brain Res. 562: 164, 1991). The present study sought to further characterize this phenomenon. Rats were treated with either saline or MK-801 (0.25 mg/kg, i.p.) for 5 days and challenged with MK-801 after 3 days off. Studies in which the D1 antagonist SCH 23390 (50 μg/kg, i.p., 15 min before MK-801) was coadministered on either the treatment days or the test day indicated that neither the development nor expression of sensitization to MK-801 required dopamine (DA) receptor stimulation. Microdialysis studies demonstrated that MK-801 challenge was associated with only a modest (10-15%) increase in DA levels in the nucleus accumbens and that this effect was not augmented in MK-801 sensitized rats. Electrophysiological studies showed that repeated administration of MK-801 resulted in supersensitivity of both D1 and D2 receptors in the nucleus accumbens. These results suggest that MK-801 sensitization does not require DA receptor stimulation for its induction but may share other features with sensitization to amphetamine and cocaine. Supported by DA-07735 (MEW), DA-04093 (FJW), MH-40832 (FJW), NARSAD (MEW), the PMA Foundation (MEW) and Institutional NRSA GM-08164 (RJB).

657.13

AMPA/KAINATE RECEPTORS IN THE NUCLEUS ACCUMBENS ARE INVOLVED IN MEDIATING CONDITIONED PLACE PREFERENCE ELICITED BY AMPHETAMINE, COCAINE, AND MORPHINE. F.G. Kaddis, R.T. Layer, N.J. Uretsky*, and L.J. Wallace. College of Pharmacy, Ohio State University, Columbus, OH 43210.

Activation of AMPA/kainate glutamatergic receptors in the nucleus accumbens (NACC) may be a component of the mechanism of drug induced reward. To test this, the antagonist DNQX was injected into the NACC just prior to administration of amphetamine, cocaine, or morphine during the training phase (acquisition) of a conditioned place preference (CPP) paradigm. Rats were then tested for CPP in the absence of drugs. In other experiments, DNQX was given just prior to testing for place preference (expression) but not during training. Bilateral intra-NACC administration of DNQX (1 µg/0.5 µl/side) inhibited acquistion of CPP induced by amphetamine (1 mg/kg) and cocaine (20 mg/kg) but not morphine (10 mg/kg). During acquisition, DNQX attenuated the locomotor stimulation elicited by amphetamine during the first but not subsequent sessions and that by cocaine during all training sessions. However, DNQX made morphine treated rats akinetic. When given prior to testing, DNQX inhibited the expression of CPP induced by amphetamine and morphine (experiments with cocaine are in progress) but did not affect locomotor activity. Our results suggest that activation of AMPA/kainate receptors is involved in the primary reward stimulation (acquisition of CPP) of psychostimulants but not opiates and in behaviors related to memory of primary reward stimulation (expression of CPP) for both classes of drugs. Furthermore, locomotor activity during conditioning is not necessary for acquisition of CPP.

DEGENERATIVE DISEASE: PARKINSON'S V

658.1

ALTERED STRIATAL MAO-B KINETICS FOLLOWING RECOVERY FROM CHRONIC DEPRENYL TREATMENT IN RATS. I.A. Terleckyj* and W.J. Nicklas, Dept. of Neurology, UMDNJ-RWJ Med. Sch., Piscataway, NJ 08854, U.S.A.

Monoamine oxidase (MAO) is the enzyme responsible for the neuronal metabolism of aminergic transmitters and exists in two isoforms, MAO-A and B. We have previously shown that daily administration of deprenyl (DEP), an irreversible MAO-B inhibitor, to rats and mice at doses acutely selective for inhibiting MAO-B results in cross-inhibition of MAO-A in the CNS (Heikkila,1990). This effect is both time and dose-dependent and has also been observed in Parkinsonian patients who received DEP for 1 week (Riederer,1986). To further examine the effects of chronic DEP administration, male Sprague-Dawley rats were given either 0.2mg/kg or 0.02mg/kg DEP s.c. for 10 consecutive days. Another group of animals received a single injection of 0.7mg/kg DEP. Animals were sacrificed 4 days following final inhibitor administration to allow for 20-30% recovery in enzyme activity. [C^{14}]benzylamine was used as substrate for MAO-B in a radioenzymatic assay to measure K_m and V_{max} in neostriatum (STR) and remaining cerebral hemispheres (CER). In both regions, V_{max} was similar between the 0.2 and 0.7mg/kg groups (STR = 1.72nmol/mg tissue/hr, CER = 2.11) which differed from the 0.02mg/kg group (STR = 2.12, CER = 2.83). In STR, MAO-A inhibition occurred only in the 0.2 mg/kg group (13%) when serotonin was used at V_{max} conditions. A significant decrease in K_m occurred in STR of animals receiving 0.02mg/kg (65μM ± 10.8) compared to naives (97 ± 22), whereas the 0.2mg/kg and 0.7mg/kg groups displayed insignificant changes. However, no alteration in K_m was observed in CER. Whether the difference in K_m reflects an allosteric modification of MAO-B or expression of a different isoform remains to be elucidated.

658.2

CYCLIC AMP, BUT NOT BASIC FGF, INCREASES THE IN VITRO SURVIVAL OF MESENCEPHALIC DOPAMINERGIC NEURONS AND PROTECTS THEM FROM MPP$^+$ -INDUCED DEGENERATION. **J. Hartikka* and H. Lübbert. Preclinical Research, Sandoz Pharma Ltd, CH - 4002 Basel, Switzerland.**

We have tried to identify substances which affect the survival of dopaminergic neurons using primary cell cultures prepared from fetal (E14) rat substantia nigra. Exposure of mesencephalic cultures to forskolin or cAMP analogues during the first three days after plating increased the dopamine uptake activity by 100 - 200%. The response of dopaminergic neurons to forskolin could be greatly enhanced by exposing the cultures simultaneously to phosphodiesterase inhibitors IBMX or Ro 20-1724. In 3-day old cultures treated with forskolin or cAMP analogues, the number of dopaminergic neurons was increased by 58%.

Exposure of cultures to 1 µM of MPP$^+$ for 24 hours at the end of a 6 - day long culture period reduced the number of TH$^+$ neurons by 50%. Cyclic AMP, but not basic FGF, was able to prevent the degeneration of dopaminergic neurons induced by MPP$^+$.

The results suggest that increased intracellular levels of cAMP protect dopaminergic neurons in situations of stress like the process of dissociation, or the exposure to neurotoxic compounds. Our results reveal novel possibilities for the treatment of Parkinson's disease.

658.3

L-DOPA TOXICITY IN CULTURES OF RAT MESENCEPHALIC DOPAMINE NEURONS. P.J. Kontur*, K.L. Marek, D.E. Redmond, Jr. and R.H. Roth. Neural Transplant Program, Depts. of Pharmacology, Psychiatry and Neurology, Yale U Sch of Med, New Haven, CT 06510.

Treatment of Parkinson's disease by transplantation of fetal brain tissue may be compromised by the effects of the continued administration of L-DOPA on the developing dopamine (DA) neurons in the graft. Cell cultures obtained from mesencephalic brain tissue of rat embyos 13 to 13.5 days of gestation were used to study the potential toxicity of L-DOPA on the development, survival and function of DA neurons. DA neurons were identified and characterized using neurochemical (HPLC analysis of monoamines, uptake of exogenous tritiated dopamine and activity of tyrosine hydroxylase) and immunohistochemical techniques. DA neurons show extensive fiber formation after visualization using an antibody to tyrosine hydroxylase. DA and 3,4-dihydroxyphenylacetic acid levels in the cultures reach a plateau after 10 days *in vitro*. Cocaine-sensitive tritiated DA uptake increased to a plateau between 3 and 7 days *in vitro* where it remained for up to 17 days. Addition of 5x10-5 or 10-4M L-DOPA to the culture media for 4 days resulted in cellular degeneration, a 50-55% decrease in DA levels and a total loss of cocaine-sensitive tritiated DA uptake. Concentrations of 10-6 and 10-5M L-DOPA caused 20-30% decreases in DA levels and 10-30% decreases in cocaine-sensitive tritiated DA uptake. The demonstration of morphological and biochemical changes in cultured DA neurons after short-term administration of L-DOPA suggests that L-DOPA may affect the development of transplanted fetal DA neurons. Supported by the G. Harold and Leila Y. Mathers Charitable Foundation, the United Parkinson Foundation and the National Parkinson Foundation.

658.4

DIHYDREXIDINE DISPLAYS HIGH POTENCY AND FULL EFFICACY AT D_1 DOPAMINE RECEPTORS IN PRIMATE STRIATUM. C.P. Lawler[1,#], V.J. Watts[1], J.H. Gilmore[1], S.B. Southerland[1], J.R. Atashi[1], H.P. Smith[1], C.A. Mathis[2], D.E. Nichols[3], and R.B. Mailman[1]. University of North Carolina[1], Chapel Hill, NC, 27514-7250, University of Pittsburgh[2], Pittsburgh PA, 15213, and Purdue University[3], West Lafayette, IN, 47907.

Dihydrexidine (DHX; trans-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydro benzo[a]phenanthridine) has been reported to be a high potency, full efficacy D_1 dopamine receptor agonist in rat striatum (*Mottola et al., in press; JPET*). We have suggested previously that such an agent may have clinical utility for the treatment of idiopathic Parkinson's disease; this hypothesis has been supported by recent *in vivo* studies demonstrating dramatic anti-Parkinsonian effects of DHX in MPTP-treated monkeys (*Taylor et al. Eur. J. Pharmacol. 199:389, 1991).* In view of these encouraging results, the present studies examined further the pharmacological properties of DHX in primate brain. *In vitro* binding studies in postmortem human brain demonstrated that DHX competed for D_1 receptor sites (labeled with ^{3}H-SCH23390) with low nanomolar potency. Affinity of DHX for D_2 receptors in human brain was assessed using competition with [^{125}I]-epidepride; DHX was significantly less potent at competing for D_2 sites than for D_1 sites. These data from antagonist binding studies suggest that, in primate brain, as in rat brain, DHX has at least ten-fold selectivity for D_1 over D_2 receptors. Functional activity of DHX in primate brain was assessed by the ability of DHX to stimulate adenylate cyclase in adult rhesus monkey putamen. DHX had both high potency and full efficacy (relative to dopamine) in stimulating cAMP synthesis; conversely, SKF38393 was only a partial agonist in monkey putamen, similar to its effects in rat brain and various cell lines expressing D_1 receptors. The potency and efficacy of these two agonists did not show large inter-species differences; this aspect of our studies fails to confirm a previous report of significantly lower efficacy of SKF38393 in primate vs. rat striatum (*Pifl et al. Eur. J. Pharmacol. 202:273, 1991*). (Supported, in part, by PHS Grants MH40537 and MH42705).

658.5

POSTSYNAPTIC SUPERSENSITIVITY IN STRIATAL D_1 DOPAMINE RECEPTORS IN MPTP-TREATED MICE. L.L. Cook[1]*, D.B. Miller[2], J.P. O'Callaghan[2], J.M. Petitto[1], D.E. Nichols[3], M.H. Lewis[1] and R.B. Mailman[1]. Brain and Development Research Center, Univ. of North Carolina[1], Chapel Hill, NC; Neurotoxicol. Div., EPA[2], RTP, NC; and Purdue Univ.[3], West Lafayette, IN.

Although much is known about the postsynaptic compensation after denervation with 6-hydroxydopamine, few studies have evaluated such effects after MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) treatment. C57BL/6 mice were used to evaluate possible functional supersensitivity of striatal D_1 receptors, since MPTP produces selective degeneration of substantia nigra-caudate neurons in this strain. Dihydrexidine (DHX) was used as a probe because it is a potent full efficacy D_1 agonist with some D_2 agonist properties (Brewster et al., J. Med. Chem., 33:1756, 1990). Female C57BL/6 mice (7-8 months of age) were administered a single SC injection of 15 mg/kg MPTP. Three weeks after dosing, mice were tested for behavioral supersensitivity, and striatal tissue was collected to measure dopamine concentrations, density of D_1 and D_2 receptors, and D_1/D_2 effects on regulation of adenylate cyclase. MPTP caused a 78.5% depletion of dopamine, but did not cause behavioral supersensitivity to apomorphine (0.45-3.0 mg/kg). Using ^{3}H-SCH23390 and ^{3}H-spiperone as ligands, the densities of striatal D_1 and D_2 receptors were found to be unchanged in MPTP-treated mice. The efflux of cAMP from superfused striatal slices was measured following stimulation by 1.0 μM DHX, both in the absence and presence of the D_2 antagonist sulpiride (30 μM). Unlike what is found in rat striatal minces, DHX alone did not increase cAMP efflux. Sulpiride significantly potentiated the DHX-induced cAMP efflux, indicating a pronounced D_1/D_2 interaction in the mouse striatum. In the presence of sulpiride, the efflux of cAMP from striatal slices of MPTP-treated mice was 56% greater than in controls, demonstrating a lesion-induced change in D_1/D_2 functional interactions. Thus, in this model, there may be both D_1 and D_1 functional supersensitivity concomitant with an absence of behavioral supersensitivity. (Supported by ES01104, MH40537, and MH42705 and the Foundation of Hope).

658.6

CHRONIC SINEMET (L-DOPA) ALTERS DOPAMINE RECEPTORS IN MPTP-TREATED MONEKYS. L. Rioux*, P.A. Frohna, J. S. Schneider and J.N. Joyce. Dept. Psychiatry, Univ. Penn. Sch. Med, and Dept Neurology, Hahneman Univ. Sch. Med., Philadelphia , PA , U.S.A..

Administration of MPTP to monkeys results in a parkinsonian syndrome that can be corrected by administration of L-DOPA (Sinemet). Chronic treatment with L-DOPA also can lead to dyskinesias in parkinsonian animals. In order to understand the involvement of the DA system in this syndrome, the present study examined the regional integrity of the DA system using quantitative autoradiography for the pre-synaptic DA system ([^{3}H]mazindol binding to DA uptake sites), D1 receptors ([^{3}H]SCH 23390), and D2 ([^{125}I] epidepride) in the basal ganglia (caudate-putamen (CPu), nucleus accumbens (Nas), globus pallidus internal (GPi) and external (GPe)) of symptomatic monkeys. The monkeys were divided into three groups: control, MPTP monkeys and MPTP monkeys receiving L-DOPA. The MPTP-treated monkeys were sacrificed 6-11 weeks after the last injection of MPTP and the L-DOPA treated monkeys 30-48 hrs after the last dose of L-DOPA. One hemisphere was used for measurement of DA and metabolites, and the other for autoradiographic studies. MPTP induced losses of [^{3}H]mazindol binding in the striatum (> 90%), slightly more in dorsal areas (98%) than ventral ones (91%), while relatively sparing the NAS (64% loss). D2-receptor density and distribution was largely unchanged by MPTP and L-DOPA treatment. In MPTP monkeys, a substantial increase of D1 receptor density was observed in CPu and GPi. In MPTP-treated monkeys receiving L-DOPA, a further increase of D1 receptor density was observed in most regions of striatum, but not GPi. Interestingly, L-DOPA induced an increase in D1 receptors in the GPe. Contrary to previous reports, D1 but not D2 receptor density is increased following MPTP. L-DOPA-induced D1 increases may be related to the development of dyskinesias. **Funded by R29 MH 4385, FRSQ fellowship, and the Benign Essential Blepharospasm Research foundation**

658.7

SYSTEMIC CHLOROQUINE PROTECTS AGAINST STRIATAL DOPAMINE DEPLETION INDUCED BY UNILATERAL INTRA-NIGRAL MPP+ INJECTION IN RATS. G.T. Golden*, G.M. Alexander, R.J. Schwartzman, G.G. Smith, D.S. Martin. Thomas Jefferson Medical College, Philadelphia, PA 19107 and VAMC, Coatesville, PA 19320.

Intracellular iron may induce Parkinson-like conditions due to enhanced formation of toxic O2 species and/or autoxidation products. Chloroquine is a lysosomotropic amine shown to reduce the sensitivity of cells to oxidative stress by preventing the mobilization of ferric iron from ferritin. Adult, male LEH rats (n=12) were tested for 'dominant' rotational behavior with amphetamine and apomorphine. Rats received chloroquine (30mg/kg) or DH20 ip for 21 days. On day 22 four rats from each group had the SN in either the dominant or the non-dominant hemisphere injected with MPP+ (15 nM in 2 ul) and two rats from each group had the SN injected with normal saline. Rotation was tested in a computerized activity monitor where CW turns, CCW turns and incomplete turns were monitored before and after SN injection of MPP+ or control vehicle. The injected and non-injected striati were analyzed for DA, 7-8 weeks post SN injection. Striatal DA levels were Chlor TX + MPP+ (Inj-70.2 ±13.2, Non-inj-75.5±4.7); Control Tx + MPP+ (Inj-57.1 ±12.2, Non-inj-75.1±9.8). The Chlor Tx + Control Vehicle and the Control Tx + Control Vehicle showed no striatal DA differences between injected and non-injected sides. We conclude that pretreatment with chloroquine protects against DA depletion produced by intra-nigral injection of MPP+. Supported in part by NIH Grant #NS27101 and the VA.

658.8

STRIATAL DOPAMINE RECEPTORS AND GABAA RECEPTORS IN DYSKINETIC AND NON-DYSKINETIC MPTP-MONKEYS TREATED CHRONICALLY WITH CY 208-243, C. Gagnon[1]*, B. Gomez-Mancilla[2], P.J. Bédard[2] and T. Di Paolo[1], [1]Sch. of Pharmacy, Laval Univ. and Dept of Mol. Endo., CHUL, Quebec, G1V 4G2; [2]Dept of Pharmacol., Fac. Med., Laval Univ., Quebec, G1K 7P4, Canada.

Two out of four MPTP-monkeys developed dyskinesias following chronic treatment with the D-1 agonist CY 208-243 (CY). ^{3}H-SCH 23390 (SCH) (D-1 antagonist), ^{3}H-spiperone (D-2 antagonist), ^{3}H-SKF 38393 (SKF) (D-1 agonist), ^{3}H-N-propylnorapomorphine (NPA) (D-2 agonist) and ^{3}H-muscimol (GABAA agonist) specific binding were compared in the striatum of these monkeys. Non-dyskinetic MPTP-monkeys were less denervated than dyskinetic animals, as shown by the higher striatal dopamine (DA) levels in non-dyskinetic monkeys. ^{3}H-SCH and ^{3}H-SKF binding were similar in the striatum of both groups of MPTP-monkeys, whereas ^{3}H-spiperone, ^{3}H-NPA and ^{3}H-muscimol binding were higher in the posterior striatum of dyskinetic monkeys compared to non-dyskinetic animals. ^{3}H-SCH/^{3}H-spiperone and ^{3}H-SKF/^{3}H-NPA ratios were lower in the posterior striatum of dyskinetic compared to non-dyskinetic MPTP-monkeys. ^{3}H-SKF/^{3}H-SCH, ^{3}H-NPA/^{3}H-spiperone, ^{3}H-muscimol/^{3}H-SCH and ^{3}H-muscimol/^{3}H-spiperone ratios were similar in the striatum of both groups of MPTP-monkeys. ^{3}H-muscimol/^{3}H-SKF ratio was increased in the posterior striatum, whereas the ^{3}H-muscimol/^{3}H-NPA ratio was decreased in the anterior striatum of non-dyskinetic compared to dyskinetic MPTP-monkeys. The degree of MPTP-induced denervation as well as the relative changes of D-1 and D-2 DA receptors as well as GABAA receptors in the striatum may explain appearance of dyskinesias in MPTP-monkeys treated chronically with CY. Supported by the Parkinson Foundation of Canada and the MRC.

658.9

EFFECT OF PARTIAL 6-OHDA LESIONS ON THE EXPRESSION OF TYROSINE HYDROXYLASE IN DOPAMINERGIC NEURONS OF THE SUBSTANTIA NIGRA AND THEIR PROJECTIONS. R.Raisman-Vozari*(1), V. Blanchard(1), M. Chritin(2), S.Vyas(1), M. Savasta(2), C. Feuerstein(2), F. Javoy-Agid(1) and Y. Agid(1). (1) INSERM U 289, 75013 Paris; (2) U 318, 38043 Grenoble, France.

In a preclinical phase of Parkinson's disease (PD) few neurological symptoms are observed despite an extensive damage of the dopaminergic system. The compensatory mechanisms involving either the activity or the amount of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, has been proposed. The present study was designed to characterize the effects of partial lesions of nigro-striatal system on the expression of TH. Unilateral lesions were produced by the injection of 1 μl 6-OHDA (8 μg/4 μl) in the substantia nigra pars compacta (SNC). Animals were sacrificed either one or 7 months after lesions. TH was quantified by immunoautoradiography in the striatum along the dorso-rostral axis of the structure. TH protein amount and TH mRNA were quantified respectively in the SNC by immunoautoradiography and in-situ hybridization using computer assisted image analysis. 50 % loss of TH positive cells was observed one month after partial lesions by cell counting. Loss in the amount of striatal TH followed the neuronal loss but with a characteristic pattern of TH distribution. The decrease of the enzyme was more pronounced in the caudal striatum compared to more rostral planes. In addition a preferential loss was observed in the lateral region compared to the medial area. Parallel quantification of TH protein and TH mRNA on surviving dopaminergic cells indicated absence of TH compensatory mechanism one month after partial denervation of the dopaminergic system. Results obtained 7 months later will be presented. Results suggest that one month post-lesion period may represents either an early phase for compensatory mechanisms or that other compensatory events underlie the observed preclinical phase of PD.

658.10

NEUROMELANIN AND MPTP-INDUCED PARKINSONISM IN MONKEYS. M.T. Herrero[1,2*], E.C. Hirsch[1], M.R. Luquin[2], J. Laguna[2], F. Javoy-Agid[1], J.A. Obeso[2], Y. Agid[1]. (1) INSERM U289, 75013-Paris, France and (2) Neurologia Experimental, Univ. Navarra, 31080-Pamplona, Espana.

Neuromelanin (NM), a by-product of catecholamine metabolism, accumulates during aging in the primate brainstem. By analogy to Parkinson's disease in which a preferential vulnerability of melanized neurons is observed, it has been hypothetized that NM may contribute to dopaminergic cell death characteristic of both MPTP toxicity and Parkinson's disease.

In order to analyse the time-dependency of NM accumulation in primates and the influence to MPTP vulnerability of the NM presence in dopaminergic neurons, we have analyzed the number of melanized and non-melanized catecholaminergic neurons in the mesencephalon of cynomolgus monkeys. 5 control animals of different ascending age (from 0 to 13 years) have been used to study the effect of age on NM-accumulation. Age matched controls were compared with 7 MPTP-treated monkeys (2 severely disabled, 2 moderately affected, and 3 treated by L-dopa) in order to study the effect of MPTP on melanized neurons. Catecholaminergic neurons from the brainstem were identified by immunohistochemistry of tyrosine hydroxylase (TH), and NM was detected by Masson staining. Each neuron was plotted and total number of neurons in each catecholaminergic region was estimated using image analysis.

A time-dependent increase both of the number of NM-containing cells and the NM content per neuron was observed. However, since some melanized cells were also preserved, these results suggest that NM may participate in MPTP toxicity, but that other factors are also needed to explain the loss of dopaminergic neurons. In MPTP-treated monkeys the loss of catecholaminergic neurons was the most severe in regions containing the high proportion of melanized neurons, SNα+β and SN pars lateralis.

658.11

ENKEPHALIN, DYNORPHIN AND TYROSINE HYDROXYLASE mRNA IN THE WEAVER MUTANT MOUSE BRAIN S. Reid*, J. Lipkin, F.M. Leslie and S.E. Loughlin, University of California Irvine, CA 92717

In the weaver mutant mouse, a subpopulation of substantia nigra cells is lost during early postnatal development. As a result, caudate putamen dopamine levels are greatly decreased and a number of striatal neurochemical characteristics are affected. In other models, loss of striatal dopamine causes changes in endogenous opioid peptides and receptors. In rats, neurotoxic lesions of the nigrostriatal projection have been shown to decrease striatal opioid receptors, to decrease striatal dynorphin expression and to increase striatal enkephalin expression. In the weaver mouse, we have recently shown that striatal delta and kappa opioid receptors are decreased as compared to controls, while mu receptors are unchanged. In the present study, we compared tyrosine hydroxylase, proenkephalin and prodynorphin mRNAs in weaver and control littermate mice using in situ hybridization and quantitative autoradiography. Tyrosine hydroxylase mRNA decreased in the substantia nigra pars compacta of weaver mice, but appeared unchanged in the olfactory bulb. While striatal prodynorphin mRNA did not differ between control and weaver brains, proenkephalin mRNA significantly increased in the weaver striatum ($p < 0.025$). Thus, enkephalin is upregulated following genetically determined, early postnatal loss of striatal dopamine. The results suggest that upregulation of enkephalin is not necessarily associated with mu opioid receptor downregulation. Parallels between opioid system changes in weaver brains and Parkinson's disease suggest novel treatment strategies for symptoms not controlled by L-DOPA, such as dyskinesia or affective disorder. Supported by NS 26761 and the American Parkinson Disease Association SCC.

658.12

THE EFFECT OF ESTROGEN UPON L-DOPA EVOKED STRIATAL DOPAMINE RELEASE IN VITRO IN MPTP-TREATED FEMALE MICE. J.L.McDermott* and D.E.Dluzen, Department of Geriatric Medicine, University Hospitals, Cleveland, OH 44105, and Department of Anatomy, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Ovariectomized (OVX) retired breeder CD-1 and C57/Bl mice ± estrogen (EB) were treated with MPTP (10 mg/kg ip, q.i.d. x 1d). At 5-10 days post-MPTP, L-DOPA (5 μ M) evoked dopamine (DA) release from superfused corpus striatal tissue fragments was determined. Estrogen treatment of CD-1 mice treated with MPTP increased L-DOPA-evoked DA release ($\bar{x}$ ± SEM - areas under L-DOPA-evoked DA release curves in pg/70 minutes: OVX/MPTP = 316 ± 53, N=12 versus OVX + EB/MPTP = 494 ± 104, N=11). Moreover, MPTP failed to alter L-DOPA -evoked DA release from that obtained in estrogen-treated CD-1 mice (OVX + EB = 511 ± 48, N=12). In contrast, estrogen treatment of C57/Bl mice treated with MPTP did not significantly alter L-DOPA-evoked DA release (OVX/MPTP = 372 ± 89, N=12 versus OVX + EB/MPTP + 303 ± 37). However, MPTP did significantly reduce L-DOPA-evoked DA release in these C57/Bl mice (OVX + EB = 579 ± 69, N=11). In both strains estrogen treatment resulted in significant increases of L-DOPA-evoked DA release (CD-1: OVX = 335 ± 58, N=12 versus OVX + EB = 511 ± 48, N=12; C57/Bl: OVX = 219 ± 46, N=12 versus OVX + EB = 579 ± 69, N=11). These results demonstrate that OVX mice of both strains show enhanced striatal DA responsiveness to L-DOPA following estrogen treatment. Interestingly, while MPTP treatment did not reduce L-DOPA-evoked DA release from OVX females of either strain, the estrogen dependent augmented L-DOPA evoked response was completely abolished in C57/Bl, but not CD-1, mice treated with MPTP.

658.13

MPTP DOES NOT MIMIC THE EFFECTS OF PARKINSON'S DISEASE ON THE ADRENAL MEDULLA OF THE MOUSE. S.L. Stoddard*, G.J. Merkel, J.A. Cook, and S.W. Carmichael. Depts. of Anatomy and Microbiology, Indiana Univ. Sch. of Medicine, Fort Wayne, IN 46805 and Dept. of Anatomy, Mayo Clinic, Rochester, MN 55905

We have previously reported that the catecholamine content of the adrenal medulla is depressed in the parkinsonian patient [Stoddard et al. *Exp. Neurol. 104*, 218-222, 1989]. This condition may be a peripheral manifestation of Parkinson's disease. Since MPTP destroys dopaminergic cells in the substantia nigra and is used to produce a model of Parkinson's disease, we wished to determine whether this neurotoxin also affects peripheral targets. Mice (C57BL, ♂, 8-wk-old, N=30) were injected with *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (20 mg/kg; 3.0 mg/ml) at 0, 4, 8, and 24 hours, and sacrificed 4, 8, 12, or 16 weeks later. Catecholamines were extracted from both the adrenal glands and striatum by alumina adsorption, quantitated by high performance liquid chromatography with electrochemical detection, and expressed as ng/adrenal pair or ng/mg striatum. Catecholamine levels in MPTP-treated adrenals were compared to levels in age-matched control adrenals (N=21) using a simple randomized two-factor ANOVA. Although a slight decrease in catecholamine content was observed at 4 weeks in the MPTP-treated animals, the difference was not significant and catecholamine levels returned to control levels over time. These data suggest that, although MPTP mimics the central effects of Parkinson's disease by decreasing the dopamine content of the striatum, this neurotoxin does not produce the concomitant depletion of adrenal medullary catecholamines that is observed in human patients with idiopathic Parkinson's disease.

658.14

L-DOPA EFFECTS ON METHIONINE ADENOSYL TRANSFERASE (MAT) AND CATECHOL-O-METHYLTRANSFERASE: RELEVANCE TO PARKINSON'S DISEASE (PD) THERAPY. R. BENSON* AND C. CHARLTON, Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

It was observed that the injection of S-adenosyl methionine (SAM) into the lateral ventricles of rodents produced symptoms similar to those seen in PD patients, namely, tremors, hypokinesia, depletion of tyrosine hydroxylase and dopamine (DA), an increase in homovanillic to DA ratio and degenerative changes in the nigrostriatum; therefore, increased biological methylation may occur in PD. L-dopa, the major therapeutic agent for PD, is a potent methyl acceptor. It utilizes SAM when it is administered. Such a utilization, along with an increase in DA, may be related to the effect of L-dopa. It follows that the lack of efficacy from prolonged l-dopa therapy may be due to a rebound phenomenon, which tends to replenish the methylation system. To test this, mice were exposed to chronic l-dopa followed by analysis of brain and plasma MAT and COMT activities.

L-dopa (100mg/kg) treatments of 1, 2, and 3 times/day for 4 days increased brain MAT activity by 0.2%, 4.9% (not significant) and 21.3%, respectively. A treatment of 3 times/day for 8 days further increased (28.4%) brain MAT activity. MAT was not detected in the plasma. Preliminary data showed that L-dopa treatments of 1 and 3 times/day for 4 days increased brain COMT activity by 27.9% and 29.1%, respectively, above that of the controls. In contrast, the plasma COMT activity was decreased by over 80%. These results indicate that high frequency and chronic l-dopa treatments will induce increases in the activities of brain MAT and COMT, while decreasing plasma COMT. Increased MAT will increase SAM, which in combination with the increased COMT will lead to the increased metabolism of L-dopa and DA. (Supported by NIH GM08037 and NS 28432)

658.15

THE BIOCHEMICAL EFFECTS OF S-ADENOSYL-METHIONINE (SAM) IN RATS: RELATIONSHIP TO PARKINSON'S DISEASE. B. Crowell, Jr.*and C. Charlton, Dept. of Physiology, Meharry Medical College, Nashville, TN 37208

S-adenosyl-methionine (SAM) is a potent methyl donor that is involved in the metabolism of several biogenic amine neurotransmitters, including dopamine (DA), norepinephrine (NE) and serotonin (5-HT). When SAM is injected into the lateral ventricle of rats, tremors, hypokinesia, and abnormal posture are observed. These impairments along with the depletion of DA, NE and 5-HT have been observed in Parkinson's Disease (PD) patients. Therefore SAM may be involved in PD. The mechanism by which SAM induces motor impairments in rats is believed to be related to a depletion of DA.

We tested this hypothesis by injecting SAM into the lateral ventricle of rats and measuring changes in DA, NE and 5-HT. Measurements were made acutely (one injection and determination at 20 post-injection) and chronically (daily injection for 14 days and determination at 16 days post-injection). Both the acute and chronic administration of SAM depleted DA by 26.6 and 41.8% and increased HVA/DA ratio by 42.3 and 47.9% respectively. NE was depleted by 30.3% following the acute administration of SAM, whereas, 5-HT was depleted by 60.1% following only the chronic administration. These results showed that the biochemistry of DA, NE and 5-HT are affected following the administration of SAM.

NE and 5-HT are not known to play direct roles in PD, but DA depletion and an increase in the HVA/DA ratio do occur in PD and in animal models of the disease. Therefore, the administration of SAM into the lateral ventricle of animals may serve as a model of PD. This recently observed biochemical effect of SAM and the already established behavioral effects indicate that excessive SAM-dependent reactions may be involved in PD. Supported by NIH GM08037 and NS 28432

658.16

EFFECT OF U-91356A, A NOVEL DOPAMINE AGONIST AND ANTIPARKINSON (AP) DRUGS ON STRIATAL ACETYLCHOLINE CONCENTRATION. V.H.Sethy*, N.F.Nichols and P.J.D. Schreur, CNS Diseases research, The Upjohn Company, Kalamazoo, MI 49001.

The potency and intrinsic activity of a dopamine agonist, U-91356A (U), has been compared with AP drugs apomorphine (A), bromocriptine (B), lisuride (L), quinpirole (Q) and pergolide (P) by measuring the striatal acetylcholine (ACh) concentration in non-reserpinized (NR) and reserpinized (R) rats. Some of the drugs have also been investigated in unilateral substantia nigra (SN) lesioned (6-hydroxydopamine) rats. In NR rats, the ED50s of Q, U, P, A and B, in descending order of potency, were 0.06, 0.21, 1.62, 1.72 and >10.0 mg/kg i.p. L failed to increase ACh levels by 50%. U was the most efficacious (100%) compound, and the relative intrinsic activities of Q, P, A and B were 80%, 63%, 45% and 30%, respectively. Reserpine (5 mg/kg i.p.) significantly ($p<0.01$) decreased ACh concentration. In R rats the, ED50s of AP drugs were several fold lower than in NR rats. In R rats, the slope of the dose-response curves for P and L were significantly different from those of U and Q. This may be due to the D2 selectivity of U and Q and non-selectivity of P and L for dopamine receptors. In addition, serotonergic and adrenergic effects of P and L may alter their dopaminergic response. The responses of Q and U for increasing ACh concentration were significantly higher on the lesioned side as compared to the intact side due to supersensitivity of D2 receptors following the degeneration of SN. The effects of AP drugs on striatal ACh concentrations indicate that U-91356A is a post-synaptic dopamine receptor agonist and may be useful for treatment of Parkinson's disease.

659.1

DECREASED EXTRACELLULAR BUFFERING CAPACITY DIMINISHES HIPPOCAMPAL SLICE SURVIVAL OF ANOXIA.
E.L. Roberts, Jr.* Department of Neurology and GRECC, VA Medical Center, University of Miami School of Medicine, Miami, FL 33136

The capacity of brain tissue to survive anoxia or ischemia may depend upon how well brain tissue buffers pH changes during and after such insults. This dependency was examined in hippocampal slices from male F-344 rats. Slices were placed into an interface chamber and bathed in an artificial cerebrospinal fluid (ACSF) of control (26 mM) or low (5 mM) bicarbonate buffering capacity. Slices were bubbled initially with gas mixtures containing 95% O_2, 5% CO_2 (control $NaHCO_3$) or 99% O_2, 1% CO_2 (low $NaHCO_3$). ACSF pH was 7.3-7.4. Slices were made anoxic (95% O_2, 5% CO_2 (control) or 99% N_2, 1% CO_2 (low $NaHCO_3$)) for approximately 9 min., then returned to the appropriate oxygen-containing gas mixture. During an experiment, extracellular K^+ activity (K^+_o) and the orthodromically-elicited CA1 pyramidal cell population spike were monitored. During anoxia, disappearance of the orthodromic population spike occurred sooner in low $NaHCO_3$ ACSF. After anoxia, slices exposed to low $NaHCO_3$ were less able to reestablish K^+_o homeostasis and did not recover population spike activity. These results support the view that sufficient HCO_3^- buffering capacity is required for recovery of ion homeostasis and synaptic transmission in brain tissue following anoxia. (Supported by NIA AG08710)

659.2

LACTATE ACCUMULATION DURING CEREBRAL ISCHEMIA: ATTENUATION BY INHIBITING EXCITATORY AMINO ACID-MEDIATED ION FLUXES AND ION PUMP ACTIVITY.
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Cerebral ischemia induces massive ionic shifts through the cell membrane resulting in cell swelling. This cell swelling has been though to be due to energy depletion. However, recently we have shown that excitatory amino acid (EAA) antagonists attenuate both ischemia-induced ionic shifts and cell swelling. Consequently we proposed that a major cause of the ionic shifts seen following ischemia is due to the stimulation of EAA-coupled ion channels resulting in an increase in energy demand in order to restore ionic balance. Therefore, inhibition of EAA-mediated ion fluxes could delay energy depletion during ischemia. To test this hypothesis, we measured cerebral lactate concentrations during the acute phase of cerebral ischemia as an indicator of energy metabolism and tested the effects of the Na+/K+ ATPase inhibitor ouabain and the EAA antagonists kynurenic acid (KYN) on the ischemia-induced lactate accumulation. Microdialysis probes were placed bilaterally in the hippocampal CA1-dentate area. One probe was perfused with Ringer's solution (control) and the other with ouabain or KYN. 30 min after the start of dialysis, ischemia was induced by decapitation and dialysate lactate concentrations were measured by HPLC. In the control probes, lactate concentration immediately increased and reaching maximum levels within 3-5 min after ischemia induction. This lactate accumulation was significantly delayed by ouabain and KYN. These results suggest that ion pumps are activated to restore ionic balance disrupted by EAA-mediated ion fluxes, resulting in increased energy demand which causes activation of glycolysis and lactate production. The attenuation of energy consumption and lactate accumulation may be one of the protective mechanisms of EAA antagonists against ischemic brain damage.

659.3

SNARF AND NEUTRAL RED INDICATE THE PRESENCE OF DISTINCT HYDROGEN ION COMPARTMENTS IN THE RAT HIPPOCAMPAL SLICE.
Tim S. Whittingham[1]*, Chii-Wann Lin[2], Alfred O. DiScenna[1] and Joseph C. LaManna[2,3]. Departments of Neurological Surgery[1], Biomedical Engineering[2] and Neurology[3], Case Western Reserve University, Cleveland, OH 44106.

We have previously reported the effects of amiloride analogs on intracellular pH estimated by the Neutral Red spectrophotometric method. We have extended these studies, comparing the pH estimates made from SNARF fluorescence to those of Neutral Red. The difference in control values and in response to anoxia or ammonium-induced acidification suggest that the two methods are monitoring separate pH compartments. Hippocampal slices were exposed to 50 μM Neutral Red or 10 μM SNARF-AM in HEPES-buffered artificial cerebrospinal fluid (ACSF) for 1 hr prior to a 30 min washout period to remove extracellular dye. The slices were transferred to an optical recording chamber and monitored for 30 min in control conditions. The ACSF was then switched to one of three experimental paradigms: 1) ACSF equilibrated with 100% nitrogen to produce anoxia; 2) ACSF containing 1 mM amiloride to block sodium-hydrogen antiport; or 3) ACSF containing 20 mM ammonium chloride. The control pH value for SNARF was 7.09 ± 0.02, compared to 7.59 ± 0.05 for Neutral Red. In response to anoxia, SNARF pH dropped 0.25 units in 10 minutes, and reached a value of 6.75 following 30 min of anoxia. In contrast, Neutral Red pH changed little in the first 10 minutes and was 7.08 after 30 min. Amiloride had no significant effect on SNARF pH during a 60 min exposure, but Neutral Red pH fell 0.8 units to 6.76. Finally, rebound acidification following exposure to 20 mM ammonium chloride caused SNARF pH to acidify by about 0.2 units with no subsequent recovery. Neutral Red pH also fell about 0.3 units, but recovered to control pH level in 10-15 minutes. Initial photographs indicate that SNARF appears to be more highly concentrated in the vicinity of the hippocampal somal layers, while Neutral Red exhibits a more uniform distribution in the slice.

659.4

METABOLIC FAILURE LEADS TO THE DETERIORATION OF THE BORDER ZONE IN REVERSIBLE FOCAL ISCHEMIA.
W.R. Selman, W. D. Lust, S. Pundik, C.M. Jenkins, S.U. Bhatti and R.A. Ratcheson*. Lab. of Exper. Neurol. Surgery, Case Western Reserve Univ., Cleveland, OH 44106

Experimental studies have shown that the benefits of reperfusion are limited to the first 2 hours following occlusion of the middle cerebral artery in spontaneously hypertensive rats (SHR). Even the border zone (BZ) surrounding the ischemic core deteriorates under these conditions. To determine what triggers the onset of irreversible damage, the metabolic status of the BZ was determined at one hour of reflow following increasing periods of focal ischemia. The MCA was occluded for 1, 2 or 4 h after which reperfusion was initiated and the brains were frozen in situ at 1 h of reflow. The brains were sectioned, lyophilized and ATP, P-creatine, glucose, lactate, GABA and glutamate were measured in the cerebral cortex adjacent to the ischemic core. While glucose recovered in the BZ of all groups, lactate levels were 2-, 4.5- and 11.8-fold over those of control and ATP levels were 61, 42 and 22 % of control at 1 h of reflow after 1, 2 and 4 h of occlusion, respectively. Accompanying these metabolic changes were significant increases in GABA and decreases in glutamate. GABA levels were 1.6-, 3.6- and 7.5-fold greater than those of control, while those for glutamate were 76, 64 and 36% of control at 1 h of reflow after 1, 2 and 4 h of ischemia, respectively. These metabolic derangements in the BZ during reflow following increasing periods of focal ischemia indicate that a persisting lesion in the metabolic machinery during reperfusion may be a factor in the evolution of border zone damage following focal ischemia.

ISCHEMIA: NEUROPHYSIOLOGY

660.1

WHOLE CELL PATCH CLAMP ANALYSIS OF MEMBRANE CHANGES DURING HYPOXIC AND NORMOXIC SPREADING DEPRESSION IN HIPPOCAMPAL CA1 PYRAMIDAL AND GLIAL CELLS.
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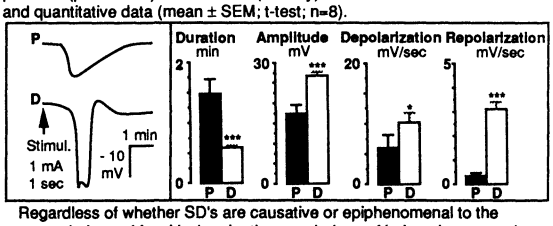
Spreading depression (SD) can be provoked in normoxic hippocampal tissue by various events. A similar if not identical phenomenon can be provoked by hypoxia. The identity of normoxic and hypoxic SD is open to question. We used the whole cell patch technique in voltage- and current-clamp modes to study SD in pyramidal and glial cells in CA1 region of hippocampal slices. Population responses were recorded by extracellular electrode near the patched cell; this extracellular potential, rather than the bath ground, was used as the voltage clamp reference. Patch pipettes contained either cesium or potassium gluconate. Glial cells were identified by lack of active responses to depolarizing voltage steps and to orthodromic volleys. SD was provoked by local irrigation of high K^+ solution or by 3-5 minute periods of hypoxia. In some cells both types of SD were provoked sequentially. We found: (1) Neural responses did not differ observably during normoxic vs. hypoxic SD. In I-clamp cells depolarized at first slowly, then rapidly to V_m near 0 mV, accompanied by complicated changes in firing properties. In V-clamp neurons held at or near resting potential exhibited an increased inward current during SD, to levels as high as 2 nA. I/V plots of responses to ramp commands during SD revealed a 60-70% decrease of membrane input impedance and current reversal at or slightly positive to 0mV. (2) The input impedance of glial cells did not decrease during SD. (3) The inward current during SD reversed to outward at holding potentials near +30 mV (with cesium pipettes). These findings suggest that at the level of the cell soma membrane there is no important difference between normoxic and hypoxic SD, and that glial membrane changes during SD are a passive response to increased extracellular K^+. [Supported by NS-17771]

660.2

DISTINCT PROFILES OF PROPAGATING SPREADING DEPRESSIONS: A DIAGNOSTIC TOOL FOR THE PENUMBRA.
M. De Ryck*, R. Marrannes, E. De Prins and G. Clincke. Janssen Research Foundation, Department of Neuropsychopharmacology, B2340 Beerse, Belgium.

A photochemical thrombotic infarct (Stroke,1989,20,1383-1390) was induced in rat parietal cortex (n=31), where two glass micropipettes recorded DC activity and K^+_o or pH_o. A platinum stimulating electrode was placed frontally. In sites 100-500 µm proximal (P) to the infarct edge, infarct-related spreading depressions (SDs) progressively increased in duration, decreased in amplitude, and developed slower repolarization slopes: they became attenuated and shallow. By contrast, in sites 1-3 mm distal (D), SDs maintained a sharp profile, while somewhat increasing in duration. However, infarct-related SDs always ceased. When electrically induced SDs were then allowed to propagate towards the infarct, they were drastically deformed in the proximal (penumbral) but not distal (healthy) tissue. See illustrated individual and quantitative data (mean ± SEM; t-test; n=8).



Regardless of whether SD's are causative or epiphenomenal to the neuropathology of focal ischemia, the morphology of induced, propagating SD's can be used as a diagnostic tool for the penumbra.

660.3

RELATIONSHIP BETWEEN DEPOLARIZATION AND HISTOLOGICAL CHANGE AFTER FOCAL ISCHEMIA IN THE RAT. Y. Takeda, M. Jacewicz*, Y. Takeda, and W. A. Pulsinelli. Dept. of Neurology and Neuroscience, Cornell University Medical College, New York, NY 10021

We examined the temporal-spatial relationship between anoxic depolarization and focal injury in halothane-anesthetized Spontaneously Hypertensive Rats. The middle cerebral and common carotid arteries were occluded temporarily for 1 h or 3 h (n=5 per group). DC potential electrodes (DCPE) were inserted 800 μm below the cortical surface at 2 mm posterior and 1-6 mm lateral to bregma (1 mm intervals). At 24 h after occlusion the brains were perfusion-fixed, sectioned and stained with hematoxylin-eosin. The DCPE sites were identified by a needle track through each burr hole. The margin between depolarized and non-depolarized brain was found at 3.4±0.5, 3.4±0.5, 3.6±0.5, 3.8±0.8 mm lateral to bregma at 15 min, 1 h, 2 h and 3 h, respectively. The location of this margin was compared with histological markers of neuronal necrosis and infarction at 24 h for each animal (Table 1). These results indicate that, while the region of depolarized tissue does not change during 3 h ischemia, the margin of eventual tissue injury is established during the same interval, and includes territory not subjected to early depolarization.

Table 1. Margin of Tissue Injury vs. Depolarization
[mm medial (+) or lateral (-) relative to depolarization margin]

Ischemic Duration	Neuronal Necrosis	Infarction
1 h	+0.5 ± 0.4	-0.6 ± 0.9
3 h	+1.4 ± 0.5*	+0.7 ± 0.6*

(* $p<0.05$ vs. 1 h)

660.4

ROLE OF POST-SYNAPTIC MECHANISMS IN ANOXIC-INDUCED DEPRESSION OF NEOCORTICAL SYNAPTIC TRANSMISSION A. S. Rosen* and M. E. Morris. Dept. of Pharmacology, Univ. of Ottawa, Ottawa, Canada K1H 8M5.

Brief anoxia (5 min) differentially depresses EPSPs and IPSPs, recorded intracellularly in rat neocortical pyramidal neurons (*Rosen & Morris 1991, Soc. Neurosci. Abstr. 17:1079*). The depression is reversible and is not caused by occlusion due to the concurrent depolarization, nor by decreased Na^+ gradient due to Na-K-pump failure.

Anoxia may affect ATP-dependent processes related to postsynaptic receptors. Quisqualate (QUIS) (100 μM) and GABA (1 mM) were pressure-ejected in the slice close to the recording site (15-40 psi, 5-400 ms, 1 pulse/min). QUIS elicited strong depolarizing responses, which decreased in amplitude by ≈ 25% in the presence of N_2. GABA elicited at V_R depolarizing responses which became biphasic at more positive V_M (reversal of late phase at -70 mV). Anoxia depressed the responses to GABA by ≈ 26%. The limited effect of N_2 on the post-synaptic responses to locally applied transmitters cannot account for the entire synaptic depression observed (70% decrease in amplitude of the monosynaptic early EPSP) and suggests a dominant (though not exclusive) role of pre-synaptic factors in determining anoxic synaptic failure.

(Supported by the Medical Research Council of Canada)

660.5

HYPOXIC SYNAPTIC DEPRESSION IS A RESULT OF ALTERATIONS AT A PRE-SYNAPTIC SITE AS REVEALED BY THE STUDY OF MINIATURE EXCITATORY POST SYNAPTIC CURRENTS. N. Hershkowitz* A. N. Katchman, S. Veregge. Department of Neurology, Georgetown Univ., Wash. D.C. 20007.

It is well established that hypoxia will result in suppression of neuronal activity by alterations at the synapse. It is less clear whether this represents a pre- or post-synaptic effect. We examined this by evaluating changes in miniature excitatory post synaptic currents (mEPSCs) as well as in the response to pressure-ejected glutamate following hypoxia in in vitro hippocampal slices using whole cell patch clamp. K-gluconate patch electrodes (V_H=-60mV) were used; hypoxia was induced by exposure to 95% N_2/ 5% O_2. We previously reported that after hypoxia there is an increase in the frequency of spontaneous transient inward currents. These currents are suppressed by kynurenic acid and occur in the presence of tetrodotoxin, identifying them as mEPSCs. The average amplitude of currents under normal conditions and in the presence of tetrodotoxin was 12.3 ± 0.7 pA (n = 17) and 13.2 ± 0.7 pA (n = 14), respectively. There was no change in average amplitude of these events after hypoxia even when amplitudes were evaluated at times during which there was nearly complete blockade of the orthodromically elicited responses. However there existed an increase in mean frequency of mEPSCs of 430%. Currents from pressure ejection of glutamate was inhibited by 10-15 % at a time when there was nearly 100% blockade of the orthodromically elicited synaptic response. These results indicate that hypoxic synaptic block occurs at a presynaptic locus and may be associated with an increase in presynaptic calcium concentration. (Supported by NINDS grant NSO1460-01).

660.6

ISCHEMIA-INDUCED CHANGES IN EXPRESSION OF HIPPOCAMPAL LTP AND PTP. N. Hori* and D.O.Carpenter, NYS Dept. of Health and School of Public Health, Albany, NY 12201

Pyramidal neurons in area CA1 of dorsal hippocampus are extremely vulnerable to ischemic episodes, and disappear 3 to 5 days after a non-lethal ischemic event. The reasons CA1 neurons are more vulnerable to ischemia than others are not certain. We have studied properties of injured but still viable CA1 neurons.

Male Wistar rats (200-250 gm) were used in all studies. Under pentobarbital anesthesia, the vertebral arteries were electrocauterized and the isolated carotid arteries were enclosed in a loop of thread. On the following day, the carotid arteries were ligated for 10 or 20 min. Animals that lost righting reflexes after ligature were used for acute brain slice experiments 24 hours later. At this time there are no morphologic indicators of neuronal death in hippocampus, although the neurons in CA1 degenerate after 3 to 5 days.

In slices of animals subjected to 10 or 20 min ischemia 24 hours earlier, field potential recordings made from area CA1 upon activation of the Shaffer collateral pathway from CA3 showed little change as compared to slices from control animals. The evoked response was not obviously reduced in amplitude, although there was a slight broadening of the population spike. Intracellular recordings from single pyramidal neurons in CA1 showed resting membrane potentials and input resistances which were similar to those in control slices.

There were, however, dramatic differences in indicators of synaptic plasticity. Post-tetanic potentiation was normal in slices of animals subjected to 10 min occlusions, but was considerably depressed in slices from animals with 20 min occlusions. Long term potentiation was diminished after a 10 min occlusion and was absent in slices from animals with 20 min occlusions. These results indicate that synaptic events, particularly those with a major presynaptic component, are the first to fail in the process of ischemic neuronal cell death. Supported by NS23807.

660.7

ANOXIA DECREASES HUMAN TEMPORAL NEURONAL EXCITABILITY BY SODIUM CHANNEL MODULATION. G.G.Haddad*, T.R.Cummins and C.Jiang. Dept. of Pediatrics, Sect. of Respir. Med. & Interdepart. Neurosci. Program, Yale University School of Medicine, New Haven, CT.

Although O_2 deprivation can be disastrous for central mammalian neurons, the underlying mechanisms are still unclear. For example, the basic mechanisms for the decrease in excitability that occurs in neocortical neurons during anoxia are not well understood. Using human brain slices and intracellular techniques, we have previously shown that excitability diminishes before any significant alteration in input resistance and membrane potential (Vm). In order to further understand mechanisms of excitability during anoxia, we asked whether O_2 deprivation alters voltage-dependent inward currents. We therefore studied freshly dissociated neurons from adult human cortex in order to effectively voltage clamp membrane currents. Using patch-clamp techniques, we isolated a TTX-inhibitable inward current (I_{Na}) by exposing cells to Cd^{++} and using Cs^+ in the pipette. I_{Na} was studied before, during and after brief exposure to anoxia (A) (PO_2 = 0 Torr) or Cyanide (CN). Neither CN nor A had an effect on the shape of the I-V curve but both reversibly decreased the magnitude of I_{Na} by 33 ± 8% and 48 ± 17% respectively (Vm = -70 mV). In addition, both CN and A had a major effect on the steady-state inactivation of I_{Na} in that the mid-point of the h_∞(V) curve was left-shifted by about 15-20 mV (n=18). ATP (2-10 mM) in the pipette eliminated the attenuation of I_{Na} to a large degree. We conclude that in human temporal neurons: a) the decrease in excitability with anoxia is related, at least in part, to I_{Na} inhibition and b) this I_{Na} inhibition depends on the decrease of intracellular ATP. We speculate that this decrease in I_{Na} during anoxia is a cellular adaptive measure to decrease excitability, minimizing the mismatch between O_2 supply and demand.

660.8

THROMBOTIC INFARCTION TRIGGERS SPREADING DEPRESSION-LIKE EVENTS IN DISTANT BRAIN REGIONS. H. Leistra, Z.C.-Feng, B.D. Watson*, M. Rosenthal and W.D. Dietrich, Dept Neurology, University of Miami School of Medicine, Miami, Fl. 33101.

In rats anesthetized with halothane/nitrous oxide, local cerebral thrombotic infarction can be induced by irradiating the intact skull with green light (560 nm) for 7 min following systemic injection of rose bengal. Initial goals were to define hemodynamic consequences of this insult by recording cerebral blood flow (CBF) with a laser doppler probe. 3 mm anterior to the infarct border, severe (approx 4X) transient hyperemic episodes (THEs) lasting 1-2 min were intermittently recorded. THE frequency declined over a 3-hr period. To define the mechanism of THEs, extracellular potassium ion activity (K^+o) and tissue oxygen tension (tPO_2) were recorded by microelectrodes implanted approx 0.5 mm below the cortical surface adjacent to the side of the doppler probe opposite to the infarct. Whenever THEs occurred, they were always preceded by a precipitous rise in K^+o (from approx 3 to over 50 mM. tPO_2 usually increased in association with these spreading depression-like events. Also characteristic of SD, K^+o clearance to baseline preceded baseline recovery of CBF. These data indicate that THEs are reactive to physiological events resembling spreading cortical depression which provoke increased demand for oxygen and blood flow. This conclusion is supported by findings that MK-801 (1 mg/kg; i.v.) inhibited subsequent SD-like episodes. These data suggest that the remote consequences of thrombotic infarction may be magnified by SD-like events or by the pathophysiological mechanisms underlying such events.

661.1

Acute Outcome Evaluation after Experimental Focal Cerebral Ischemia. Masaaki Uno, M. Christopher Wallace* Cerebrovascular Research Lab., Univ. of Toronto.

This study was designed to evaluate outcome methodology in 3 models of acute permanent focal cerebral ischemia. Fisher 344 rats were used in 3 different models: proximal middle cerebral artery occlusion (p-MCAO) via craniectomy, distal MCAO with ipsilateral common carotid occlusion (ipsi-CCO) and intravascular MCAO(I-MCAO) with a 4-0 suture. Sacrifice was performed at time intervals of 30 minutes to 4 hours after ischemia. Outcome methods included: tetrazolium Chloride perfusion fixation (TTCP) (n=45), Neutral Red (NR) injection (n=20) or 3H-forskolin binding (FK-b)autoradiography (n=25). There was well correlation between infarction volume demonstrated by TTCP (89.3±31.4), and histology (87.5±19.0) with p-MCAO at 4 hours. Similar results were obtained with cortical infarction (66.4±23.3, 69.9±19.3) with distal MCAO and ipsi-CCO. FK-b in caudate dropped (112±36 vs 187±22pmol/g) at 30 minutes post ischemia. Discrepancy between TTCP (87.3±34.8) and histology (42.2±63.4) was found after I-MCAO. NR demonstrated no changes in this model at 4 hours. Acute outcome evaluation affords continual monitoring of physiologic variables. Care must be exercised in choosing the appropriate model and acute outcome methodology.

661.2

GLOBAL ISCHEMIA IN RATS IMPAIRS SPATIAL WORKING MEMORY, BUT NOT SPATIAL MAPPING. J. E. Kelsey* and L. M. Genova. Dept. Psych., Bates College, Lewiston, ME 04240.

The intention of this study was to examine the nature of the memorial deficits produced by global ischemia and to examine the hypothesis that these deficits are produced by glutamate excitotoxicity. Global ischemia was produced in 10 male Wistar rats by bilateral cauterization of the vertebral arteries and occlusion of the carotid arteries for 30 min. Ten control rats did not have their vertebral arteries cauterized or their carotid arteries occluded. In Experiment 1, the ischemic rats were not impaired in their capacity to find a platform hidden below the surface of the water in a circular water tank. In contrast, in Experiment 2, the same ischemic rats were impaired in a spatial DNMTS task that required them to remember which arm of a Y-maze they had previously entered. Moreover, the choice accuracy of the ischemic rats decreased dramatically as the retention interval between forced and choice runs increased from 30 to 120 s, whereas the accuracy of the controls was unaffected. In Experiment 3, injections of the NMDA receptor antagonist, dextromethorphan (20 mg/kg i.p.), 1 hr before and 1 hr after the onset of ischemia did not affect the impairment produced by ischemia in the Y-maze. These results indicate that CA1 hippocampal damage produced by global ischemia produces a deficit in working memory, i.e., the capacity to remember where they have been (Experiment 2), but not in the capacity to form a spatial map, i.e., to localize where they are (Experiment 1). Moreover, Experiment 3 indicates that this deficit was not caused by glutamate excitotoxicity.

661.3

ASSESSMENT OF THE EFFECTS OF VARIOUS DURATIONS OF CEREBRAL ISCHEMIA FOLLOWED BY REPERFUSION ON THE PERFORMANCE OF GERBILS IN THE MORRIS WATER MAZE. R.P. Wiard, M.S. Carroll, O.G. Beek and B.R. Cooper*. Div. of Pharmacology, Burroughs Wellcome Co., RTP, NC 27709.

The effect of different durations of cerebral ischemia produced by bilateral carotid occlusion for 5, 10, or 15 minutes was determined on performance of a spatial memory test by male Mongolian gerbils. After recovering from the lesion for 21 days, the gerbils were trained in a Morris water maze to swim to a hidden platform which was located in a fixed location. Following 10 daily tests in the Morris water maze the animals were sacrificed and their brains were examined for hippocampal damage. The relationships between the duration of the ischemic insult, the extent of hippocampal damage, and the degree of impaired performance in the memory task was defined. In a second study, the ability of hypothermia (30°C) was studied using both histological and behavioral measures. Maintaining low body temperature during ischemia markedly decreased both indices of ischemic damage.

661.4

EFFECTS OF INCOMPLETE GLOBAL FOREBRAIN ISCHEMIA IN RATS ON SUBSEQUENT PERFORMANCE IN A NOVEL WATER MAZE. G.J. Kant* and F.C. Tortella. Dept. Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

The present studies were conducted to evaluate a novel water maze task for studying the behavioral effects of ischemia-induced neural injury. A four vessel occlusion procedure was used to produce temporary (15 min) forebrain ischemia in rats. The maze was a traditional type of maze (not an open Morris maze), set inside a small wading pool such that rats had to learn and remember to swim through a series of doors to reach an exit platform. Both swim time and incorrect door choices were recorded. In the first study, ischemic rats trained to swim a maze prior to surgery had poorer performance post-ischemia than non-operated controls or operated non-ischemic rats with respect to both the previously-learned maze and the learning of a new maze configuration. In a second study where rats were not trained prior to surgery, both ischemic and operated non-ischemic rats required more time to learn the water maze task than did non-operated controls. In a 3rd study that reduced ischemia to 5 min, no apparent differences between control and ischemic animals were seen.

661.5

EARLY NEURONAL DAMAGE IN THE NEWBORN PIGLET INDUCED BY HYPOXIA AND ELEVATED INTRACRANIAL PRESSURE. C.S. Easley, A.E. Kopelman, F.S. Wartman, and T.M. Louis*. Depts. of Pediatrics and Anatomy/Cell Biology, East Carolina University School of Medicine, Greenville, NC 27858-4354.

Perinatal asphyxia occurs in 5% of all births and can result in permanent brain damage or death. Neurologic damage is caused by brain hypoxia and ischemia. Despite its clinical importance, few animal models of early hypoxic ischemic brain damage are available. To study this problem,we examined the effect of hypoxia and elevated intracranial pressure (ICP) on brain histopathology. We produced hypoxia in isoflurane- anesthetized piglets by administering a 1:1 mixture of air and nitrous oxide for 2 h. To produce brain ischemia, warm saline was injected through a burr hole into the epidural space under the right parietal bone. ICP was maintained at 20 mm Hg above the mean systemic blood pressure for 20 min. We reduced the elevated ICP and survived the piglets for 8 h. We studied the hippocampal histopathology using a silver impregnation method highly selective for degenerating neurons. Damaged neurons were found in the CA3/CA4 and hilar regions of the hippocampus. Only the piglets exposed to a combination of ICP and hypoxia showed silver impregnated neurons. The hypoxic ICP piglet should prove useful as a model for determining the mechanisms of hypoxic ischemic brain damage in the newborn. Partially supported by ECU-SOM Biomedical Research Support Grant 2 S07 RR05812-12.

661.6

RECOVERY OF SENSORIMOTOR FUNCTION FOLLOWING PHOTOTHROMBOTIC OCCLUSION OF THE DISTAL MIDDLE CEREBRAL ARTERY IN RATS. C.G. Markgraf*, M. Castro, A.S. Wilkens, E.J. Green, P.M. McCabe, W. D. Dietrich, N. Schneiderman and M.D. Ginsberg. Cerebral Vascular Disease Research Center and Dept. of Psychology, University of Miami School of Medicine, Miami FL 33101

We have developed a photothrombotic model of distal middle cerebral artery occlusion (dMCAO) in rats, using the interaction of a laser light and rose bengal dye. This model is advantageous in that it produces more consistent infarcts and is more clinically relevant than the mechanical dMCAO model. The present study sought to determine the long-term behavioral consequences of the photothrombotic model. Sensorimotor and cognitive behaviors were examined following dMCAO in 10 Sprague-Dawley rats and 10 sham operated rats. Beginning on Day 2 post-ischemia (PI) and continuing to Day 30 PI, all rats were tested twice weekly using a battery of 5 tests that measure aspects of sensorimotor integration. Subsequently, from Day 35-43 PI, animals were trained in a water maze task. Histological analysis at 8 weeks revealed that the dMCAO group had a mean ±S.E.M. infarct volume of 98.36 ± 28.0 mm^3. Significant deficits were seen on the two most sensitive tests: elicited limb placing and posture reflex. The severity of the deficits were positively correlated with the extent of the ischemic damage for these two tests. Sensorimotor function recovered to control levels on the posture reflex test by PI Day 20, but limb placing performance did not show complete recovery by PI Day 30. There were no deficits in muscle strength or in learning the spatial mapping water maze task. Behavioral deficits are seen to be reflective of the various cortical areas damaged, and the different recovery patterns might be attributed to the different demands of the tasks. These results help establish the dMCAO model as a useful and relevant rodent model of focal cerebral ischemia. Supported by NS 05820.

661.7

EFFECTS OF FOCAL CEREBRAL INFARCTION OF THE FRONTAL CORTEX ON A VIBRISSAE - DEPENDENT BEHAVIORAL RESPONSE IN RATS. A.J.Pazos, E.J. Green*, W.D. Dietrich, P.M. McCabe, C.P. Earl, B.D. Watson, B.E. Hurwitz, M.D. Ginsberg and N. Schneiderman. Depts. of Psychology and Neurology, University of Miami, Coral Gables, FL 33124.

Previous metabolic studies in this laboratory have demonstrated a decrease in the uptake of 2-deoxyglucose in the cortical barrel field 5 days following thrombotic infarction of the frontal cortex. The present study sought to document the behavioral correlates of this "functional diaschisis" by assessing the effect of a photochemical infarction of the frontal cortex on an appetitively motivated response in a T-Maze using a vibrissal deflection cue.

Adult male Wistar rats were randomly assigned to one of two groups. Rats in the first group were trained to turn right in the T-Maze in response to a right vibrissal stimulation, and left in response to no vibrissal stimulation; rats in the second group were trained to perform the opposite response. Rats received 50 randomly alternating daily trials (25 right turn trials and 25 left turn trials) until the behavioral criterion was achieved (80% or better correct on both the left and right turn trials).

Following training, focal insults to the left frontal cortex were produced in half of the animals in each training group through the interaction of a photosensitive dye (rose bengal) injected into the rats' tail vein, and the direct irradiation of the cranium with a light beam (Watson et. al., 1985). The other half of the animals underwent the same procedure, except saline was injected in place of rose bengal (shams). Behavioral testing starting 24 hours after the insult revealed substantial deficits in the left and right turning of lesioned animals relative to pre-insult levels. These deficits recovered to pre-insult levels within 3-4 days following the insult. Shams exhibited no behavioral deficits. These results indicate that frontal cortex infarctions are associated with significant behavioral deficits in the performance of a task that requires cortical barrel field integrity. Supported by NS 05820.

661.8

EFFECT OF LIPOPOLYSACCHARIDE ON VON WILLEBRAND FACTOR AND FACTOR VIIIC PRODUCTION IN RATS WITH AND WITHOUT RISK FACTORS FOR STROKE. D.A. Doron*#, E. Heldman#, A.Siren^, Harvey B. Pollard# and John M.Hallenbeck&.#LCBG, NIDDK,&SB, NINDS, National Institutes of Health Bethesda, Maryland 20892 and ^Department of Neurology Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814.

We have previously reported that following a provocative dose with Lipopolysaccharide (LPS), rats with the risk factor for stroke hypertension developed more stroke events than normal rats. We have also demonstrated that hypertensive rats (SHR) produce more TNF-α, in blood and cerebrospinal fluid than normotensive rats (WKY) when challenged with LPS injected iv or icv. It is therefore possible that monokines, may act by changing levels of intravascular mediators of thrombosis and coagulation such as von Willebrand factor (vWF) and factor VIIIc. To test this hypothesis we asked whether this factors were differentially affected by LPS in SHR and WKY. We report here that hypertensive rats have higher levels of vWF than control rats after a single injection of LPS. When SHR rats were injected with a single iv dose of 1.8 mg/kg of LPS, vWF concentration increased in a time and dose dependent manner, reaching a peak after 2 hr and with a maximum response at a dose of 1.8 mg/kg. When the response of the hypertensive rats to a challenge with LPS was compared with the normotensive rats, the SHR animals showed significantly higher levels of vWF compared to WKY rats, after a 1.8 mg/kg of LPS was injected either iv or icv. No significant differences were found at lower doses. LPS injected iv at a concentration of 1.8 mg/kg had an inhibitory effect on factor VIIIc activity which resulted in an increase in the vWF/VIIIc ratio. These and other data strongly suggest that the effect of LPS in vivo is not direct on the endothelial cells but most likely mediated through the release of monokines by activated macrophages and microglias.

661.9

BEHAVIORAL, PHYSIOLOGICAL AND PHARMACOLOGICAL ASPECTS OF STIMULUS-INDUCED POST-ANOXIC MYOCLONUS IN THE RAT. P.H. Schwartz*, R.R. Matsumoto, M.J. Hussong, C. Nguyen, P. Vo, and D.D. Truong. Dept. of Neurol., Col. of Med., Univ. of Calif., Irvine, CA 92717.

We investigated the nature of acoustic-induced myoclonus (AIM) in rats that had undergone cardiopulmonary arrest and resuscitation, an insult that gives rise to spontaneous myoclonus and AIM. After 3 days to four months AIM was measured using two methods: visual scoring and quantitative measurement of whole-body AIM force. AIM was non-habituating, regardless of the length of time after the insult, within sessions; the force of the response increased then decreased between sessions run on a single day and decreased slowly over sessions run on sequential days. In addition, AIM was supranormal, compared to normal controls, shortly after the insult but declined to subnormal several weeks later, regardless of prior testing. The waveform of the force of AIM was significantly different from control animals: there was a decreased latency to peak force and an increase in the initial response. In addition, a secondary longer-latency response was present that was not seen in controls. AIM was decreased by the anti-myoclonic drugs clonazepam (0.5 - 1.0 mg/kg), 5HTP (50 - 300 mg/kg), TFMPP (0.5 - 2.0 mg/kg), and valproate (100 - 300 mg/kg). These data indicate that cardiac arrest in the rat gives rise to a model of human post-hypoxic myoclonus (PHM) that can be further studied to understand the mechanisms of PHM and its therapeutic treatment.

661.10

MICE BRAIN REGIONAL METABOLITES AND BLOOD VOLUMES (BV) AFTER 30 SEC OF GLOBAL ISCHEMIA IN A SMALL ANIMAL CENTRIFUGE (SAC). A. R. Shahed*, S. Galindo, Jr., J. A. Barber and P.M. Werchan. Operational Tech. Corp. Krug Life Sci. and Armstrong Laboratory, Brooks AFB, TX 78235.

Recently we have used a SAC to study the effect of high +Gz (head-to-foot) exposure on cerebral metabolism and to understand the mechanism of gravity induced loss of consciousness (G-LOC). G-LOC has been proposed to result from a critical reduction in cerebral blood flow (CBF) during +Gz exposure. We have shown that a 30 sec +35 Gz exposure causes G-LOC and global ischemia in mice (Soc. Neurosci. Abstr., Vol. 17(2):1262, 1991). We proposed that G-LOC may occur to conserve energy and minimize acidosis during +Gz exposure. Measurement of CBF in the dynamic environment of SAC is difficult. The brain is heterogeneous in structure, function and CBF. Therefore the objective of the present study was to measure BV and regional energy metabolism during +Gz stress. **Methods:** Fully awake mice were exposed to +35 Gz for 30 sec in the SAC (to induce global ischemia) and brain sampled at various time points during and after deceleraration by microwave fixation. The brain was dissected in 5 regions and tissue extracts were analyzed for metabolites and total iron to determine the BV. Control mice were exposed to 0.5 Gz for 30 sec and the brain sampled. **Results:** Data show that the level of lactate increased and glucose, Cr-P and ATP decreased significantly in all regions after 30 sec except the cerebellum (CB). The level of metabolites did not return to control levels 60 sec after deceleration. BV decreased more than 50% in all regions except the mid brain (25 %) and CB (no change). There was a pronounced hyperemic response in the hippocampus, brain stem and mid brain 30 sec after deceleration. **Discussion:** It is concluded that high +Gz exposure effects regional metabolism uniformly. The presence of BV, although reduced, indicates trapped blood in the brain circulation. We propose that during +Gz exposure trapped blood may prolong the onset of G-LOC after CBF ceases.

661.11

COLLAGENASE-INDUCED INTRACEREBRAL HEMORRHAGE IN SPONTANEOUSLY HYPERTENSIVE RATS. D.R. Bramlett, T.L. Sailer, J.T. Simmonds, R.R. Notvest, J.T. Haskins*, J.A. Moyer. CNS Division, Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543-8000

Intracerebral hemorrhage is an important liability in the treatment of stroke. The collagenase-induced intracerebral hemorrhage model utilizing Sprague-Dawley rats was previously determined to be an effective and reproducible animal model for assessing damage as a function of brain edema (Rosenberg et al, Stroke 21:801,'90). In the present study, spontaneously hypertensive rats (SHR) were used because of the inherent hypertensive liabilities encountered in this species and their relevance to the clinical situation. To determine a time course, 2ul of 0.5 unit bacterial collagenase (Type VII) in saline was infused into the left caudate nucleus of adult male SHR rats. Groups were evaluated for water content at 6, 12, 24, 48, 72 and 168 hours post-infusion. Significant increases in water content occurred at 24 and 48 hours in the left hemisphere. The 48 hour timepoint was chosen for a dose response determination. Rats were infused with saline or bacterial collagenase (0.1, 0.5, or 1.0 unit) and brains were evaluated for water content. The edema values measured in the left hemisphere were 78.9, 79.2, 79.7, and 80.0 percent respectively. Groups treated with 0.5 and 1.0 unit collagenase had significantly more edema than the saline control group. The collagenase-induced intracerebral hemorrhage model provides significant and reproducible results in SHR rats and may be a useful model for the pharmacological assessment of therapeutic agents for the treatment of stroke.

661.12

"NEURONAL RESCUE" DURING CHRONIC BRAIN ISCHEMIA. G.A.S Park*, T. Fortin, B. Pappas, J. Saunders, J.C. de la Torre. University of Ottawa, Carleton University, National Research Council, Ottawa, Ontario Canada K1H 8M5.

Alzheimer-like deficits (ALD) can be induced in aged rats by provoking chronic brain ischemia (CBI) using a "reversible" 3-vessel occlusion (3-VO) technique developed by us.[1] CBI for 1 to 9 weeks results in selective CA1 hippocampal cell damage, spatial memory deficits, increased glial fibrillary acidic protein (GFAP) reactive astrocytosis and membrane phospholipid synthesis changes. To test whether interrupting CBI can prevent ALD by protecting CA1 neurons, 12 month moderately aged rats underwent 3-VO for 9 weeks.

After 3-VO, special plastic carotid artery occluders were removed to allow normal brain recirculation in Group DO rats at 1, 2 or 3 weeks and occluders were left in place in Group CO. A third group of rats (NO) had no vessels occluded. At 6 and 9 weeks, rats were tested on the Morris water maze to assess spatial memory acquisition. Following this, in vivo changes of phospholipid synthesis in the hippocampus using ^{31}P- NMR spectroscopy were measured. Prior to sacrifice at 9 weeks, CBF was measured in cortex and hippocampus using hydrogen clearance. After sacrifice, morphometry of CA1 neurons and hippocampal GFAP density was done.

Results indicate that a significant number of Group DO rats had no impairment of memory acquisition in the Morris water maze when deoccluded 1 and 2 weeks after 3-VO (as compared to normal Group NO). CA1 cell damage and reactive astrocytosis was also reduced in Group DO and this was associated with nearly pre-ischemic CBF levels in cortex and hippocampus. NMR spectroscopy indicated that phospholipid synthesis did not increase in the hippocampus of most DO rats. By contrast, non-deoccluded rats (Group CO) and 3 week DO rats showed memory impairment, increased CA1 cell damage and reactive astrocytosis while CBF remained depressed from pre-ischemic levels. CO rats also showed phospholipid synthesis increase in the hippocampal area.

We conclude that "neuronal rescue" is possible when CBF is normalized after 1 or 2 weeks CBI. These findings may be relevant in Alzheimer's disease and other dementias. (Supported by the Ontario Mental Health Foundation).

[1]de la Torre JC, Fortin T. Brain Res. Bull. 26:365, 1991

662.1

ANOXIA-INDUCED IMPAIRMENT OF GABAERGIC INHIBITION IN MATURE AND DEVELOPING RAT NEOCORTEX *IN VITRO*. Heiko J. Luhmann*. Inst. of Neurophysiology, Univ. of Cologne, D-5000 Cologne 41, FRG.

Hypoxia-induced functional deficits in excitatory and inhibitory synaptic transmission were analyzed in somatosensory cortical slices of young (P5-8), juvenile (P14-18) and adult (>P28) rats. Transient hypoxia was induced by switching the aerating gas in the interface-type recording chamber from 95% O_2 / 5% CO_2 to 95% N_2 / 5% CO_2. Extracellular field potential (FP) responses to orthodromic electrical stimulation and paired-pulse responses were recorded in supragranular layers to evaluate excitatory and inhibitory transmission, respectively. In adult cortex, hypoxia caused a reversible 45% reduction in the amplitude of FP responses and a complete loss of paired-pulse inhibition. The intracellularly recorded f- and l-IPSP was reduced in amplitude to 42 and 25%, respectively. The EPSP amplitude decreased to only 64%, suggesting a selective impact on inhibitory function. Spontaneous IPSPs, recorded in a bathing solution including APV and CNQX with electrodes containing 2 M K-nitrate, were suppressed by 20% in the early phase of hypoxia. The peak conductance of the f- and l-IPSP decreased from 140 to 92 nS and from 28 to 15 nS, respectively, and both IPSPs showed a significant shift in their reversal potentials towards more positive values. Prolongation of hypoxia induced a massive anoxic depolarization by 30-78 mV associated with complete loss of spontaneous and evoked synaptic activity. Upon reoxygenation spontaneous IPSPs were transiently increased by 118%, and the conductance of the f- and l-IPSP was markedly suppressed to 39 and 1.4 nS, respectively. All parameters recovered within the observation period and even repetitive hypoxic insults did not cause irreparable functional deficits when the interval between two subsequent anoxic periods was >60 min. In young and juvenile cortex, hypoxia-induced modifications in synaptic transmission were less clearly pronounced and post-anoxic hyperexcitability could not be observed. These data indicate a selective vulnerability of the inhibitory system to transient hypoxia in adult neocortex.

662.2

PHOTOCHEMICALLY-INDUCED LESION OF THE RAT RETINA: QUANTIFICATION OF CHOLINERGIC AND GABAERGIC NEURONAL DEGENERATION. G. Lombardi*, Fulvio Moroni[x], S. Faussone[xx] and F. Moroni. Department of Pharmacology, [x]Institute of Ophthalmology, [xx] Department of Anatomy and Histology, University of Florence, 50134 Florence, Italy.

Intravenous injections of the flourescein rose bengal dye and intense focal illumination have been used for the development of reproduceable models of cerebral and retinal ischemia (Mosinger J. L. and Olney J. W., Exp. Neurol., 105, 110-113, 1989). We quantified the photochemically-induced neuronal damage to obtain a reliable model to test drugs possibly active against ischemia. Rats were injected with 80 mg/Kg of rose bengal and one eye was exposed to cold light (peak absorption 560 nm) for different periods (5, 15, 30 min). The animals were then sacrificed at different times (1, 4 h and 2, 7 days) after the lesion. At light microscopy the retinae appeared edematous, the microvessels were enlarged and numerous neurons showed pyknotic nuclei; vacuoles were also present especially in the inner nuclear and in the ganglion cell layer. The extent of retinal damage was grossly related to the time of illumination. In other experiments the retinae were used for the determination of GAD (glutamic acid decarboxylase) and ChAT (choline acetyltransferase) activity, two enzymes whose decrease was considered directly related to the extent of retinal damage. The activity of the enzymes GAD and ChAT significantly decreased in a time dependent manner: no changes were present 1 h after the lesion, but 4 h later GAD and ChAT activity decreased by 50% and 40% respectively in retinae illuminated for 5 min and by 70% and 60% in those illuminated for 15 min, indicating a diffuse degeneration of the amacrine cells. This damage remained unchanged for at least 7 days.

662.3

DELAYED GABA NEURON LOSS IN THE SUBSTANTIA NIGRA FOLLOWING ISCHEMIA DEPENDS ON THE EXTENT OF STRIATAL INJURY. B.T. Volpe*, M. Cohen and M. Saji. Dept. of Neuro. and Neurosci., Cornell Univ. Med. School, at The Burke Inst. for Med. Res., White Plains, NY 10605.

Transient forebrain ischemia by the method of four vessel occlusion reproducibly damages the striatum and the hippocampus. However, it has been shown that the substantia nigra reticulata (SNr) is not damaged acutely. In order to test whether delayed injury occurred in the SNr of animals exposed to 20 minutes of ischemia, the survival period was prolonged, the extent of striatal injury was measured, and in situ hybridization for glutamic acid decarboxylase (GAD) identified GABAergic neurons in the SNr. Five of 13 animals sacrificed 1 week to 3 months after ischemia demonstrated focal GABA neuron loss in the medio-dorsal SNr accompanied by an increase in non-neuronal elements. Each of these 5 animals had ischemic damage in the caudate nucleus (CN) and lateral globus pallidus (LGP). The remaining 8 animals had no SNr damage, and the ischemic injury was confined to the CN alone. Seven animals were sacrificed 2-3 days after ischemia and none of these had SNr damage. Three of these 7 animals had ischemic damage in the CN and LGP, 4 of 7 animals had ischemic injury in the CN alone. All animals demonstrated severe CA1 hippocampal injury. These data suggest that ischemic injury to CN and LGP are necessary to induce GABA neuron loss in the SNr. Animals must survive longer than 3 days for the SNr injury to become evident. Disinhibition via transneuronal pathways may be one of several possible pathophysiologic mechanisms which would account for delayed injury distal from the site of immediate damage.

662.4

EFFECTS OF ANOXIA ON DENTATE GRANULE CELLS. M. Patil, A. DiScenna and D. Durand* Applied Neural Control Lab., Dept. of Biomedical Engineering, Case Western Reserve University, Cleveland, OH - 44106.

Dentate granule cells are considered more resistant to the effects of anoxia, and hence have not been as extensively studied as CA1 or CA3 cells (Aitken & Schiff, 1986). This work was aimed at investigating the response of granule cells to anoxia.

Intra- and extracellular recordings were obtained *in vitro*, from dentate granule cells of adult rat hippocampus, in control conditions and after exposure to 95% N_2. Anoxic response was observed within 2 min exposure to nitrogen. Extracellular recordings in anoxia showed hyperpolarization (about 10 mV) and a reduction in the amplitude of the population spike, which recovered to 40% of the initial value. Intracellular recordings showed an initial, transient hyperpolarization (3 - 4 mV in 4 of 7 cells examined) followed by a prominent depolarization (50 - 70 mV) in 21 of 23 cells. The onset of depolarization varied between 2-15 min after application of nitrogen, and rates of depolarization were also variable. Dendritic strength-duration data indicated an increase in the orthodromic firing threshold (in all 10 cells tested). This effect was reversible (wherever recovery was possible, n = 6). A simultaneous decrease in the EPSP amplitude was measured (in 10 cells: 100% in 4 cells, > 40% in 6 cells). Somatic excitability, as indicated by the response to 100 msec depolarizing current pulses, increased at the onset of depolarization in 6 of 8 cells, but was abolished during further depolarization (in all 4 cells tested). Somatic impedance decreased between 10 - 30% during anoxia. Post-anoxic hyperpolarization was also observed in 4 of 6 cells.

The roles of NMDA receptors and ATP metabolism are being investigated to understand the mechanisms underlying anoxic effects such as the large depolarization observed in the granule cells.

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662.5

ALTERED DENDRITIC MORPHOLOGY IN DiI-LABELED CORTICAL NEURONS DEPRIVED OF OXYGEN AND GLUCOSE *IN VITRO*. M.C. Bateman*, M.P. Goldberg Dept. of Neurology, Washington Univ. School of Medicine, St. Louis MO 63110.

Postsynaptic dendritic swelling is a characteristic feature of acute excitotoxic injury, but such change is difficult to visualize by conventional microscopy in living tissue. Stewart et al. (Ann Neurol 1991; 30: 758) observed excitatory amino acid-induced dendritic varicosities in fixed chick spinal cord stained with the lipophilic carbocyanine tracer, dil. We used a similar method to examine acute morphologic changes in unfixed neurons exposed to hypoxic conditions.

Primary neocortical cultures at 13-15 days in vitro were labeled by incubation in buffered saline containing either crystalline dil or a dil suspension (.5-5 μg/ml in 0.2% EtOH) (Honig and Hume, J Cell Biol 1986; 103: 172). This procedure resulted in intense fluorescent label completely filling a small proportion of the cultured cells within minutes (suspension) or hours (crystals), allowing visualization of dendritic morphology with little staining in the background glial monolayer. Immediately following 45-60 min deprivation of oxygen and glucose, phase contrast microscopy showed swelling in neuronal somata. By dil fluorescence, neurites demonstrated a pattern of focal swelling, or beading, ranging from fine varicosities between dendritic spines to dilatations several times the diameter of the neurite. A similar pattern of neurite swelling was observed following 5 min exposure to 500 μM glutamate or NMDA. Some varicosities were seen following washing alone. Neurite swelling could be attenuated by inclusion of 10 μM MK-801 in the incubation medium. Although the relationship of acute dendrite swelling to long-term neuronal injury remains to be established, such rapid alterations in dendritic structure might contribute to early failure of synaptic transmission in cerebral hypoxia-ischemia.

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662.6

SELECTIVE VULNERABILITY OF MOSSY FIBER TARGETS IN THE RAT HIPPOCAMPUS AFTER TRANSIENT FOREBRAIN ISCHEMIA. M. Hsu* and G. Buzsáki. Ctr. for Molec. & Behav. Neuroscience, Rutgers University, Newark, NJ 07102.

The vulnerability of different cell types in the rat hippocampus to forebrain ischemia induced by the 4-vessel occlusion method was assessed by immunocytochemistry using an antibody against the 72kDa heat shock protein (HSP72). Our results showed a robust induction of HSP72-like immunoreactivity (HSP72-LI) in the vulnerable CA1 pyramidal cells as well as hilar neurons; the ischemia-resistant cell populations--CA3 pyramids and dentate granule cells--remained unstained. Although the only hilar neurons implicated to be vulnerable to ischemia are the mossy cells and somatostatin neurons, many different hilar cell types were also stained in our preparation. The most striking finding, however, was the strong HSP72-LI in a subpopulation of interneurons in area CA3 and their counterparts in the hilus. In CA3, their cell bodies and long horizontal spiny dendrites, oriented parallel to the mossy fibers and perpendicular to the CA3 apical dendrites, were restricted to the stratum lucidum of CA3; in the hilus, the horizontal spiny cells were located in the subgranular zone. Both the horizontal spiny cells in CA3 and the hilus were present also at more posterior and ventral locations where no other cell types expressed HSP72-LI. We propose that a determining factor in the susceptibility of these neurons to ischemia is the density of mossy fiber innervation they receive. Furthermore, their early impairment after ischemia may be related to delayed neuronal death in the CA1 pyramidal cells.

662.7

QUANTITATIVE EM IMMUNOCYTOCHEMISTRY REVEALS POSTISCHEMIC CHANGES IN THE DISTRIBUTION OF NEUROACTIVE AMINO ACIDS IN THE RAT HIPPOCAMPUS. R. Torp[1], F.F. Johansen[2], N.H. Diemer[2], B. Arvin[3], B.S. Meldrum[3] and O.P. Ottersen*[1] 1;Dept. of Anatomy, University of Oslo, N-0317 Oslo, Norway. 2;Inst. of Neuropathology, University of Copenhagen, DK-2100 Denmark. 3;Inst. of Psychiatry, London, SE5 8AF,UK.

Forebrain ischemia (20min) was induced in rats by the 4VO method. Ultrathin sections through CA1 and CA3 were obtained after different survival times (0, 1, 40 and 150h of reflow) and subjected to quantitative immunogold cytochemistry using antisera raised against glutamate, glutamine, GABA and homocysteic acid. The main observations were: 1; The concentration of glutamate in pyramidal cell bodies and dendrites are substantially reduced during ischemia (probably reflecting a loss to the extracellular space) but in surviving cells it returns to normal after 40h of reflow. Excitatory-type terminals showed only a minor decrease in the level of glutamate. 2; The glutamate/glutamine ratio in astrocytes was strongly increased at 0h (reflecting a metabolic block) but returned to pre-ischemic values after 1h. 3; The level of GABA in interneurons was elevated at 0 and 1h. 4; A homocysteic acid-like substance appeared in astrocyte processes in CA3 at 40h and in CA1 at 150h. The possibility that the latter change is related to the development of delayed neuronal death is currently under investigation.

662.8

EFFECTS OF O_2 AND GLUCOSE DEPRIVATION ON HUMAN AND RAT NEOCORTICAL NEURONS IN-VITRO. C. Jiang* & G. G. Haddad, Section of Respiratory Medicine, Dept. Pediatrics, Yale University School of Medicine, New Haven, CT 06510

The neocortex is extemely sensitive to O_2 and glucose deprivation. Such stress causes rapid loss of cortico-encephalographic activity and unconsciousness. To study the underlying mechanisms for the loss of neuronal electrical activity during O_2 and glucose deprivation, intracellular recordings were performed on neocortical neurons in humans and rats using in-vitro brain slices. Intracellular labelling and electrophysiological characterization showed that most neurons (layers 4 and 5) were pyramidal cells with a regular-spiking or a burst firing pattern. Anoxia induced a depolarization that was apparent mostly after 7 min in rats and 10 min in humans following a transient hyperpolarization (2-5 mV, 1-3 min). Rheobase markedly increased and spontaneous EPSPs were suppressed during anoxia, while membrane resistance (Rm) was only modestly (22%) reduced. Total deprivation of glucose depolarized neocortical neurons by 28±14 mV after 1-2 hours, while combined deprivation of both glucose and O_2 resulted in a major depolarization (mean≈50 mV) within 5-10 min in both human and rat neurons. Extracellular K^+ activity (K^+_o) increased by 1-2 mM during anoxia (20 min) or glucose deprivation (30 min), but deprivation of both O_2 and glucose elevated K^+_o by about 25 mM. We conclude that 1) rat neocortical neurons seem to be inherently more resistant to anoxia than brainstem neurons; 2) membrane excitability of neocortical neurons is markedly reduced during anoxia and this is not due only to a decrease in Rm; and 3) anaerobic glucose metabolism in the human neocortex is important for neuronal survival during anoxia.

662.9

INVOLVEMENT OF THE NMDA RECEPTOR IN ANOXIA-AGLYCEMIA INDUCED DAMAGE IN THE HIPPOCAMPUS. S. Papas*, V. Crépel and Y. Ben-Ari, INSERM Unit 29, 123 Bd. de Port-Royal, Paris, 75014, France.

Although there is increasing evidence that the N-methyl-D-aspartate (NMDA) receptor is involved in the induction of anoxic/ischemic injury, its exact role is unclear. For example, protective effects of NMDA antagonists have been found to vary with the ischemic model used. We have used two protocols to examine the involvement of the NMDA receptor in the inhibition of synaptic transmission induced by anoxia and aglycemia (AA) in CA1 of the hippocampal slice.

In initial studies, the protective effects of a non-competitive NMDA antagonist, MK-801, against AA induced damage was examined. Inhibition and recupertion of synaptic transmission was determined by the disappearance and percent recovery of the CA1 field EPSP in the 30 min period after AA insult. Rat hippocampal slices incubated in MK-801 (0.1 μM, 1 μM, or 10 μM) for 15 min prior to various periods (3 min to 4 min 30 s) of AA (95%N_2/5%CO_2, glucose replaced with sucrose) showed greater percent recovery of EPSP amplitude than did control slices incubated in Krebs. Slices treated with 0.1 μM MK-801 had greater EPSP recuperation following 3 min 30 s AA (29 ± 13% vs 10 ± 10% in controls, $p<0.05$). Larger concentrations of MK-801 showed protective effects in more than one time period of AA. In a second investigation, NMDA currents were enhanced during AA by incubating slices in Mg^{++} free Krebs. Slices subjected to AA in the absence of Mg^{++} showed a smaller percent recovery of EPSP amplitude following 2 min (34 ± 15% vs 81 ± 11% in controls, $p<0.05$) and 2 min 30 s (25.5 ± 14% vs 77 ± 10% in controls, $p<0.005$) of AA. Thus, the NMDA receptor appears to play a role in the inhibition of synaptic transmission induced by anoxia and aglycemia in the hippocampal slice.

662.10

HYPOXIA OR GLUCOSE DEPRIVATION ACTIVATES TWO SEPARATE KAINATE RECEPTOR SUBTYPES IN HIPPOCAMPAL SLICES. B.M. Rigor*, C.A. West, and A. Schurr. Dept. of Anesthesiology, Univ. of Louisville Sch. of Med., Louisville KY, 40292.

The exposure of rat hippocampal slices to conditions that are known to reduce energy supplies (hypoxia or glucose deprivation, GD), make them hypersensitive to the excitotoxins glutamate and N-methyl-D-aspartate (NMDA). Thus, slices deprived of either oxygen or glucose show an increase in the degree of neuronal damage (as measured electrophysiologically) if they are concomitantly exposed to low concentrations of NMDA. This increased neuronal damage could be abolished by supplying slices either with antagonists such as 2-amino-5-phosphonovalerate (APV) or 7-chlorokynurenate, or by perfusion with high [Mg^{2+}] or low [Ca^{2+}].

Here we studied the effects of hypoxia or GD combined with exposure to kainate (KA) on the degree of hippocampal neuronal damage. KA was 3-5 times more potent than NMDA in enhancing either hypoxic- or GD-induced neuronal damage in hippocampal slices. The KA-enhanced hypoxic neuronal damage could be attenuated by APV, kynurenate, elevated [Mg^{2+}] or depletion of Ca^2. The KA-enhanced GD neuronal damage was unaffected by APV or high [Mg^{2+}] but completely abolished by Ca^{2+} depletion.

These results indicate that KA receptor channels gate Ca^{2+} in addition to Na^+. Moreover, the results strongly suggest the existence of two distinct KA-receptor subtypes which are differentially activated by hypoxia or GD.

662.11

QUINOLINATE: A MODULATOR OF GLUTAMATE RECEPTORS' AGONISTIC ACTIVATION IN HYPOXIC RAT HIPPOCAMPAL SLICES. A. Schurr*, C.A. West, and B.M. Rigor. Dept. of Anesthesiology, Univ. of Louisville School of Medicine, Louisville KY, 40292.

Excitatory amino acids (EAAs) in the central nervous system are involved both in neurotransmission and excitotoxicity. Quinolinic acid (QUIN) is an endogenous tryptophan metabolite and an excitotoxin, and has been shown to cause Huntington's disease-like striatal lesions. The hypoxic rat hippocampal slice preparation and its electrophysiology were employed to study QUIN's modulatory role in the activation of the N-methyl-D-aspartate (NMDA) and kainate (KA) subtypes of the glutamate (GLU)-receptor. The degree of neuronal damage in this preparation was used to measure the excitotoxic potency of several GLU ligands.

When given at sub-toxic doses, QUIN potentiated the excitotoxicity of GLU, NMDA, and KA in hypoxic hippocampal slices. While the NMDA competitive antagonist DL-2-amino-5-phosphonovalerate (APV) blocked the QUIN-potentiated NMDA toxicity, 7-chlorokynurenate (7-Cl-KYN), a competitive antagonist of the NMDA-receptor glycine modulatory site, did not. The non-toxic analogue of QUIN, 6-methyl-QUIN, potentiated NMDA toxicity as effectively as QUIN itself. We concluded that QUIN has a specific modulatory binding site on both the NMDA and the KA receptor complexes, different from the glycine modulatory site on the NMDA receptor. We postulate that QUIN can increase both the excitatory and the excitotoxic efficacy of EAAs by potentiating the agonistic activation of GLU receptors.

662.12

GLUTAMATE ANTAGONISTS REDUCE DELAYED NEURONAL DAMAGE AFTER TRANSIENT ISCHEMIA IN THE ORGANOTYPIC CULTURE OF RAT HIPPOCAMPUS. C. Shin*, J. Wilson and Y. Tamaki, Epilepsy Research Laboratory, Duke and VA Med. Ctrs., Durham, N.C. 27705.

Organotypic cultures of rat hippocampus could prove useful as an *in vitro* model of ischemia since the cytoarchitecture and intrinsic synaptic connectivity are preserved. We used hypoxia-hypoglycemia to simulate ischemia and tested the effectiveness of the glutamate antagonists in reducing the delayed ischemic damage in the pyramidal neurons of the organotypic culture.

Cultures were exposed to 2-deoxyglucose substituted HBSS equilibrated with 95% N_2-5% CO_2 atmosphere. D-APV and CNQX were applied, singly and combined, either during and after or only after the ischemic period. Neuronal injury was assessed 24 hours later by assigning a severity index (0=none, 4=severe) to propidium iodide fluorescence, which predicts later histology.

With 30 minutes of ischemia, CA1 selective neuronal injury was obtained (CA1, 2.2 v. CA3, 0.7; n=6, $p < 0.03$). Both D-APV (100 μM, n=8) and CNQX (100 μM, n=8), applied during and after the ischemic period reduced the neuronal injury index in CA1 to 0.1 ($p < 0.005$) and to 0.0 in CA3 ($p < 0.05$, Mann-Whitney U). With 45 minutes of ischemia, more extensive injury was produced, but again with more severe involvement of CA1. D-APV, but not CNQX, showed protective effect. Concomitant application of D-APV and CNQX produced an effect similar to D-APV alone. Drugs applied only after the 45 minute ischemia afforded no significant protection.

These results show that CA1 selective neuronal injury is seen after transient ischemia in the organotypic cultures and that both NMDA and AMPA receptor mediated mechanisms contribute to the process. The organotypic culture could provide a very useful model to dissect out the mechanisms underlying the delayed neuronal damage induced by transient ischemia.

662.13

ASSESSMENT OF ISCHEMIC DAMAGE TO ORGANOTYPIC CULTURES OF RAT HIPPOCAMPUS USING PROPIDIUM IODIDE FLOURESCENCE DW Newell, S Hsu, ATMalouf*, VM Papermaster, IE Franck Department of Neurological Surgery, University of Washington School of Medicine, Seattle, WA 98195

The objective of this study was to determine if the time course of selective damage to the hippocampus by ischemia could be assessed using propidium iodide staining as an index of membrane integrity. Organotypic cultures were prepared from rat pups (4-6 days old) and grown on glass cover slips in roller tubes for 2 weeks. Cultures were then placed in balanced salt solution with no glucose in an anerobic chamber for 30 minutes (artificial ischemia). Control cultures were placed in balanced salt solution containing glucose for an equivalent time period. Both groups were maintained in the presence of propidium iodide dye and periodically examined using an inverted flourescent microscope and images were digitized and analyzed using Optimus software. Immediately following artificial ischemia all exposed cultures showed marked swelling of the entire pyramid cell layer and dentate compared to controls. There was an increase in the delayed appearance of flourescence in the pyramidal cells in CA1, which was significantly different from controls at 18 hours following ischemia. Selective vulnerability to ischemia in the hippocampal subfields was also observed in the following order CA1>CA3>Dentate. In conclusion the organotypic cell culture model offers new insights into the time course and selectivity of ischemic cell damage in the hippocampus.

662.14

The development of agonist-specific regional vulnerability in organotypic hippocampal culture accounts for sensitivity to in vitro ischemia and hypoglycemia. R. C. Tasker* and J. J. Vornov, Dept Anesthesiology, and Neurology, The Johns Hopkins School of Medicine, Baltimore, MD 21205

We have described models of hypoglycemia and ischemia in organotypic hippocampal culture which preserve the characteristic regional vulnerability observed in animal models. Hypoglycemia is achieved by incubation with 2-deoxyglucose to block glycolysis. Potassium cyanide is added to also block oxidative phosphorylation to model ischemia. The distribution of injury is distinct in each case. NMDA receptor antagonists are protective in both, even if added 30 minutes after the end of the insult. We now report distinct patterns of agonist-specific injury during *in vitro* development of cultures.

Hippocampal slice cultures were prepared from 7 day-old rat pups and examined after 2, 5, or 12 days in vitro. Propidium iodide was used to directly observe membrane injury in the living cultures. At 24 hours, cultures were examined histologically. NMDA (3, 10, 100 μM, 30 min exposure) in 2 and 5-day-old cultures caused injury either localized to CA1 alone or to CA1 and CA3. Severity of histologic damage was greater with increasing age of the culture. Glutamate (1 mM, 30 min) caused injury in CA1 and CA3 in 2 day, but not 5 day-old cultures. Hypoglycemic injury had the same pattern of development as NMDA toxicity, while vulnerability to ischemia paralleled vulnerability to glutamate. These developmental patterns suggest enhanced postnatal sensitivity to glutamate neurotoxicity, distinct from direct NMDA receptor-mediated injury.

ISCHEMIA: GLIA

663.1

GLIAL AND NEURONAL CHANGES IN THE HIPPOCAMPUS FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA IN THE RAT. W.R.Woodward*, C.J.Pepin, D.D.Copeland, N.Lessov, C.K.Meshul, T.E.Williams, F.P.Eckenstein. Depts. of Neurology, Pharmacology, and Cell Biology and Anatomy, Oregon Health Sciences Univ. and VA Medical Ctr., Portland, OR 97201

The Siesjõ 2-vessel occlusion model of global forebrain ischemia in rat was used to investigate the time course of changes in specific populations of hippocampal glial cells and neurons following ischemic injury. Astrocytes and microglial cells were immunostained with an anti-basic fibroblast growth factor (bFGF) monoclonal antibody and the OX42 monoclonal antibody respectively, while two subpopulations of pyramidal neurons were stained with monoclonal antibodies to the calcium binding proteins, calbindin and parvalbumin. In the 7 days following ischemia, there is a significant increase in the number of bFGF-staining astrocytes in CA1 as well as in the intensity of bFGF-staining in astrocytic nuclei. Concurrently, there is a substantial rise in glial fibrillary acidic protein (GFAP)-immunoreactivity. Changes in bFGF following ischemia are consistent with a role in promoting astrocyte proliferation and in regulating gene control of astrocyte function. In addition the microglial marker, OX42, shows a large elevation that is restricted to CA1 and the hilus of the dentate, areas of hippocampus which are most vulnerable to ischemic injury. The increase in OX42 staining reveals the importance of microglia in resolving the damage to hippocampus following injury. In contrast to the glial proliferation, there is a greater than 90% loss of viable neurons in CA1. In the 7 days following ischemia, the subpopulation of CA1 neurons immunoreactive for calbindin were completely eliminated, whereas the parvalbumin-staining neurons were spared, consistent with a possible protective role for parvalbumin but not calbindin in these neurons. Supported by NIH Grant NS17493, the Oregon Heart Association and the Veterans Administration.

663.2

ASTROGLIAL AND MICROGLIAL REACTIONS IN EARLY STAGES OF ISCHEMIA. C.A. Pardo[1]*, L. Monsein[2], V. Mathews[2], P. Barker[2] and R.N Bryan[2]. Neuropathology Lab[1]. and Neuroradiology Div.[2] The Johns Hopkins University School of Medicine, Balto., MD 21205.

The development of astroglial and microglial reactions and macrophage migration were studied in models of regional ischemia in baboon models, including: complete ischemia (CI) by irreversible occlusion of the middle cerebral artery (MCA); and incomplete ischemia (IIR) by reversible occlusion of MCA followed by reperfusion. GFAP and vimentin as well as macrophage/microglial markers (e.g., HLA-DR, CD68, LN4, and ED2) were used to study cell response following ischemia. In both models, early astroglial and microglial activation and macrophage migration were observed in the perinecrotic region as early as six hours postischemia but were most prominent in IIR. Astrocytes showed hyperplastic reactions and extension of glial processes and, in cortical ischemic regions, astrocytes issued long processes resembling radial fibers. These findings suggest that glial cells have an important role during early stages of ischemia.

663.3

CLUSTERING OF LIVE AND DEAD ASTROCYTES IN CULTURE: MODULATORY ROLE OF GAP JUNCTIONS. Robert S. Goldman*. Department of Neurology, Medical College of Wisconsin, Milwaukee, WI 53226.

Iodoacetic acid (IAA), which interferes with anaerobic metabolism, killed rat hippocampal astrocytes in culture in a dose dependent manner. With intermediate doses of IAA cell death was consistantly observed to occur in a distinctly non random spatial distribution; the dead cells formed clusters. Using a two color fluorescence assay, and video microscopy, the spatial distribution of live and dead cells was studied over an area of 5x5 mm. Using a modification of nearest neighbor analysis the extent of clustering was quantified. The clustering effect was robust; the probability of cell death was higher than would be expected based on a random spatial distribution up to hundreds of cells away from a dead cell; and was higher than expected up to hundreds of microns from a dead cell. Although the live and dead cells could represent two subpopulations, the astrocytes stained uniformly GFAP positive and A2B5 negative, suggesting that they represent a single population. Exposure to IAA in the presence of the gap junction blocker octanol (500uM) significantly reduced, though did not entirely eliminate, the clustering. This evidence suggests that the non random spatial distribution of cell death is due, in part, to diffusion of a toxic molecule(s) through gap junctions. Previous studies have indicated that IAA-induced astrocyte death is related to elevation of intracellular calcium, raising the possibility that the toxic molecule may be either calcium itself or inositol trisphosphate.

663.4

APPEARANCES OF PHAGOCYTIC CELLS AND REACTIVE ASTROCYTES IN THE CA1 AREA OF GERBIL HIPPOCAMUS FOLLOWING ISCHEMIA. Y. Uchiyama1, T. Nitatori1, Y. Karasawa2, H. Araki2*, and E. Kominami3. Dept. of Anat.1, Sch. of Med., Iwate Med. Univ., Iwate, 020 Japan, Lab. of Pharmacol.2, Taisho Pharmaceutical Co., Saitama, Japan, and Dept. of Biochem.3, Juntendo Univ. Sch. of Med., Tokyo, Japan

We examined by immunohistochemistry behaviors of glial cells in the CA1 area of gerbil hippocampus after brief ischemia. Three days after the ischemic operation immunodeposits for cathepsin H (CH), a lysosomal cysteine proteinase and cystatin ß (Cß), its endogenous inhibitor appeared in small cells located in the CA1 molecular layer. At 7 days strongly immunopositive cells for CH and Cß occupied the CA1 pyramidal layer. By electron microscopy, numerous microglia-like cells having debris of pyramidal neurons were seen in the CA1 pyramidal layer at 7 days, suggesting that CH- and Cß-immunopositive cells are microglia-like cells. To determine the origin of the microglia-like cells in the pyramidal layer, fluorescent latex particles were injected from the femoral vein of gerbil 7 days after the ischemic operation. Two days after the injection, cells showing fluorescence of latex particles appeared in the CA1 pyramidal layer and the same cells were confirmed to demonstrate immunoreactivity for CH or Cß. The results suggests that CH and Cß are excellent markers for microglia-like cells in the central nervous tissue, which originate from peripheral blood monocytes. At 14 days both GFAP and vimentin immunopositive astrocytes and their processes occupied the CA1 pyramidal layer.

663.5

INVOLVEMENT OF SODIUM CHANNELS IN ANOXIC INJURY IN MAMMALIAN CENTRAL NERVOUS SYSTEM WHITE MATTER: ULTRASTRUCTURAL CORRELATES J.A. Black*, P.K. Stys, B.R. Ransom, and S.G. Waxman. Dept of Neurology, Yale Univ Sch of Med, New Haven, CT and Neuroscience Research Center, VA Med Ctr, West Haven, CT 06516

The *in vitro* rat optic nerve has provided a reliable, quantitative model of white matter anoxic injury. Previous studies have correlated optic nerve dysfunction following anoxia with ultrastructural changes in the nerves,which include mitochondrial swelling, cytoskeletal dissolution, and the appearance of empty spaces adjacent to axons within the myelin. Electrophysiological evidence suggests that anoxia results in a cascade of events leading to reverse operation of the Na^+-Ca^{2+} exchanger, and subsequent entry of calcium into the axons (Stys et al. 1992).

Optic nerves were maintained in Ringers containing 1 μM tetrodotoxin (TTX) during anoxia (60 min). At the end of the anoxic period, the nerves maintained in TTX-Ringers were ultrastructurally similar to control optic nerves maintained in oxygenated, normal Ringers for an equivalent length of time. These experimental nerves exhibited slight swelling of some mitochondria, but neurofilaments, microtubules, and paranodal loops maintained their integrity. Empty spaces between the myelin sheath and axon were not apparent in the nerves maintained during anoxia in TTX-Ringers. At rare nodes, accumulations of membranous profiles were observed in the nodal axoplasm. Reoxygenation in normal Ringers did not substantially change the ultrastructure of the experimental nerves. These observations provide morphological evidence for the involvement of sodium channels in anoxic injury in white matter and, together with our previous work, support the idea that anoxic injury involves sodium influx that leads to reverse operation of the Na^+/Ca^{2+} exchanger. [Supported in part by VA and NIH]

663.6

EFFECTS OF EXTRACELLULAR CALCIUM ON ULTRASTRUCTURE IN ANOXIC INJURY IN MAMMALIAN CENTRAL WHITE MATTER. S.G. Waxman*, J.A. Black, B.R. Ransom and P.K. Stys, Dept. of Neurology, Yale Univ. Sch. of Med., New Haven, CT 06510 and Neuroscience Research Center, VA Med. Ctr., West Haven, CT 06516

We have developed a reproducible, quantitative model of anoxic white matter injury using the rat optic nerve *in vitro*. Optic nerve function rapidly fails when the nerve is exposed to anoxia. Ultrastructural changes in anoxic optic nerves include large, distended myelin sheaths associated with vacuoles adjacent to the axon, mitochondrial swelling, loss of microtubules, and detachment of terminal paranodal oligodendroglial loops. Reoxygenation of the nerve is associated with partial recovery of function and restoration of nodal integrity and disappearance of internodal vacuoles.

Loss of optic nerve function, as measured by the area under the compound action potential (CAP), is dependent upon the molar concentration of extracellular calcium ($[Ca^{2+}]_o$). Optic nerves maintained in Ca^{2+}-free Ringers throughout the anoxia period (60 min) regain 100% of the compound action potential following 60 min in normal, oxygenated Ringers. Ultrastructurally, optic nerves in Ca^{2+}-free Ringers at the end of the anoxia period show few pathological changes. While mitochondria were generally swollen and disrupted, microtubules were intact and few internodal vacuoles were present. Paranodal loops generally maintained their integrity, though paranodal detachment was occasionally observed in some larger axons. Following 60 minutes of reoxygenation in normal Ringers, the nerves subjected to anoxia in Ca^{2+}-free Ringers exhibited similar ultrastructure to control nerves. These observations support the idea that, during anoxia, myelinated fibers are damaged by an influx of calcium. [Supported in part by the VA and NIH]

ISCHEMIA: TEMPERATURE EFFECT

664.1

BRAIN VERSUS BODY TEMPERATURES IN BLOODLESS PROFOUNDLY HYPOTHERMIC DOGS. M.L. Leavitt*, J. Bailes, E. Teeple, A. Elrifai, M. Taylor, T. Shih, J.C. Maroon, K. Ciongoli, C. Devenyi and B. Bazmi. Allegheny General Hospital, Pittsburgh, PA 15212 and Cryomedical Sciences, Inc., Rockville, MD 20850.

The purpose of this study was to compare temps. measured from the brain (B), esophagus (E) and subcutaneous tissues of the back (SQ) in profoundly hypothermic dogs (N=14) to better understand the appropriate level of hypothermia needed for cerebral protection. Flether-anesthetized dogs were cooled externally to E=24.3°C (B=18.1°, SQ=11.3°) at which time bleeding was started. Following total exsanguination, internal cooling was achieved by continuous circulation of a cold oxygenated solution (Cryomedical Sciences, Inc.) for 3 hr. at E<10°C. Rewarming and return of the blood were next performed. Prior to blood substitution, E=30°, B=22.8° and SQ=12.6°C. Temperatures recorded from the 3 sites were not significantly different from each other during asanguineous perfusion. Throughout the first 46 min. of rewarming, E was greater than B and SQ; B was consistently closer to SQ than to E. The maximal disparities between E to B and E to SQ were 9.8°C (p<.001) and 8.0°C (p<.001) respectively at 18 min. of rewarming at which time the SQ to B disparity was only 1.7°C. There were no significant differences between the 3 sites upon rewarming to 33.2°. Thus, significant differences were noted between E vs B and SQ during rewarming when there was similarity between B and SQ. These data suggest that SQ temp. may be a better index of B temp than core (E) temp. during rewarming and the risk caused by direct brain monitoring could be avoided by use of a SQ probe.

664.2

SERUM LEVELS OF CREATINE KINASE (CK) IN HYPOTHERMIA AND COMPLETE BLOOD SUBSTITUTION. A.Elrifai, J.Bailes, E.Teeple, M.Leavitt, S.Shih, M.Taylor and J.Maroon*. Allegheny-Singer Research Inst., Allegheny General Hospital & Cryomedical Sciences, Inc., Pittsburgh, PA 15212.

Recent studies have shown that creatine kinase (CK) & its brain fraction (CK-BB) activity in CSF is a good prognosticator of cerebral damage after an insult & also post profound hypothermia. To evaluate a less invasive course in a dog model to study the effect of profound hypothermia with blood substitution (J Neurosurg 74:781-788, 1991), serum CK & CK-BB were determined at 1, 2 & 3 days post-op. Neurologic Deficit Scores (NDS: 0= normal, 1= minimal abnormality, 2= weakness, 3= paralysis, 4= coma, 5= Death) were determined for 19 animals, of whom 13 survived long term & 6 died at 1-3 days post-op. Animals were grouped based on the NDS score. Maximum serum CK & CK-BB occurred at the first day post-op.

n= ( )	NDS	Day 1	Day 2	Day 3
CK-BB	=0	102± 41(6)	40± 14(5)	27± 5(6)
IU/L	≥1	136± 30(7)	121± 72(3)	57± 31(5)
P		N.S.	N.S.	N.S.
CK	=0	2.0± 1.8(6)	1.6± 1.5(5)	0.5± 0.5(6)
IU/L(10^4)	≥1	13.1± 7.1(13)	11.9± 6.4(8)	3.2± 1.2(7)
P		N.S.	0.03	0.01

Serum CK & CK-BB value may prove to provide a predictive scheme for neurological outcome however its reliability needs to be tested in a larger series.

664.3

HYPOTHERMIC PROTECTION AGAINST ISCHEMIC INSULT: ASSESSMENT USING HISTOLOGICAL, BEHAVIORAL AND ELECTROPHYSIOLOGICAL MEASURES. S.M. Nurse*, R.S. Neuman and D. Corbett. Division of Basic Medical Sciences, Faculty of Medicine, Memorial University of Newfoundland, St. John's, NF, Canada, A1B 3V6.

A 5 min period of global ischemia produces extensive cell loss in certain brain regions, such as the CA1 subfield of the hippocampus. We investigated the ability of mild brain hypothermia [induced at the initiation of a 5 min carotid artery occlusion and terminated upon reperfusion] to prevent histological, behavioral and electrophysiological changes associated with ischemia.

This short interval of intraischemic hypothermia provided dramatic protection of CA1 neurons. Behavioral deficits, such as increased exploratory activity in an open field maze, were attenuated by the hypothermic treatment. Finally, periods of forebrain ischemia normally result in an extensive reduction of the extracellular field potential recorded in stratum radiatum. Hypothermic intervention prevented this decline in the fEPSP. Preservation of hippocampal function, as assessed by field potentials recorded in CA1, directly parallels the degree of behavioral and histological protection.

Supported by the Medical Research Council of Canada.

664.4

TEMPERATURE CHANGES ASSOCIATED WITH FOREBRAIN ISCHEMIA IN THE GERBIL. F. Colbourne *, S. M. Nurse and D. Corbett, Fac. Med., Memorial University of Newfoundland, St. John's, NF, Canada A1B 3V6.

Mild hypo- or hyperthermia, especially during occlusion, can markedly affect ischemic outcome. We have systematically examined brain, skull and rectal temperatures in gerbils (n=10) subjected to 5 min of bilateral carotid artery occlusion. During surgery skull and body temperatures were maintained with heating blankets. Brain, skull and rectal temperatures were monitored during ischemia and brain temperature was recorded for 3 h into the postischemic period. Intraischemic brain temperature fell by ~ 1.5°C even though skull and rectal temperatures remained at 37°C. Since skull and brain temperature can become dissociated after brief periods of anesthesia, we examined the relationship between skull and brain temperature during extended anesthesia. An 85 min period of postischemic halothane anesthesia did not reduce CA1 cell loss (Kuroiwa et al. 1990) if brain and skull temperatures were maintained at 37°C. In cases when brain temperature fell below skull temperature CA1 cell loss was attenuated. Skull temperature does not reliably reflect brain temperature during periods of prolonged anesthesia.

664.5

THERMAL SENSITIVITY OF HYPOXIC RESPONSES IN NEOCORTICAL BRAIN SLICES. K. Hiramatsu, N.F. Kassell *, K.S. Lee. Dept. of Neurosurgery, University of Virginia, Charlottesville, VA22908.

Electrophysiological responses to transient hypoxia were studied in neocortical brain slices from adult gerbils. Evoked responses and DC potentials were recorded in layer III of the frontoparietal cortex under normoxic and hypoxic conditions. The excitatory synaptic component of the evoked wave form was identified by its sensitivity to calcium and DNQX (6,7-dichloroquinoxaline-2,3-dione). Under normoxic conditions, excitatory synaptic responses were reduced in a temperature-dependent manner. At lower temperatures, the delays to synaptic loss and hypoxic depolarization were prolonged significantly. Synaptic recovery from a fixed period of hypoxia was enhanced greatly when transient hypoxia was administered at reduced temperature. These findings demonstrate that glutamatergic synaptic transmission is reduced under hypothermic conditions, but that these responses are sustained for a longer period of time during hypoxia. The data also demonstrate directly that hypothermia protects against hypoxic damage to excitatory synaptic mechanisms in the cortex.

664.6

DELAYED HYPOTHERMIA DOES NOT CONTRIBUTE TO NEUROPROTECTION IN GERBILS. C.A. Hoffman and C.A. Boast* Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543.

It has repeatedly been shown that hypothermia following ischemia can provide neuroprotection; therefore, the interpretation of the neuroprotective effect of many drugs is confounded by drug-induced hypothermia. One technique to eliminate this problem has been to maintain animals at a normothermic temperature for 8 hours after the ischemic episode. The present experiments were conducted to determine whether a shorter duration of temperature maintenance was sufficient to eliminate this confound. Female gerbils were placed on a heating pad and subjected to a 5 minute bilateral carotid occlusion under halothane anesthesia. Immediately after surgery a rectal probe was inserted, the gerbil was restrained in a refrigerator and the probe was connected to a temperature maintenance system (YSI controlling indicator with attached heat lamp and fan). Temperature was either maintained at 32°C for the entire 6 hour period, or at 36.5°C for 2 hours, followed by reduction to 32°C for 4 hours. Brains were harvested four days later and hippocampal damage was rated on a 0-4 scale. Six hours of hypothermia (32°C) produced significant neuroprotection, reducing the median brain damage rating to 2, while delaying the onset of hypothermia for two hours produced no neuroprotection (a brain damage rating of 3). From these data we conclude that hypothermia beginning two hours post occlusion does not provide neuroprotection, allowing for: 1) a shorter period of temperature maintenance when drugs are administered within the first 2 hours after ischemia; and 2) no temperature maintenance requirement for compounds administered after this two hour period.

664.7

MILD HYPOTHERMIA : EFFECT ON INFARCT SIZE AND BRAIN NITRIC OXIDE PRODUCTION IN FOCAL ISCHEMIA. Abraham Kader,Rosario R. Trifiletti,Robert A. Solomon and Robert R.Goodman *. Departments of Neurology and Neurosurgery, The Neurological Institute of New York, Columbia University College of Physicians and Surgeons. New York, NY 10032.

The effect of mild hypothermia on cerebral injury was evaluated in a rat model of permanent (R) middle cerebral artery (MCA) occlusion. The MCA occlusion was performed in rats at temporalis muscle temperatures of 33°C or 36.5°C.Twenty-four hours after MCA occlusion, rats were sacrificed and the percent infarcted (R) hemisphere determined in brain sections with 2,3,5-triphenyltetrazolium chloride (TTC). We found a percent infarcted volume of 8.2 ± 2.2% (n=8) in the 33°C group as compared to 19.6 ± 1.6 % (n=13) in the 36.5°C group, indicating significant reduction in infarct size by mild hypothermia (P<0.01).

To begin to explore factors accounting for hypothermic neuroprotection, we determined markers of nitric oxide production (NO2- and cGMP) after 10 minutes of ischemia at 33°C or 36.5°C. We found an increase (ipsi- vs. contralateral MCA cortex) in brain nitrite of 136 ± 102% (n=7) in the 36.5°C group as compared to -8 ± 21% (n=5) in the 33°C group (P< 0.05). cGMP was increased (in ipsi- vs. contralateral MCA cortex) by 51 ± 21% (n=7) in the 36.5°C group as compared to 17 ± 18 % (n=6) in the 33°C group.(P<0.05). No significant differences in nitrite or cGMP were found in ipsi- or contralateral cerebellum, which is not rendered ischemic in this model. These data suggest that the mechanism of hypothermic neuroprotection may be due in part to decreased production of nitric oxide in ischemic brain.

664.8

INFLUENCE OF THERAPEUTIC HYPOTHERMIA ON THE RELEASE AND UPTAKE OF EXCITATORY AMINO ACIDS. A.M. Palmer*, P.J. Robichaud and L. Coppula. Departments of Psychiatry and Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

Large increases in the extracellular concentrations of excitatory amino acids (EAAs) and a large depletion of the energy charge are considered to play a major role in mediating the secondary brain injury that occurs following cerebral ischemia. Modest decrements in brain temperature (to 30-36°C) during ischemia substantially attenuate neuronal damage in vulnerable brain regions and improve neurologic outcome (*J Cereb Blood Flow Metab* 7: 729-138; 10: 57-70). This study aims to elucidate the mechanism by which moderate hypothermia prevents the excitotoxic rise in extracellular EAA concentration that occurs during cerebral ischemia (*Stroke* 20: 904-910). The superfused efflux of preaccumulated D-[^{3}H]aspartate was determined in minislices of rat cerebral cortex (flow: 1.6 ml/min; collection time: 0.5 min) and uptake of D-[^{3}H]aspartate determined in cerebrocortical homogenates. The energy charge of these preparations was depleted by applying cyanide, an inhibitor of electron transport. The superfused efflux of preaccumulated D-[^{3}H]aspartate and the Na^+-dependent uptake of D-[^{3}H]aspartate was determined at both normothermic (37°C) and hypothermic (32°C) temperatures. Cyanide (10 μM - 10 mM) increased the efflux of D-[^{3}H]aspartate evoked by 50 mM K^+ and inhibited D-[^{3}H]aspartate uptake in a dose-dependent fashion. However, neither of these changes was influenced by hypothermia. In contrast, cyanide-induced increase in the basal efflux of D-[^{3}H]aspartate was attenuated (by 60-66%) by reducing the incubation temperature from 37° C to 32°C. Thus, therapeutic hypothermia does not influence the release and uptake of EAAs but rather attenuates cyanide-induced efflux of EAAs by another mechanism, probably by militating against cell lysis. (This work was supported by NINDS Grant 1P20NS30318-01).

664.9

TEMPERATURE - DEPENDENT CYCLOHEXIMIDE PROTECTION OF HIPPOCAMPAL ISCHEMIC DAMAGE. G.D. Miller* and J.N. Davis, VA Neurology Laboratory and Duke Univ., Durham, NC.

Neurons in the CA_1 region of the hippocampus undergo delayed neuronal death after brief periods of ischemia. Recent reports suggested that inhibition of protein synthesis with either cycloheximide (CHX) or anisomycin (ANISO) protected these neurons from ischemia. However many variables including temperature, serum glucose and plasma glucocorticoids can influence CA_1 neuronal damage and could be affected by CHX or ANISO administration. We studied 4 groups of gerbils all exposed to 5 minutes of bilateral carotid occlusion and then were sacrificed 5 days later. One group received ANISO (50 mg/kg starting 1 hr. before ischemia, n=8), another, CHX (2.5mg/kg, n=6), a third received sham injections (n=13) while the fourth received CHX (n=5) and was kept in a warm environment. Plasma glucose and cortisol levels did not differ amongst the groups. Similarly pre-ischemic and ischemic temperatures were the same. By contrast the post-ischemic temperatures were depressed in the animals treated with CHX and ANISO kept at room temperature. The animals receiving CHX and kept in a warm environment had post-ischemic temperatures comparable to sham injected animals. The ANISO and CHX treated animals with post-ischemic hypothermia had significant protection of CA_1 neurons compared to sham injected animals. However the CHX injected animals kept in a warm environment did not show protection. We conclude that the reported effect of CHX and ANISO administration on CA_1 neuronal damage may be mediated by post-ischemic hypothermia induced by the protein synthesis inhibitors. (Supported by NS 06233 and the Veterans Administration)

664.10

MILD HYPOTHERMIA HAS EFFECTS ON CORTICAL AMINO ACID EFFLUX BUT DOES NOT PROTECT AGAINST PERMANENT FOCAL ISCHEMIA. E.H.Lo*, R.Newcomb, N.Panahian, N.Maidment, G.K.Steinberg. Center for Imaging and Pharmaceutical Research, Harvard Medical School, Boston MA 02129; Neurex Coorporation, Palo Alto; and Department of Neurosurgery, Stanford University, Stanford, CA 94305.

The effect of hypothermia on relationships between the spatial distribution of damage and alterations in neurotransmitter levels may be dependent on the severity of the ischemic insult. In this study, we examined the effects of mild brain hypothermia (33C, n=7) versus normothermia (37.5C, n=7) in a rabbit model of permanent focal ischemia (4 hr occlusion of left anterior, middle cerebral, and internal carotid arteries). In vivo microdialysis was used to measure brain extracellular environments in anterior ventrolateral and dorsal cortex of the ischemic hemisphere. These locations were defined in previous studies to correspond with regions of dense and boundary zone ischemia respectively. Glutamate was significantly increased by about 1 hr after the onset of ischemia (p<0.05). Alanine, GABA and taurine levels were also changed. Phospho-ethanolamine was increased after about 1 hr. In general, absolute concentrations of amino acids were lower in the hypothermic animals. Lowered temperature appeared to eliminate glutamate efflux in the ventrolateral probes. However, there was significant variability in glutamate efflux between animals; some animals with little or no efflux still demonstrated significant histological damage. Hematoxylin eosin stains revealed no hypothermic neuroprotection in the cortex or striatum. The lack of protection may correspond with the sustained release of glutamate in the dorsal cortex . The reason for decreased efflux of glutamate in the dense ischemic region of the ventrolateral cortex is not clear. The variability in glutamate release suggests that other mechanisms of injury may also be involved in severe ischemia. It is concluded that permanent focal ischemia may result in too severe an insult for hypothermic neuroprotection.

665.1

SELECTIVE ALTERATION OF CYTOSOLIC FREE CALCIUM IN CELL BODIES AND GROWTH CONES OF DIFFERENTIATED PC12 CELLS. G. Gibson* and L. Toral-Barza. Cornell Univ. Med. Coll. at Burke Medical Research Inst., White Plains, NY 10605

Our previous studies indirectly demonstrated that the nerve cell body and terminal responded differently to hypoxia. To directly test that possibility, the responses of cytosolic free calcium ($[Ca^{2+}]_i$) to K^+ depolarization, hypoxia (KCN), nifedipine and ω-CgTX were compared by fura-2 imaging of the cell bodies and growth cones of NGF-differentiated PC12 cells. Under resting conditions $[Ca^{2+}]_i$ was lower in the growth cones than the cell bodies. The response to K^+-depolarization was more rapid in the growth cone than in the cell body. Although nifedipine did not alter resting $[Ca^{2+}]_i$, it partially impaired the K^+-induced increase of $[Ca^{2+}]_i$ in the growth cone and almost totally blocked the response in the cell body. ω-CgTX nearly abolished the response to K^+ in the growth cone, but did not affect the K^+-induced increase in $[Ca^{2+}]_i$ in the cell body. Hypoxia increased $[Ca^{2+}]_i$ less in the growth cone than in the cell body and neither calcium antagonist altered the hypoxia-induced change. The combination of hypoxia and K^+ depolarization increased $[Ca^{2+}]_i$ more than either alone and the elevation was greater in the cell body. ω-CgTX greatly reduced the exaggerated increase in $[Ca^{2+}]_i$ due to K^+ in hypoxic growth cones, but nifedipine had only minimal effects. ω-CgTX did not alter this response in hypoxic cell bodies, but nifedipine greatly attenuated the K^+ response. Thus, any hypothesis to explain altered neuronal function during hypoxia must include regional differences in cellular calcium regulation and a differential response to calcium antagonists.

665.2

DECREASE IN INS(1,4,5)P_3 3-KINASE mRNA EXPRESSION IN FOCAL CEREBRAL ISCHEMIA. G. Y. Sun*, P. Wixom, T. A. Lin, T. N. Lin, R. T. Zoeller and C. Y. Hsu. Depts of Biochem. and Anatomy & Neurobiology, Univ. Missouri, Columbia, MO and Div. of Restoratory Neurology, Baylor College of Medicine, Houston, TX.

Disturbance of intracellular calcium homeostasis is thought to be an important mechanism underlying the ischemia-induced neuronal cell death. Ins(1,4,5)P_3 3-kinase and 5-phosphatase are responsible for metabolism of Ins(1,4,5)P_3, the second messenger for mobililization of intracellular calcium stores. Our recent studies have indicated a specific decrease in the Ins(1,4,5)P_3 3-kinase activity due to focal ischemia in rats induced by ligation of the right middle cerebral artery. Enzyme activity was further decreased during reperfusion after prolonged ischemic insult. In this study, changes in mRNA expression of Ins(1,4,5)P_3 3-kinase was examined. In situ hybridization with frozen coronal sections using oligomer probes indicated high levels of expression in the hippocampal CA1 neurons, dentate gyrus and cerebral cortex. Ischemic insult resulted in a decrease in mRNA expression in the neocortical area similar to the area of infarct. Northern blot analysis revealed no change in mRNA level in the right cortex during the ischemic insult but a decrease was found at 14 hr after ligation. Results indicate that besides the decrease in enzyme activity, focal cerebral ischemia also induced a decrease in the mRNA expression of Ins(1,4,5)P_3 3-kinase which occurred some time during the reperfusion period.

665.3

ISCHEMIA-INDUCED REGIONAL ALTERATIONS IN PKC ACTIVITY ARE SENSITIVE TO MODERATE CHANGES IN INTRAISCHEMIC BRAIN TEMPERATURE. R. Busto,* M.Y.-T. Globus, E. Martinez , I. Valdés , and M.D. Ginsberg. CVD Research Center, Univ. of Miami, Sch. of Med., Miami, FL, 33101.

Extracellular glutamate release and histopathological outcome following ischemia are sensitive to intraischemic brain temperature. Since PKC is implicated in neurotransmitter release and glutamate receptor mediated events, the relationship between intraischemic brain temperature and PKC activity was evaluated. Twenty min of 2-vessel occlusion plus hypotension was induced in rats whose intraischemic brain temperature was maintained at 30°C, 37°C, or 39°C. Using a PKC enzyme assay kit, cytosol and membrane PKC activity were determined in hippocampal, striatal, cortical, and thalamic homogenates at the end of ischemia and at 15min, 30min, 1h and 24h of recirculation. A reduction in cytosol and membrane PKC activity (43-50% and 45-49%, respectively; $p < 0.05$) was observed in the hippocampus, striatum and cortex of normothermic rats as early as 30min of recirculation. PKC activity remained diminished at 24h in the hippocampus and striatum but normalized in the cortex. No changes were documented in the thalamus. Ischemia-induced reductions in PKC activity were abolished in hypothermic animals. Conversely, postischemic PKC activity was significantly lower in the hippocampus and cortex of hyperthermic animals compared to normothermic ($p < 0.01$). Results indicate the ischemia-induced changes in PKC activity is a temperature sensitive process, suggesting that it may be involved in the effects of temperature on ischemic outcome.

665.4

CORRELATION OF CALBINDIN AND NEURONAL ANOXIC RESPONSES. M.E. Morris*, K.G. Baimbridge, H. El-Beheiry, D.S.K. Magnuson, G.V. Obrocea and A.S. Rosen. Dept. of Pharmacology, Univ. of Ottawa, Ottawa, Canada K1H 8M5.

The Ca^{2+}-buffering capacity of neurons which contain calbindin-D28K (CaBP) has been proposed to play a protectant role against excitotoxicity. It is hypothesized that cells with different early responses to anoxia may show differences in CaBP distribution and vulnerability. Experiments were carried out to correlate CaBP presence and anoxic-evoked changes in membrane parameters of identified neurons in neocortex, hippocampus (CA1/2) and mesencephalon of rat brain. Slices, perfused and equilibrated with 95% O_2/5% CO_2 at 34°, were exposed to 3-10 min of N_2 during intracellular recording and injection of Lucifer Yellow, followed by fluorescent CaBP antibody labelling. In CA1 N_2 evoked sustained depolarizations (17 ± 3.4 mV for 7 trials from V_M -68 ± 5.4 mV) in 4/5 pyramidal cells, characterized as CaBP+; 3 neurons which responded with hyperpolarization (≤10 mV from V_M -68 ± 2.8 mV) and showed burst activity before and during anoxia, were CaBP-. Although the neurons of other brain regions were depolarized by N_2, they were mainly CaBP-. These preliminary data suggest that, at least in the highly vulnerable CA1 area, differential responses may reflect differences in the Ca^{2+} buffering capacity of individual neurons/groups and explain different susceptibilities within that region.

665.5

EXPRESSION OF PROTEIN KINASE C ISOZYMES AND THE EFFECT OF HYPOXIA ON PKC IN RAT GLIAL CELLS. K. Kumar*, B. Kim, H. Rupp, & B. V. Madhukar. Departments of Pathology, and Pediatrics and Human Development, Michigan State University, E. Lansing, MI 48824.

Hypoxia and ischemia produce a number of intracellular changes conducive to the activation of Protein Kinase C (PKC) that has a number of isozymes. To study the presence of various isozymes of PKC in glial cells, and the effect of hypoxia on PKC, immunofluorescence (IF) studies were performed on glial cell cultures of the rat cortex. The antibodies tested were against α, β, γ and ε isozymes, and the catalytic domain (cd), of PKC. The relative degree of IF for each group of cells was compared by using an automated laser cytometric device. The data indicate that rat glial cells express at least three isozymes of PKC, ε, β, and α (in descending order of intensity). The IF for PKC - cd was more intense than for any of the isozymes. The glial cells were subjected to hypoxia by exposure to 100% N_2 for varying periods of up to 72 h. IF studies were performed as above. Even though this was not a quantitaive study, a decrease in the PKC - cd IF was demonstrated following 72 h of hypoxia. Decrease in the activity of PKC may lead to altered protein phosphorylation reactions, thereby playing critical roles in neuronal death due to hypoxia.

665.6

DIFFERENCE IN ALTERATIONS BETWEEN Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE AND CALCINEURIN IN THE HIPPOCAMPUS AFTER TRANSIENT FOREBRAIN ISCHEMIA.

M. Morioka*, K. Fukunaga, S. Nagahiro, Y. Ushio and E. Miyamoto. Dept. of Neurosurgery and Pharmacology, Kumamoto University School of Medicine, Kumamoto 860, Japan.

After transient ischemia of the brain, the increase of the intracellular Ca^{2+} level is observed, which may trigger the intracellular process of neuronal death. We have investigated the regional and temporal alterations of Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) and calcineurin (CaN) (Ca^{2+}/CaM-dependent protein phosphatase) after transient forebrain ischemia. The immunoreactivity and enzyme activity of CaM kinase II decreased in all regions of the hippocampus at the early stage (6-12 hrs) after ischemia, but gradually recovered during the course of the time except for the CA1 region. Furthermore, an increase in Ca^{2+}/CaM-dependent activity was detected until 3 days after ischemia in all regions tested, suggesting that the concentration of intracellular Ca^{2+} increases. In contrast to CaM kinase II, immunohistochemistry and regional immunoblot analyses revealed that CaN was preserved until morphological degeneration of CA1 neurons. These results suggest differences between CaM kinase II and CaN in regard to regional and temporal loss after ischemia and the occurrence of the imbalance of the Ca^{2+}/CaM-dependent protein phosphorylation-dephosphorylation.

665.7

REGIONAL DEPRESSION IN CALCIUM/CALMODULIN DEPENDENT PROTEIN KINASE II (CaM-KII) IN FOCAL ISCHEMIA. S.K. Hanson, J.C. Grotta, M.N. Waxham, R. Earls, R. Strong, N. Dafny*. Dept. of Neurology and Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, TX 77030

Change in CaM-KII activity was evaluated in a rat model of focal ischemia. Activity in homogenate preparation of cortex measured by phosphorylation of synthetic peptide substrate was decreased by 76% ($p<0.0001$) in histologically defined infarct core (n=12) when compared with similar cortical regions in sham operated controls (n=4). Decreases in histologically defined infarct borderzone (defined at one hour of ischemia) were less dramatic (34%, $p=0.0007$). These changes were immediate so that depression in activity after 5 minutes was comparable to that seen after one hour of ischemia with no reperfusion. Reperfusion is associated with incomplete return of activity at 24 hours even in regions with no apparent histologic damage. CaM-KII downregulation is extremely sensitive to a focal ischemic insult and changes in activity are proportional to the amount of histologic damage.

665.8

BLOCK OF Ca^{2+} INFLUX DURING ISCHEMIA INHIBITS DOWN REGULATION OF Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II AND PROTEIN KINASE C. J. Aronowski, J. C. Grotta[1] and M. N. Waxham*. Depts. of Neurobiology & Anatomy and [1] Neurology, Univ. Texas Medical School, Houston, TX 77030.

Excitotoxicity is generally considered to be a major factor for ischemia induced cell damage in vulnerable regions of the brain. Ca^{2+} influx evoked by glutamate through N-methyl-D-aspatate-gated ion channel (NMDA) as well as voltage-gated Ca^{2+} channels (VDCC) during ischemia induces undefined sequences of Ca^{2+}-dependent biochemical events which may lead to impairment of neuronal homeostasis and subsequent cell death. Recently we reported that 20 min. of global ischemia in rats induced by bilateral carotid artery occlusion caused dramatic decreases of Ca^{2+}/calmodulin-dependent protein kinase II (CaM-KII) and protein kinase C (PKC) activities in both hippocampus and cerebral cortex. This inhibition was detected immediately after ischemia and was not reversible for at least 2 h and only partially 7 days after ischemia (J. Neurochem. 58, 1743).

Here we describe effects of Dextrorphan (DEX), the well defined noncompetitive NMDA channel antagonist which also express VGCC antagonistic properties, on changes in activity of both CaM-KII and PKC induced by reversible global ischemia in rats. 15 mg/kg of intravenous DEX, 5 min before induction of 20 min of ischemia, significantly inhibited down-regulation of both kinases. The observed protection was detected immediately after ischemia as well as after 24 h of reperfusion. There was no effect of DEX on kinase activity observed after 2 h of reperfusion. Pretreatment, but not postreatment of animals with DEX subjected to 20 min of ischemia also resulted in histological protection of hippocampal and cortical neuronal tissue.

665.9

ANOXIA LIMITS DEPOLARIZATION INDUCED CALCIUM UPTAKE IN THE RAT HIPPOCAMPAL SLICE. I.S. Kass*, A.E. Abramowicz, G. Chambers and J.E. Cottrell. Anesthesiology Dept., SUNY Health Science Center, Brooklyn, NY 11203.

High cytosolic calcium levels are thought to lead to neuronal damage after anoxia. We found that Ca-45 uptake during anoxia was far less than the uptake measured during depolarization. We therefore examined whether anoxia could inhibit depolarization induced Ca uptake.

Hippocampal slices were subjected to either anoxia ($95\%N_2$-$5\%CO_2$), depolarization (12.5 uM veratridine) or a combination of the two. Ca-45 uptake was measured in the CA 1 region.

Calcium uptake increased from 9.0 ± .2 to 10.7 ± .2 nM/mg dry wt. ($P < .001$) during 15 min of anoxia. Veratridine for 10 min caused a 3 fold increase in Ca uptake to 30.2 ± .7. If anoxia was started 5 min before the onset of veratridine depolarization and continued throughout the depolarization (total anoxia = 15 min, veratridine = 10 min) then there was only a 2 fold increase in calcium uptake to 18.4 nM/mg.

Thus anoxia reduced the depolarization induced calcium uptake indicating that there might be a mechanism by which anoxia can inhibit the voltage sensitive calcium channel.

665.10

ELECTRODES BASED ON ETH-129 SHOW ASTROCYTIC Ca^{2+} RISES DURING SPREADING DEPRESSION. R.P. Kraig* & C.D. Lascola. Dept. of Neurology, Univ. of Chicago, Chicago, Il 60637.

Spreading depression (SD), a benign yet massive perturbation of brain, transiently converts astrocytes into reactive species (**1**). A rise in pH_i, associated with SD (**2**), may allow this glial metamorphosis to occur but what might trigger it is unknown. A rise in $[Ca^{2+}]_i$ stimulates enhanced anabolism, including proliferation, in other cells and thus, might trigger reactive astrocytosis (RA).

Ca^{2+}-ISMs based on ETH-129 were used to determine astrocytic $[C_{a2+}]_i$ changes during SD in anesthetized rats. SD was induced by electrical stimulation and astrocytes were identified by electrophysiologic criteria (**2**). Ca^{2+}-ISMs had a linear response and sensitivity for changes in pCa^{2+} to almost 8 and at least 10, respectively, in 100 mM K^+. Membrane potentials fell from 80-110 mV while $[Ca^{2+}]_i$ rose from the 100 nM range by orders of magnitude during SD. Both parameters returned to baseline after SD.

Resting astrocytic $[Ca^{2+}]_i$ in the nM range and rise with SD is consistent with that seen *in vitro* (**3**) and supports the validity of this data compared to previous work (**4**) (based on ETH-1001) that may have been erroneous due to an excessive Ca^{2+}-leak into cells. Perhaps, a coupled rise in pH_i and $[Ca^{2+}]_i$ synergistically increase astrocytic anabolic activity in RA.

1. Kraig et al., J. Neurosci, '91; 2. Chesler & Kraig, J. Neurosci, '89; 3. For rev. see Cornell-Bell & Finkbeiner Cell Calcium, 1991. 4. Kraig, Soc. Neurosci (abst.), '89.

ISCHEMIA: CLINICAL STUDIES

666.1

BRAIN FUNCTIONAL ABNORMALITIES IN CHRONIC ESSENTIAL HYPERTENTION. MJ Mentis, JA Salerno, B Horwitz, Murphy DGM, SI Rapoport*, MB Schapiro. Lab. Neurosci., Natl Inst on Aging, NIH, Bethesda MD.

To determine if chronic essential hypertension is associated with abnormal brain functioning, we performed a correlational analysis on the results from 17 hypertensive men (mean age± s.d.= 68±8yr) and 25 age-and sex-matched normotensive control subjects, who were studied with high resolution positron emission tomography (PET) using 18-fluorodeoxyglucose when off medication for at least 2 weeks. Hypertensives had been medically-treated for at least 10 yrs, and had no apparent end-organ involvement. All subjects were free from other medical, and cognitive disorders. A correlational analysis was performed to count the number of significantly different correlation pairs (sdc) between the two groups in the vascular areas of the brain. Correlations were performed from each vascular area of interest to all brain areas. There were significantly more sdc's with a smaller hypertensive value in the carotid distribution compared to the basilar, and within the carotid area, in the middle-anterior watershed area compared to the middle and anterior cerebral artery areas. These results suggest that well controlled hypertensives without clinical cognitive or other deficits, have abnormal brain correlations in the carotid artery area, and within this territory mostly in the middle-anterior artery watershed area. This latter pattern of functional abnormality - mainly in the watershed area - suggests a low flow state (ie episodes of hypotension) rather than high pressure as the immediate cause.

666.2

ANXIETY DISORDERS FOLLOWING STROKE. CS Castillo, SE Starkstein*, JP Fedoroff, TR Price, RG Robinson, Univ. of Iowa Coll. Med., Iowa City, Ia 52242

A series of 309 admissions to a stroke unit were examined for anxiety symptoms. Patients were diagnosed with DSM-III-R generalized anxiety disorder (GAD) symptom criteria. They were divided into groups of no anxiety (59.2%), worried but not fulfilling GAD criteria (13.9%), and GAD (26.9%). Patients were then divided into depressed and non-depressed groups based on the existence of DSM-III major or minor (dysthymic) depression. These groups were not significantly different in their background characteristics or the severity of physical impairment. Anxiety plus depression (74% of all GAD patients and 19% of all patients) was associated with left cortical lesions while anxiety alone was associated with right hemisphere lesions ($X^2=4.9$, $df=1$, $p=.043$). Patients with GAD had a greater frequency of posterior right hemisphere lesion than patients with right hemisphere lesions without GAD ($F=3.5$, $df=2,46$, $p=.038$) Two year follow-up at 3,6,12 and 24 months revealed a stable prevalence of GAD at each of follow-up intervals of approximately 30%. Patients who were initially not worried had a mean prevalence of 18% during the 2-year follow-up. The initially GAD (IGAD) patients had a mean follow-up prevalence of 44% while those that were initially worried had a mean prevalence of 30%. Prevalence in the follow-up depressed patients was 36.5% while in the non-depressed it was 18%. Initially not depressed IGAD (11% prevalence) patients had no cases on follow-up. Initially depressed IGAD maintained the highest follow-up prevalence, 49%. New onset GAD at follow-up averaged 58% of all GAD cases seen over 2 years and 80% also had depression. Findings suggest that anxiety disorder (independent of depression) is not related to background characteristics or to severity of impairment but is influenced by brain structures injured. Post-stroke GAD has a high incidence.

666.3

Motor Control of the Hand in Humans with Upper Motor Neuron Dysfunction Due to Stroke

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Upper limb volitional motor control impairment, in particular, the loss of fine precise movement of the hand and fingers is common following stroke. The evolution of recovery from stroke has a well described pattern with the majority of functional improvement occurring in the first 3 months after onset. Functional outcome measures have demonstrated the variable nature of clinical assessments and the inherent variability of clinical signs in the hemiplegic population.

We studied the features of motor control of the upper limbs in 29 hemiplegic subjects by recording surface EMG from the pectoralis major, deltoids, biceps brachii, triceps brachii, wrist extensor and flexor muscles of both sides. This simultaneous recording of motor activity provides a picture of the pattern used to perform the series of voluntary and reflex maneuvers. The hemiplegic subjects were divided on the basis of clinical observed volitional wrist movement into four groups: (1) no movement; (2) trace movement; (3) movement with limited range; (4) full range movement. In all of these groups, characteristic features of motor control are described, including grousps 1 and 2.

666.4

QUANTITATED EEG (QEEG): A TOOL TO DIAGNOSE OR MONITOR PATIENTS WITH ANEURYSMAL SUBARACHNOID HEMORRHAGE. P. Newlon*, M. Sumas, F. Schinco, B. Limperis, & S. Gudeman. East. Va. Med. Sch., Norfolk, Va., 23507.

EEG was recorded from 16 normal subjects and serially from 16 patients with cerebral aneurysms (14 had subarachnoid hemorrhage (SAH); 15/16 aneurysms were surgically repaired). The EEG was quantified (Cadwell Spectrum 32) and analyzed with regard to absolute and relative power, symmetry, and coherence over 4 frequency bands and 21 sites. In general, patients showed increased slowing, frontal power asymmetry, and increased coherence over temporal sites. Focal abnormalities in delta power and coherence were characteristic of an aneurysmal source in 12 preoperative studies. Anterior communicating artery aneurysms were linked with fronto-temporal absolute power, while coherence abnormalities in anterior, central and posterior sites were seen with internal carotid, middle cerebral and posterior communicating artery aneurysms, respectively. In 3 patients, QEEG suggested aneurysmal sources at a time when angiographic data were either negative or inconclusive. Abnormalities were also seen in patients with incidental aneurysm findings, suggesting that the QEEG does not simply reflect the presence of SAH. Postoperatively, 9 of 15 patients developed vasospasm by clinical or radiographic criteria. In 3 of 5 patients who received pre- and post-spasm QEEG studies, novel focal abnormalities in QEEG could be seen prior to the onset of vasospasm, and the brain regions involved were appropriate to the type of functional deficit that ultimately emerged.

Although preliminary, these results suggest that quantifiable changes in EEG power distribution over the scalp may be useful both in diagnosis of aneurysms in patients with SAH, and in the detection or prediction of impending vasospasm. Further studies are aimed at determining the reliability and validity of this technique to detect subclinical cerebral vasospasm and allow early intervention.
This work was supported in part by the Departments of Neurosurgery and Clinical Neurophysiology at Sentara Norfolk General Hospital.

INFECTIOUS DISEASES

667.1

BALB/c SUBSTRAIN DIFFERENCES IN SUSCEPTIBILITY TO TMEV-INDUCED DEMYELINATING DISEASE AND POSSIBLE MECHANISMS. S.M.Nicholson*, J.L.Hoeft, L.Dech, C.Waltenbaugh, and R.W.Melvold. Dept. of Microbiology-Immunology and Institute for Neuroscience, Northwestern University, Chicago, IL, 60611.

We report differences among four BALB/c substrains in susceptibility to Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease (TMEV-IDD), a model for human multiple sclerosis. TMEV-IDD is believed to result from bystander damage to myelin inflicted as part of a delayed type hypersensitivity response directed against chronic, low level infection of the CNS by TMEV. We have found BALB/cByJ and BALB/cCum mice to be resistant to the development of TMEV-IDD, while BALB/cAnNCr and BALB/cJ are intermediately susceptible. We are attempting to define the genetic and immunological mechanisms involved in this differential susceptibility. Studies in our laboratory have shown that some resistant strains can be converted to a susceptible phenotype by the application, prior to infection with TMEV, of immunopotentiating regimens such as low dose cyclophosphamide or γ irradiation or administration of anti-I-J antibodies. Low dose irradiation efficiently converted resistant BALB/cByJ mice to a susceptible phenotype, indicating that the resistance of BALB/cByJ to development of the immune responses associated with development of TMEV-IDD is due to an active regulatory mechanism rather than an intrinsic inability to develop the destructive responses. Low dose cyclophosphamide was far less effective in producing the susceptibility in BALB/cByJ. Experiments have been initiated to identify the cellular mechanisms involved in the protective regulation.

667.2

IL-1 AND TGF-B1 CONTROL ASTROCYTOSIS IN HUMAN BRAIN. A. da Cunha+, J.J. Jefferson#, W.R. Tyor$, J.D. Glass&, F.S. Jannotta@ and L. Vitkovic+* +NIAID, #NCI, NIH, Bethesda, MD 20892; Dept $Neurology and &Pathology, The Johns Hopkins Univ, Baltimore, MD 21205; @George Washington Univ Med Ctr, Washington DC.

Astrocytic hyperplasia, an increase in number, and hypertrophy, an increase in size are common neurocellular manifestations of brain pathology. Published data indicate that interleukin-1 (IL-1) is elevated in experimental models of brain pathology; IL-1 causes proliferation of astrocytes under experimental conditions in vitro and in vivo and transforming growth factor beta 1 (TGF-B1) causes hypertrophy of astrocytes in vitro. The precise causes of hyperplasia and hypertrophy of astrocytes in brain of individuals with a variety of diseases are unknown. These astrocytic changes and IL-1 and TGF-B1 immunoreactive products (IRPs) were morphometrically measured in frontal cortex and subcortical white matter of 22 individuals who died with various diseases. The data demonstrate that the number and the size of astrocytes were correlated with amounts of IL-1 and TGF-B1, respectively, but not vice versa. These correlations were statistically significant. Further, these cytokines and the cellular changes of astrocytes were co-localized in all examined tissues. Thus, these cytokines appear to control astrocytosis.

667.3

INCREASED 3-HYDROXYANTHRANILIC OXYGENASE IMMUNOREACTIVITY IN MEASLES VIRUS-INFECTED MICE. C.L. Eastman*, F. Du, [1]T. Andersson, [2]A. Löve and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD, [1]Karolinska Institute, Huddinge (Sweden) and [2]Univ. Iceland, Reykjavik (Iceland).

Intracerebral injection of BALB/c mice with the hamster neurotropic (HNT) strain of measles virus produces a bilateral hippocampal lesion remarkably similar to that observed following injection of quinolinic acid (QUIN). Neurodegeneration can be observed in Nissl-stained sections five days after injection, can be prevented by the NMDA receptor antagonist MK-801, and is preceded by marked hippocampal astrogliosis. Since the endogenous NMDA receptor agonist QUIN is produced by astrocytes in the CNS, it is likely that QUIN metabolism may be altered during HNT infection. In the present study, 3-hydroxyanthranilic acid oxygenase (3HAO), QUIN's biosynthetic enzyme, was assessed immunocytochemically in control and HNT-infected mouse brains. Three days after HNT injection, many 3HAO-immunoreactive hypertrophic astrocytes were evident in the CA1 region of the hippocampus. By 7 days post-injection, hypertrophied 3HAO-containing astrocytes were observed throughout the hippocampus. The increase in 3HAO immunoreactivity is consistent with the hypothesis that enhanced production of QUIN may contribute to the neurodegeneration associated with HNT infection.

Supported by USPHS grant NS 16102.

667.4

CNS QUINOLINIC ACID INCREASE IN MURINE RETROVIRAL ENCEPHALOPATHY. C. A. Wiley*, M. P. Heyes and R. M. Nagra. Univ. of California, San Diego, Department of Pathology. La Jolla, CA 92093-0612.

Quinolinic acid (QUIN) is an agonist of *N*-methyl D-aspartate receptors and is a known excitotoxin. Increased concentrations of QUIN in cerebrospinal fluid have been implicated in the spongiform encephalopathy associated with human and simian immunodeficiency virus infections, but tissue levels of this compound are not known. Infection with a molecularly cloned neurotropic Murine Leukemia virus ***pNE-8***, or a temperature sensitive mutant ***ts-1*** lead to extensive neuronal and oligodendroglial infection and spongiform degeneration. Microglial activation and infection was only observed in ts-1 infecetd mice. Serum QUIN levels in both infections were similar to those of non-infected mice, however, QUIN concentration in the brainstem, cerebellum and spinal cord were increased by 4 to 10 folds in ts-1 infected mice. Microglial infection by ts-1 may be the major source of elevated QUIN levels. These murine models are ideal for studies of the pathogenesis of CNS damage in retroviral infection.

667.5

NEUROTOXICITY OF LENTIVIRUS DERIVED TAT PEPTIDE FRAGMENTS IN RAT STRIATUM. M.Hayman, G.W. Arbuthnott*,G. Harkiss. Preclin. Vet. Sciences, & Vet. Pathology, Univ. of Edinburgh, Summerhall, Edinburgh EH9 1QH. U.K.

Lentiviruses including VISNA in sheep and HIV in humans cause neuronal damage in spite of their not having been seen to replicate in neurones. We have studied the time course and dose dependence of neurotoxicity following direct injection of amino-acid sequences from proteins derived from both viruses into the striatum of rats.

We estimated the neurotoxic effect by measuring the volume of reactive microglial immunostaining in the striatum one week after the toxin injections. The minimal dose resulting in a measurable lesion was between 1 & 5 ug (0.8 - 4nM) of the peptide from VISNA.

Preliminary results suggest that while the cysteine residues can be modified without change in the toxicity the arginine doublet in the centre region is necessary.

Since these peptides derive from the tat sequences in the viral peptides and are thus expected to be passed on to neighbouring cells, secretion of them by infected glia could both damage nearby neurones and recruit more microglia thus explaining the neuropathological pattern seen in infected animals.

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667.6

GENETICALLY DETERMINED HERPES SIMPLEX VIRUS 1 (HSV 1) RESISTANCE IN OLIGODENDROCYTES (OL) MAY PLAY A ROLE IN LIMITING SPREAD OF HSV 1 IN THE CENTRAL NERVOUS SYSTEM (CNS): DELAY OF IMMEDIATE EARLY (ICP4) AND EARLY (ICP8) ANTIGEN SYNTHESIS IN HSV 1 RESISTANT OLs FROM C57BL/6J AND BALB/cByJ MICE. *E.E. Thomas[1],A.Lau[2], L.F. Kastrukoff[2]* [1]Division of Microbiology, Department of Pathology, British Columbia's Children's Hospital and [2] Division of Neurology, Department of Medicine, University of British Columbia, Vancouver, Canada.*

Primary murine OL cultures from 3 inbred strains show HSV 1 replication differences, which correlate with in vivo infection (A/J, susceptible; BALB/cByJ, moderately resistant; C57BL/6J, resistant). The nature of the in vitro resistance at the cellular level was investigated using an indirect immunofluorescense assay (IFA) with monoclonal antibodies to ICP4, ICP8 and gC HSV antigens. OLs from HSV C57BL/6J mice showed restricted expression of all antigens: only small amounts of ICP4 and ICP8 (10% of cells) and no gC was detected until 72 hrs postinfection (p.i.) in contrast to OLs from A/J mice in which 80-100 % of cells expressed all three proteins 72 hrs p.i. Balb/c showed an intermediate protein expression pattern: 50% of cells were ICP4 pos., 30-50% ICP8 pos. and 20% gC pos. 72 hrs p.i. HSV adsorption (using ^3H labelled HSV) to the 3 OL strains was also studied, and no difference in adsorption pattern was noted. These results suggest the existence of an early, postadsorption HSV replicative block in C57BL/6J OLs and a less restricted early replication block in Balb/cByJ OLs. Electronmicroscopy studies confirm this observation. The selective differences in HSV resistance mediated by OLs, reflect differences in virus host cell interactions, and likely contribute to differences in mortality, viral spread, and pathological differences in the CNS of the different mouse strains.

667.7

VIRUS DNA SEQUENCES AND CNS DISEASE IN NEONATAL HERPES SIMPLEX INFECTION. P. Gressens[1], C. Langston[2], W.J. Mitchell[1] and J.R. Martin[1]* [1]NIH, Bethesda, MD; [2]Texas Children's Hospital, Houston, TX

This study uses polymerase chain reaction (PCR) to detect herpes simplex virus (HSV) genomic sequences in formalin fixed paraffin sections from perinatal HSV-1 and -2 encephalitis autopsy tissues and correlates these results with immunohistochemistry (IHC) and histological lesions. Brain sections from 10 mice with acute HSV-2 infection and from 20 uninfected controls were used to establish PCR conditions of specific HSV DNA amplification and detection. Primers which bracketed a 110 BP fragment of the HSV-2 thymidine kinase gene , 30 cycles of amplification and Southern blot hybridization with an appropriate oligonucleotide probe were selected as they gave 100% sensitivity and specificity in repeated tests with control mouse tissues. 56 neural (NT) and non-neural tissue (N-NT) blocks from 10 neonates were tested; previous IHC studies showed that in N-NT of 6 neonates antigen was HSV-2 and in 3 it was HSV-1 in type; 1 case, dead 7 weeks after clinical encephalitis, was negative for HSV antigen. HSV antigen-positive N-NT contained HSV DNA sequences, confirming the sensitivity of this PCR protocol. In NT, 22 blocks displayed histological lesions of possible viral origin but the IHC detected HSV antigen in only 9 blocks; in contrast, we obtained a specific band of amplified HSV DNA in all these blocks, including 1 neonate dying 7 weeks after birth. 15 NT and N-NT blocks were histologically normal and HSV antigen-negative; all, except 2 blocks from the 7 week old neonate, contained HSV DNA sequences. All PCR results were reproducible. PCR detects HSV DNA in CNS areas that contain no detectable HSV antigen but are histologically abnormal; this suggests that these histological lesions are virus-induced.

667.8

NEURONAL DEATH IN MOUSE SPINAL GANGLIA FOLLOWING HERPES SIMPLEX VIRUS TYPE-2 (HSV-2) INFECTION D.B. Henken*[1], M.E. Goldstein[2] & J.R. Martin[1], NIH, NINDS, LENP[1], LNC[2], Bethesda, MD, USA, 20892.

In this study, we examine whether HSV-2 infection causes neuronal death in mouse dorsal root ganglia (DRG). The right hind footpads of anaesthetized female BALB/c mice were inoculated with 10 μl of MS strain HSV-2 ($9.3x10^6$ pfu/ml) or medium. One month later, infected (n=4) and sham-inoculated (n=3) mice were perfusion-fixed. Spinal columns, including paired spinal ganglia, were decalcified and serial paraffin sections (10 μm) were stained with cresyl violet. Neuron numbers, somal areas and ganglion volumes for both the infected and the contralateral uninfected lumbar 4^{th} and 5^{th} DRG were determined for each mouse. Following HSV-2 infection, between 50 and 70% of neurons disappeared 1 month following inoculation. Neuron numbers in sham-inoculated and contralateral control ganglia were equivalent. Neuronal death did not target a specific subpopulation of neurons, based on somal size. Ganglionic shrinkage did not occur as a result of HSV-2 infection; neurons were replaced by large numbers of inflammatory cells. These results show that, in addition to other previously described host alterations following viral infection, HSV-2 infection results in neuronal death in the affected host ganglia. This is the first in vivo quantitative documentation of HSV-2 induced neuronal death.

667.9

ANALYSIS OF MYELIN PROTEINS IN POST MORTEM AIDS BRAIN. J.R. Möller, P.G. Durr, R.H. Quarles, B.D. Trapp, C. Power and J.T. Prince* NINDS, NIH, Bethesda, MD 20892 and Dept. of Neurology, Johns Hopkins University, Baltimore, MD.

Subcortical white matter samples from five AIDS patients and four age matched controls were histochemically and biochemically studied for changes in myelin proteins. The pathology observed histologically in the AIDS patients included diffuse subcortical white-matter pallor, as visualized by Luxol Fast Blue staining.

Tissue sections were stained with antibodies to myelin basic protein (MBP), proteolipid protein (PLP), myelin-associated glycoprotein (MAG) and with HNK1. No alterations were found in the distribution of the myelin proteins, including regions of diffuse white-matter pallor. Gliosis identified by GFAP staining was increased in HIV-infected individuals as compared to controls. Electron microscopy of subcortical white matter from HIV-infected patients revealed no indication of demyelination.

Samples for biochemical analysis were adjacent to areas examined histologically and were homogenized in 1% SDS and boiled for 10 min. Coomassie blue stained gels and Western blots were utilized for quantification of MBP, PLP, MAG, 2', 3'-cyclic nucleotide 3'-phosphodiesterase (CNP) and GFAP. No major quantitative changes in the myelin proteins in the samples from AIDS patients were found. These results do not provide morphological or biochemical evidence for extensive myelin loss in subcortical white-matter of AIDS patients.

667.10

TWO PATTERNS OF BLOOD-BRAIN BARRIER ABNORMALITIES IN THE ACQUIRED IMMUNE DEFICIENCY SYNDROME. C.K. Petito* and K.S. Cash, Dept. of Pathology, Cornell University Medical College, New York, NY, 10021.

Histological changes suggesting abnormalities in the cerebral vasculature in AIDS brains include the paravascular location of HIV-related microglial nodules, calcific vasculopathy in children, and white matter endothelial changes. Accordingly, blood-brain barrier (BBB) abnormalities were studied in formalin-fixed paraffin-embedded brains of AIDS patients with HIV encephalitis (HIVE) (n=17) and without HIVE (n=16); non-immunosuppressed patients served as controls (n=22). Immunohistochemical detection of fibrinogen(FIB) and IgG was used as a marker of vascular permeability. The immunoreactivity in the centrum semi-ovale was blindly graded as 0 - 4+. The sex ratios and post-mortem intervals were similar in all groups ($p > 0.05$) but the age of the 2 AIDS groups were significantly younger than controls (43.2 and 40.9 versus 62.5 yrs; $p < 0.05$). The two AIDS groups had significantly higher immunostaining for FIB and for IgG than the control group ($p< 0.001$ and $p < 0.0001$ respectively), but did not differ from one another. In addition, when the results were expressed as "negative" (0 or 1+ staining) or "positive" (2+-4+0, the frequency of positive staining for both FIB and IgG was higher in the 2 AIDS groups whereas the the frequency of negative staining for both proteins was higher in the controls ($p < 0.002$). Although more than 95% of HIV-related microglial nodules were negative for serum proteins, all focal lesions with tissue necrosis, including lymphoma, opportunistic infections and rarely, HIV contained extravasated serum proteins. This study shows that a diffuse BBB breakdown is present in approximately 50% of all AIDS patients at the time of autopsy and may be seen in the absence of any other brain pathology, including HIVE. These BBB abnormalities may be important in mediating some of the tissue damage that accompanies HIV infection of the brain such as the diffuse myelin pallor and gliosis which is common in HIV-infected patients. In addition, the BBB breakdown may not only facilitate viral entry into brain, but also increase the entry of blood-borne cytotoxic cytokines. Supported by NIH Grant R01-NS27416.

667.11

HISTOLOGIC LESIONS IN THE CENTRAL NERVOUS SYSTEM OF HIV-1 INFECTED MACAQUES. D. M. Anderson, H. D. Liggitt, L. Frumkin, M. Agy, W. Morton, D. Bowden*. Regional Primate Research Center, SJ-50, Univ. of Washington, Seattle, WA 98195.

We describe the occurrence of histologic lesions within the central nervous system of monkeys following infection with HIV-1 virus, the etiologic agent of human AIDS. Eight infant pigtailed macaques (*Macaca nemestrina*) were inoculated intravenously with a mixture of infected autologous peripheral blood mononuclear cells (PBMC) and free virus of three HIV-1 strains: LAI, JR-CSF, and JR-FL. A comparison group was infected with SIV. At 2, 5, 14, and 24 weeks following infection, animals were killed and examined histologically for lesions in the central nervous system.

Initial examination of the brains of two animals sacrificed 5 weeks after infection with HIV-1 showed choroiditis in both animals and extensive perivascular infiltrates in the occipital cortex of one. Choroid infiltrates were composed predominantly of lymphocytes; perivascular infiltrates were composed primarily of cells of macrophage origin with occasional astrocytes present. HIV-1 was isolated from PBMCs taken at the necropsy of each animal.

Tests for virus in the tissues are being conducted by immunohistochemistry and in-situ hybridization. Comparison of lesions in animals infected with HIV-1 with those of animals with SIV are in progress. We describe the first documented histologic lesions within the central nervous system in a macaque species following infection with HIV-1.

(Supported by USPHS grant RR00166 and Comparative Medicine Training Grant RR07019.)

667.12

ENHANCEMENT OF HIV TRANSCRIPTION BY SUBSTANCE P AND CYTOKINES IN HUMAN AND RAT GLIAL CELLS. Sundar, K.S., P. Sista, N. Quan, L. S. Kamaraju, P. Simson*, and J. M. Weiss. Department of Medicine and Psychiatry, Duke University Medical Center, Durham, NC 27710.

Asymptomatic HIV seropositive subjects often exhibit evidence of CNS infection with HIV. Progression of the infection with HIV in the brain is suggested by the clinical manifestations of neurological disorders of AIDS dementia complex. The factors that could modulate HIV replication in the brain have not been investigated. We investigated the effects of cytokines on HIV transcription in human glioblastoma cells (1321N1) and primary rat astrocyte cultures, as well as the effect of substance P (SP) on HIV transcription and IL-1 production in human macrophage cells. HIV-LTR was linked to the cDNA of chloramphenicol acetyl transferase enzyme (CAT) and increased synthesis of CAT enzyme transcription was considered indicative of HIV transcription. The results indicate that: 1) SP dose-dependently enhanced HIV transcription in the macrophage, with the minimal effective dose at 10^{-10} M and SP also induced the synthesis of IL-1; 2) IL-1 (10-100 pg/ml) and TNFa (100-1000 pg/ml) significantly enhanced HIV transcription; and 3) IL-1 and TNFa in concert with each other synergistically enhanced HIV transcription. These results suggest that neural and immune mediators may promote the progression of HIV infection in the brain and contribute to neuropathology of AIDS.

667.13

FRAGMENTS OF THE ENVELOPE PROTEIN (GP120) FROM THE HUMAN IMMUNODEFICIENCY VIRUS ARE NEUROTOXIC TO CNS CULTURES

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Previous studies have shown that purified envelope protein (gp120) from the human immunodeficiency virus produces neuronal cell death in hippocampal cultures derived from fetal mice (Brenneman et al., *Nature* 280: 345, 1988). Subsequent studies have indicated that cortical neurodystrophy and delays in developmental milestones occur in developing rats administered gp120 (Hill et al., *Soc. Neurosci. Abstrs.* 16: 615, 1990). Purified gp120 was radiolabeled and injected subcutaneously into one day old rats. The pups were either frozen and sectioned for *in vivo* autoradiography, or the brains were homogenized and assayed by FPLC for the presence and distribution of labeled gp120 and its proteolytic fragments. *In vivo* autoradiography revealed that radioactive material reached the brain and diffused into adjacent brain tissue. FPLC analysis revealed four major peaks of radioactivity. The chromatographic fractions corresponding to these peaks were assayed for neurotoxicity on dissociated cerebral cortical cultures. All four peaks exhibited neurotoxic activity that could be prevented by co-treatment of the test cultures with 1 nM peptide T. These data suggest that low molecular weight (<800 Dalton), neurotoxic gp120 fragments may play a role in the etiology of "NeuroAIDS".

667.14

EARLY DETECTION OF PROGRESSIVE CNS INVOLVEMENT IN HIV INFECTION. K.L.Coburn*, N.C.Moore, H.P.Katner, K.A.Tucker, W.S.Pritchard[1], D.W.Duke[2]. Mercer Univ. School of Medicine, Macon, GA 31207; [1]R.J. Reynolds Tobacco Co.; [2]Florida State Univ.

AIDS often is accompanied by progressive encephalopathy resulting in 'subcortical' dementia, but it is uncertain how early in HIV infection the brain involvement may begin. This study recorded EEG and ERP (auditory oddball) data from healthy controls and from patients at worsening stages of HIV infection. Neither digital frequency analysis nor nonlinear dynamical (chaos) analysis of the EEG showed differences between healthy controls and any patient group. ERP sensory components also did not differ between groups, but cognitive components showed progressive delays and amplitude reductions corresponding to increasingly severe clinical stages of HIV infection. The earliest changes were among asymptomatic HIV+ patients, suggesting that this test is a sensitive indicator of early subclinical CNS damage and may be of value in decisions regarding early aggressive antiviral therapy and in monitoring its effectiveness.

667.15

ENHANCED CELL MEMBRANE TRANSPORT AND ANTI-HIV-1 ACTIVITY OF ANTI-rev ANTIBODIES FOLLOWING CATIONIZATION. **W.M. Pardridge, U. Bickel, J. Buciak, J. Yang, and C. Markham*. Departments of Medicine and Neurology and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA, 90024.**

The replication of the human immunodeficiency virus (HIV) in acquired immune deficiency syndrome (AIDS) is dependent on the function of the rev protein. In order to develop anti-rev antibodies capable of transport across the brain capillary barrier, i.e., the blood-brain barrier (BBB), and into lymphocyte intracellular spaces, a rabbit polyclonal antiserum directed against a 16-amino acid synthetic peptide encompassing the active site of the rev protein of HIV-1 was prepared. The antibody fraction was affinity purified, and cationized with hexamethylenediamine and carbodiimide. The isoelectric point (pI) of the native anti-rev antibodies was 5.2 and the pI of the cationized anti-rev antibodies averaged 8.5 based on isoelectric focusing studies. The cationization was performed with site protection using synthetic peptide, and radioimmunoassay experiments showed that the affinity of the anti-rev antibody for the synthetic peptide was virtually unchanged following site protected cationization. The cationized and native anti-rev antibodies were radiolabeled and the cationized antibodies were found to undergo enhanced uptake into either human lymphocytes or bovine brain capillaries as compared to the native anti-rev antibodies which showed minimal cellular uptake. Cationized anti-rev antibodies, but not native anti-rev antibodies or cationized nonspecific antibodies, resulted in a 36% decrease in ^{3}H-thymidine incorporation in the human lymphocytes, but a 90% decrease in HIV-1 replication in human peripheral blood lymphocytes grown in primary tissue culture.

667.16

INHIBITION OF GP120 BINDING TO GALACTOSYL CERAMIDE AND HIV INFECTION BY POTENTIAL ANTI-HIV COMPOUNDS. S. Bhat[@], T. Otsuka[#], A. Srinivasan[#] and D.H. Silberberg[@], [@]Dept. Neurol. Univ. Penn. Med. Ctr. and [#]The Wistar Institute, Philadelphia, PA 19104.

In an attempt to find compounds capable of inhibiting the binding and infection by HIV in neural cells, we studied the effect of benzopurpurin and related compounds on the binding of gp120 to GalCer and sulfatide. By using an HPTLC binding assay, we show that the binding of gp120 to GalCer and sulfatide is inhibited by benzopurpurin and related compounds. These compounds also inhibit the binding and entry of HIV into the neural cell line, SK-N-MC. We also show that this method can be used for screening of potential anti-HIV compounds.

668.1

METABOLIC CHANGES IN CARDIAC AND SKELETAL MUSCLE OF DYSTROPHIC HAMSTERS WITH HYPERTROPHIC CARDIOMYOPATHY. P.L. Johnson, R.K. Handa, M.P. Gupta, and S.K. Bhattacharya*, Surgical Research Lab, University of Tennessee, Memphis, TN 38163.

Membrane-mediated excessive intracellular Ca accumulation plays a fundamental pathogenetic role in hereditary muscular dystrophy. To study the dystrophic muscle metabolism, glycogen, glucose-6-phosphate (G6P), hexokinase (HK), G6PDH, pyruvate, lactate, and creatine phosphate (CrP) were quantitated in ventricular myocardium (VM) and rectus femoris (RF) muscle of CHF-148 normal albino hamsters (NH) and CHF-146 dystrophic hamsters (DH) with hypertrophic cardiomyopathy. We observed a marked decrease in glycogen, G6P and CrP, concomitant with a significant increase in HK and G6PDH in VM and RF of DH. The stimulation of HK and G6PDH, with reduced glycogen and G6P, highlighted the shift of muscle metabolism in DH towards the pentose shunt pathway (PSP). Unaltered pyruvate and lactate levels, together with lower G6P in dystrophic RF (79%) and VM (70%), strongly suggest that the metabolic path in DH is selectively favored towards PSP for the generation of NADPH, reentering the glycolytic pathway at the site of glyceraldehyde-3-phosphate, and thus conserving one ATP in the process. A 75% decrease of CrP in dystrophic VM, compared to a 22% decrease of ATP, suggests that CrP acts as a buffering system for the maintenance of ATP. These findings concur with our report of decreased cellular energy charge in DH (Soc. Neurosci. Abstr., 17:1609,1991). We conclude that muscle metabolism in DH is directed towards the conservation and generation of ATP through the CrP/Cr system, due to an increased energy demand to pump excessive intracellular Ca^{2+} from degenerating myofibers. **(Supported by NIH Grant R01-AR38540)**

668.2

ROLE OF ADP PHOSPHORYLATING, GLYCOLYTIC AND KEY MITOCHONDRIAL ENZYMES IN CELLULAR ENERGETICS OF CARDIAC AND SKELETAL MUSCLE OF DYSTROPHIC HAMSTERS WITH CARDIOMYOPATHY. S.K. Bhattacharya, P.L. Johnson*, M.P. Gupta, and R.K. Handa, Surgical Research Lab, University of Tennessee, Memphis, TN 38163.

Generation of energy rich biomolecules such as ATP and creatine phosphate (CrP) is crucial for physiologic functioning of heart and skeletal muscle. Because of our recent findings of diminished ATP and CrP levels in the cardiac and skeletal muscle of CHF-146 strain dystrophic hamsters (DH) with hypertrophic cardiomyopathy and hereditary muscular dystrophy, we investigated some of the key enzymes of ADP phosphorylation, glycolytic pathway, Krebs cycle, and mitochondrial respiratory chain. Biochemical assays were performed in post 10,000g supernatants of ventricular myocardium (VM) and rectus femoris (RF) muscle of CHF-148 normal albino hamsters (NH) and DH. In dystrophic VM, a significant decrease in the activity of glycolytic enzymes like pyruvate kinase (PK), aldolase (ALD) and lactate dehydrogenase (LDH) was observed by 29%, 39% and 37%, respectively. Activity of creatine phosphokinase (CPK) was reduced by 37% in dystrophic VM. Several mitochondrial key enzymes such as succinate dehydrogenase (SDH), citrate synthetase (CS), NADH-cytochrome-*c*-reductase (NCCR), succinate cytochrome-*c*-reductase (SCCR) and NADH ferricyanide reductase (NFR) were also reduced in dystrophic VM by 34%, 31%, 27%, 52% and 19%, respectively. Similarly, dystrophic RF revealed significantly lower PK (34%), LDH (29%), ALD (47%), CPK (33%), adenylate kinase (33%), SDH (37%), CS (29%), NCCR (48%), SCCR (21%) and NFR (23%), compared to NH. These data strongly suggest a severe impairment of glycolytic pathway, as well as mitochondrial energy metabolisms at the level of the Krebs cycle and electron transport chain system in dystrophic VM and RF, contributing to marked depletion of energy rich biomolecules and progressive muscle wasting in DH. **(Supported by NIH Grant R01-AR38540)**

668.3

VIMENTIN (VM) mRNA EXPRESSION IN NORMAL & DISEASED HUMAN SKELETAL MUSCLE. A.K.Misra, N.K.Menon*, S.S.Schreiber and W.K.Engel. Neurology Svc, VA Outpatient Clinic & Dept.of Neurology, USC Sch. of Med, Los Angeles, CA 90013.

Intramyofiber VM, a cytoskeletal intermediate filament protein, is expressed in fetal myotubes during myogenesis, presumably important in their early development. As maturation proceeds, desmin replaces VM in the myofibers. In normal mature muscle, VM reappears in myofibers regenerating following injury or disease. We studied VM mRNA expression in biopsied muscle samples of normal(1), Duchenne muscular dystrophy(DMD,1), scapulohumeral muscular dystrophy(SHD,1), myotonic muscular dystrophy(MyD,1), sporadic adult-onset myotubular myopathy(SAOMM,1) and polymyositis(PM,2). 10u cryosections were hybridized in situ with a 35-S labeled cRNA probe and analysed by emulsion autoradiography. Serial sections were stained with the modified trichrome. All samples showed VM mRNA in Schwann cells, fibroblasts and blood vessels. In normal and MyD, no intramyofiber VM mRNA was seen. In contrast, intramyofiber VM mRNA was seen in 1) regenerating-degenerating myofibers in PM, DMD and SHD., 2) a few myofibers with central nuclei in SAOMM. VM reexpression in diseased myofibers suggests a VM-associated regenerative phenomenon, which may be amenable to therapeutic manipulation.

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668.4

SELECTIVE ACTION OF INTRATHECAL BACLOFEN IN SPASTIC PARESIS. G.L. Almeida, M.L. Latash, R.D. Penn*, D.M. Corcos, and G.L. Gottlieb. Universidade Estadual de Campinas/Conselho Nacional de Pesquisa, Campinas, SP 13100, Brazil, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612, University of Illinois, Chicago, IL 60612.

Effects of intrathecal baclofen were studied in six patients with spasticity due to spinal cord injury and two subjects with hemi-syndromes and spasticity due to brain trauma. Kinematic and EMG patterns were recorded during attempts at single-joint elbow or ankle voluntary movements and isometric contractions. Reflex responses to passive ankle dorsiflexion and Babinski's reflex were also recorded. Intrathecal injection of baclofen effectively suppressed spastic signs in all the spinal cord injury patients. Suppression of muscle tone and exaggerated reflexes was accompanied by a general improvement of the patterns of muscle activation. This involved the elimination of clonus and a decrease in the co-contraction of antagonist and distant muscle groups. In one of the brain trauma patients, baclofen suppressed the spastic signs and H-reflexes bilaterally. Voluntary movements in his "bad side" improved, while movements in the "good side" remained unchanged. Baclofen was ineffective in another brain trauma subject. It did not induce any negative changes in the voluntary motor performance in both "good" and "bad" sides of this subject. The subject displayed similar peak speeds, torques, and EMG patterns before and after the drug. We hypothesize that adaptive changes in the spinal cord to a traumatic lesion include an increase in the number and/or affinity of the GABA-ergic receptors leading to the selective action of intrathecal baclofen.

668.5

THE PROGRESSION OF MOVEMENT DISORDERS IN PATIENTS WITH RSD. C.J. Hunker* and M. Backonja. Dept. of Neurology, U. of Wisconsin, Madison, WI 53792.

Neuropathic pain syndromes with sympathetic involvement traditionally known as reflex sympathic dystrophy (RSD) often present associated muscle weakness, tremor, dyskinesia, dystonia, and synkinesia as primary movement disorders in affected limbs (Schwartzman & Kerrigan, 1990; Hunker and Backonja, 1991). These data supported a CNS pathogenesis; a formerly contested issue. The progression of pain features to 'non-affected' body parts has been anecdotely reported in humans and demonstrated empirically in animal models. A similar course for movement control aberrations has not been investigated. Maximal and controlled voluntary contractions were examined in 25 RSD patients via isometric wrist and thumb/index finger tasks along with accelerometer recordings from pain-affected and non-affected limbs at six to twelve month intervals. In each case, non-affected limbs weakened over time even in the absence of pain and tended to follow the same muscle weakness distribution as the pain-affected extremity. Muscle force control abnormalities in the form of tremor, dyskinesia, and dystonia were also recorded from contralateral limbs in 60% of the patients. The progressive involvement of contralateral limbs with the RSD-associated movement disorders further support the putative CNS pathophysiological substrate.

668.6

MYASTHENIA GRAVIS PATIENT PLASMA REDUCES DEPOLARIZATION-DEPENDENT UPTAKE OF CALCIUM INTO ISOLATED NERVE TERMINALS OF THE RAT. S.J. Hewett*, A.M. McLane, and W.D. Atchison, Dept. Pharmacol./Toxicol. and Neuroscience Program. Michigan State Univ. E. Lansing, MI 48824.

Myasthenia gravis is an autoimmune, neuromuscular disease, in which the postjunctional nicotinic acetylcholine receptor is generally considered to be the pathogenic site. It has generally been assumed that disruption of presynaptic processes contributes little to the muscle weakness in these patients. Recent evidence indicates that some patients with myasthenia gravis also have anti-presynaptic membrane receptor antibodies for somatic motor nerves (*J. Neurol. Sci.* 102, 39-45, 1991). We discovered unexpectedly that plasma from a patient with myasthenia gravis reduced $^{45}Ca^{2+}$ uptake into isolated nerve terminals. The present study was thus undertaken to determine whether acute application of plasma from several patients with myasthenia gravis would reduce depolarization-dependent uptake of $^{45}Ca^{2+}$ into rat cortical synaptosomes. Net influx of ^{45}Ca during 10 sec of K^+-induced depolarization (42.5 mM KCl) was significantly reduced by plasma from three patients with myasthenia gravis as compared to their non-disease plasma-treated paired control. Conversely, uptake of $^{45}Ca^{2+}$ in the presence of non-depolarizing concentrations of KCl (5mM) was not reduced significantly by plasma from the myasthenic patients. Lactate dehydrogenase release from synaptosomes incubated with plasma from patients with myasthenia gravis was not increased over controls indicating the effect was not due to decreased synaptosomal viability. These studies, to our knowledge, are the first to demonstrate direct alterations in nerve terminal function following incubation with myasthenic plasma. Supported by the Muscular Dystrophy Association, the Myasthenia Gravis Association- Detroit Chapter and NIH grant NS20683. WDA is supported by RCDA K04-ES00178.

668.7

POSTURAL STABILITY AND VARIABILITY IN TARDIVE DYSKIENSIA.
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Stereotypic motions are a characteristic feature of tardive dyskinesia (TD). These motions can arise in a variety of tasks including bi-pedal posture. This study examined the structure of the center of pressure profile in 3 groups (rhythmical TD, non rhythmical TD, normal control) of trials. Our findings suggest that: a) there is structure to the usually construed random pattern of the "normal" center of pressure; b) that some of the *developmentally disabled tardive dyskinetic subjects* exhibited a rhythmical center of pressure pattern that is consistent with a limit cycle attractor organization; c) normal center of presure has a higher dimensionality; and d) that variability of the center of pressure can only be interpreted as reflective of stability when the same attractor dynamics are evident in organizing the posture. These findings suggest: (1) that center of pressure variability needs to be interpreted in relation to dimensionality of the supporting dynamic; (2) significant limitations to traditional interpretations of the relation between center of pressure variability and posture stability; and (3) new ways to analyze human postural dynamics.

668.8

BROAD A BAND DISEASE: A NEW CONGENITAL MYOPATHY ASSOCIATED WITH LEBER'S CONGENITAL AMAUROSIS.
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A two year-old boy with Leber's congenital amaurosis displayed diffuse hypotonia with delayed motor milestones, depressed deep tendon reflexes and normal sensation. Histological and histochemical evaluation of biopsied thigh muscle showed no abnormality. One-μm plastic sections and electron microscopy showed numerous foci of broadening or smearing of the A band with loss of a distinct I band. Z-lines were normal except for a fine waviness in these areas. The inter-Z-line sarcomere had a staining density intermediate between normal A and I bands, and the thick filaments in these lesions appeared misaligned. The smaller lesions involved a single sarcomere of a single myofibril, while larger lesions extended laterally across 3-4 myofibrils and longitudinally along 3-4 sarcomeres. There were 24 such lesions in 10 random 3400 μm^2 fields, but none in 9 other hypotonic patients with "unstructured" myopathies. These findings suggest an abnormality of the M-line or of the structural protein connectin. These findings differ from those of previously-described congenital myopathies, and represent the first described morphological abnormality of muscle in a patient with Leber's congenital amaurosis.

668.9

ULTRASTRUCTURAL PATHOLOGY OF SKELETAL MUSCLE IN PROGRESSIVE SYSTEMIC SCLEROSIS.
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Upon careful examination the majority of patients with progressive systemic sclerosis (PSS) are found to have muscular proximal weakness and wasting, elevation of plasma level of muscle enzymes and alterations in EMG. Using light microscopy two different pathologic entities have been described, simple myopathy and myositis. Ultrastructural studies on the muscle lesions have emphazised the vascular changes. In this work we report the whole spectrum of changes observed by electron microscopy in the study of needle muscle biopsies from five patients with positive diagnoses of PSS and a muscle compromise.The changes observed were: a varied degree of fiber atrophy, fiber necrosis, mitochondrial alterations, vacuolation of sarcotubular system, lysosomal proliferation, presence of filamentous and concentric laminated bodies. Capillary alterations included hypertrophy of endothelium with lumen occlusion, autophagic vacuoles and widening and reduplication of basement membrane. Cell infiltration included lymphocytes , macrophages and mast cells. This work indicates a multifactorial pathogenesis, neural, vascular and autoimmune for the muscle damage in PSS.

This work was supported by CDCH of UCV (03-2709/92), Fundación Polar and The British Council Venezuela).

668.10

ELECTROMYOGRAPHIC CHANGES COMPARING MEDIAL AND LATERAL SURGICALLY TRANSFERRED RECTUS FEMORIS MUSCLE IN CHILDREN WITH CEREBRAL PALSY
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Children with cerebral palsy characteristically present with muscle contractures which result in gait abnormalities including excessive hip flexion, and internal hip rotation and adduction. Surgical procedures on rectus femoris muscles may be performed to correct these problems. The purpose of this procedure is usually to reduce the effect of the rectus femoris on hip flexion and reduce the internal rotation and adduction of the hip. The desired result is a reduction in the over activity of the spastic muscles for improved range of motion. Rectus femoris muscle transfer procedures include release and reattachment either medially to the sartorious or laterally to the iliotibial band. The purpose of this study was to examine the electromyographic (EMG) and dynamic knee flexion changes following surgical transfer of the rectus femoris medially or laterally. Rectus femoris EMG and percentage time to peak knee flexion were the critical variables observed. EMG was collected through a telemetry system. Dynamic range of motion was recorded using a VICON system with six cameras. Data were collected pre- and post-operatively and analyzed and plotted using a PDP 11/73 computer. Sixty-six spastic hemiplegic, diplegic or quadriplegic patients (4.1 to 18.1 years of age) were observed allowing analysis of 119 lower limbs. A 2 x 2 (type of surgical procedure by pre- and post-surgical gait measures) analysis of variance was performed for percentage of rectus femoris EMG and time (as percentage of gait cycle) to peak knee flexion. A .05 significance level was used for all comparisons. Significant interactions for type of procedure by pre-post rectus femoris EMG, $F(1,117) = 4.8$, $p > .05$, and time to peak flexion, $F(1,117) = 8.9$, $p = .004$, were revealed. Although neither average percentage of EMG nor time to peak flexion was reduced to normal values using either procedure the medial transfer procedure produced more favorable average outcome than the lateral transfer procedure.

668.11

SPONTANEOUS RIGIDITY RECORDED IN A MUTANT RAT (TAIEP) WITH IMMOBILITY EPISODES.
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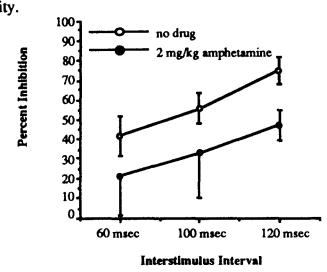
Here we tested whether the immobility episodes of TAIEP rats were associated with muscular rigidity in both flexor and extensor muscles, and whether the rigidity was related with a decrease in striatal dopamine (DA). To verify this, the spontaneous electromyographic activity of the Gastrocnemius-Soleus (GS) and Tibialis-Anterior (TA) muscles was recorded in the mutant rats (n=4), and also, during the immobility episodes. DA was assayed by HPLC. Both muscles showed spontaneous tonic activity periods lasting more than 8 min., in duration. The average firing frequency for GS was of 21.2±1.1 Hz and for TA, 38.6±3.5 Hz. This tonic activity was not detected in any animal of the control group (n=5). During the immobility the firing frequency of the muscular fibers increased to more than 100 Hz, returning to basal levels afterwards. The high firing frequency was observed only during the immobility episodes. DA content in the mutant rats (n=6) was 55% higher than the age-matched controls (n=6). In conclusion, the immobility episodes were associated with muscular rigidity in both flexor and extensor muscles, but the rigidity was not accompanied by a decrease in striatal DA.

669.1

AMPHETAMINE DISRUPTS PREPULSE INHIBITION OF THE ACOUSTIC STARTLE REFLEX: A REPLICATION D.A. Ott* A.J. Diehl, and R.J. Mandel. Dept. of Psychology, Univ. of Illinois, 603 E. Daniel St., Champaign, IL 61820

It has been reported that amphetamine inhibits the ability of rats to use a low amplitude warning stimulus (prepulse) to inhibit their acoustic startle response. It appears that the prepulse inhibition phenomenon can be modulated by dopamine agonism specificially in the nucleus accumbens and schizophrenic humans show a similar pattern of deficit in the same task (for review see Geyer et al., *Brain Res. Bull.* 25:485-498, 1990). Here, we report a replication of one of Geyer et al.'s findings; that amphetamine (AMPHET) disrupts prepulse inhibition. The figure below represents the data from 8 normal female Sprague-Dawley rats ± 2 mg/kg AMPHET sulfate i.p. These data are reported to demonstrate that the effect of systemic AMPHET can be obtained in a different laboratory using an entirely different appartus but similar experimental parameters. Dose-response experiments are underway to compare the AMPHET ED_{50}'s for prepulse inhibition versus locomotor activity.



669.2

A QUANTITATIVE TECHNIQUE FOR COMPARISON OF GYRAL AND SULCAL PATTERNS FROM MRI BRAIN SURFACE RENDERINGS. A. J. Bartley,* D. W. Jones, D. R. Weinberger. Clinical Brain Disorders Branch, IRP, NIMH, Washington, DC 20032.

High-fidelity surface rendering algorithms applied to finely spaced (1.5mm) GRASS MRI scans produce faithful representations of the gyral and sulcal patterning of the brain *in vivo*. Typically visual assessment and rating are used to compare these renderings. We have explored the possibility of employing 2-dimensional cross-correlation techniques to obtain quantitative measures of similarities between brain renderings. The cross-correlation method generates a 2-D map of a similarity measure at all possible relative offsets of two images. The peak of this map gives both the relative position of maximal similarity and an objective measure of the magnitude of this similarity on a scale of -1.0 to +1.0.

We have applied this procedure to surface renderings from brain MRI's of normal twins. Surface rendering and cross-correlation calculations were performed on a MacIIci with readily available software. The cross-correlation maps were computed by means of a fast Fourier transform (FFT) algorithm. Our results indicate that the cross-correlation technique is reliable and useful for comparing gyral and sulcal patterns between brain regions and across individuals. The cross-correlation results were found to discriminate twin pairs from unrelated individuals more accurately than visual assessment of the same rendered images by 8 raters experienced in MRI analysis. This method may also have broader application in comparing images derived from a variety of modalities, e.g., microscopy, autoradiography, PET, etc.

669.3

ERYTHROCYTE TRANSKETOLASE ACTIVITY IN DEPRESSION. B.I. Diamond, M.F. Casanova, R.L. Borison* and T.H. Nguyen. Med. Coll. of Georgia, Augusta, GA 30912-3800.

Although Beriberi and Wernicke's serve as primordial examples of thiamine deficiency, the symptoms observed in these conditions manifest themselves only after a severe and protracted vitamin B1 depletion. Fatigue is the most common symptom in those cases with mild thiamine deficiency. It is therefore noteworthy that both thiamine deficiency as well as fatigue are frequently seen in the psychiatric patient population. This study explores for the presence of thiamine deficiency in depression, a disease often characterized by loss of energy and chronic tiredness. Thirty-one patients with major depression (DSM IIIR criteria) and 13 age-matched controls participated in the study. Physical examination and laboratory screening tests were used to identify any concomitant medical conditions and confounding nutritional abnormalities. Erythrocyte hemolysates were analyzed spectrophotometrically for the activity of NADH-dependent transketolase both in the presence (TPP) and absence (TK) of thiamine pyrophosphate. Repeated blood withdrawals were performed for a total of 150 assays. No significance between group differences were found for either the TK activity or the TPP effect (t test, $p>0.05$). Our results suggest that fatigue in major depression may be related more to the dysphoric mood than to a nutritional deficiency.

669.4

MANIA: RESPONSE TO LITHIUM ACROSS THE AGE SPECTRUM R.C. Young*, B. Kalayam, G. Tsuboyama, P. Stokes, S. Mattis, and G.S. Alexopoulos. New York Hospital-Cornell Medical Center, Westchester Division, White Plains, NY 10605.

Manic states are relatively common among geriatric psychiatric inpatients as well as among younger adult patients. While lithium salts are the first line treatment across the age spectrum, the extent to which optimum treatment conditions change with age has not been adequately investigated. We have therefore begun to study geriatric and younger adult inpatients treated with lithium salts. Twenty-four (n=24) inpatients meeting Feighner criteria for mania were monitored prospectively. They ranged in age from 23 to 89 years and were predominantly female. Manic Rating Scale (MRS) scores declined over 3 weeks of lithium treatment. Greater decrements in MRS were associated with higher plasma lithium levels ($p<.02$). Patients with lower Dementia Rating Scale scores had higher MRS scores ($p<.02$). These preliminary findings suggest that concentration-effect relationships for lithium exist across the age spectrum. The implications of cognitive dysfunction for management of manic psychopathology require further study.

(MH42522-01A2)

669.5

IPRINDOLE TREATMENT INCREASES EXTRACELLULAR CONCENTRATIONS OF NA AND DA BUT NOT 5-HT IN THE PREFRONTAL CORTEX : EVIDENCE THAT IPRINDOLE INCREASES AVAILABILITY OF SYNAPTIC CATECHOLAMINE IN VIVO. T. Koyama*, M. Abe, T. Ohmori, S. Matsubara and I. Yamashita. Dep. of Psychiatry and Neurology, Hokkaido Univ. Sch. of Med., Sapporo, 060 Japan.

The effects of systemic administration of iprindole, clinically effective antidepressant which dose not inhibit amine uptake or exhibit monoamine oxidase inhibitor activity in vitro, on the in vivo extracellular concentrations of NA, DA and 5-HT were examined by brain microdialysis in the medial prefrontal cortex of freely moving rats. Iprindole treatment induced substantial increases in NA and DA outputs in a dose-dependent manner within the range of iprindole tested (25-50 mg/kg). The 5-HT efflux did not change after iprindole administration. The results suggest that iprindole increases availability of synaptic NA and DA in vivo by the different mechanism from the inhibition by itself of amine transport or catabolism. Therefore the results are consistent with the possibility that iprindole enhances the release mechanism of NA and DA, or that there are unknown metabolities of iprindole with amine uptake-or catabolism-blocking potential. Although further study is needed to clarify these possibilities, this finding is consistent with the hypothesis that antidepressants that increase synaptic NA or DA levels cause

669.6

BLUNTED DESENSITIZATION OF EPINEPHRINE-INDUCED PLATELET AGGREGATION IN DEPRESSED PATIENTS. J.E. Piletz* and D. Chikkala. Dept. of Psychiatry, Case Western Reserve Univ. & MetroHealth Med Center, Cleveland, Ohio 44109.

Elevated radioligand binding to platelet *high affinity* α2-adrenoceptors ($\alpha 2AR_H$) has been reported in depression. Herein we determined if depressed patients also (1) exhibit hypersensitivity to epinephrine-induced platelet aggregation, and/or (2) differ from controls in their desensitization of epinephrine aggregation in vitro. Desensitization is an important cellular regulatory response, and dysregulation of receptor function has been proposed in depression. Incubating unstirred platelets with 20 uM epinephrine desensitizes subsequent aggregation when the platelets are stirred. *No changes* in undesensitized aggregation were observed in depressed patients (n=20) vs. healthy controls (n=18). Moreover, there were no significant correlations between undesensitized aggregation and pre-epinephrine $\alpha 2AR_H$ Bmax (p^{125}I-clonidine saturations). By contrast, the extent of epinephrine-induced desensitization was *blunted* in depressed patients ($p \leq .05$ at 4,20,30 and 60 min post-epinephrine) and the $extent_{max}$ of desensitization was negatively correlated with the pre-epinephrine $\alpha 2AR_H$ Bmax ($r=-.48$, $p=.02$). Blunted desensitization in depression was most apparent during the first 0.5-2 min. post-epinephrine before stirring was initiated; in patients the extent of aggregation actually increased slightly during this period. Thereafter, a normal time course of desensitization was observed in depressed patients; the mono-exponential decay in epi-aggregation was not different in depressed patients vs controls ($t_{½}$ avg = 10.6 min vs 7.8 min). The data suggest that blunted receptor desensitization might give rise to up-regulated platelet $\alpha 2AR_H$ binding sites in depression. If blunted desensitization also occurs in the brain, this could underlie certain aspects of depression.

669.7

AUDITORY BRAINSTEM RESPONSE IN GERIATRIC DEPRESSION. B. Kalayam MD, CA Shamoian MD*, RC Young MD. Cornell Univ Medical College, New York, NY.

Depressed geriatric patients with onset of major depression in late-life (LOD) have more auditory disturbances compared to those with onset of depression in early-life (EOD) and elderly controls (EC) [Int. J. Geriatr. Psychiat 6:131-136;1991]. It has been proposed that central auditory changes may account for increased auditory disturbances in LOD patients. The findings from an investigation using auditory evoked response to examine lesion-sites is reported.

Nineteen subjects consisting of LODs (n=7), EODs (n=7) and ECs (n=5)have been studied. Differences were found between groups on rate-dependent latency shifts during ABR testing when stimulus rate was increased from 11 to 80 clicks/sec. LODs had greater increase for interwave intervals I-V and III-V compared to EODs ($p < .005$; ANOVA) and ECs ($p < .035$; ANOVA).

In neurologic disorders, abnormalities in rate dependent latency shifts on ABRs are attributed to occult degenerative changes in brainstem auditory pathways. Our preliminary findings suggest that: 1) LODs can be distinguished from EODs and ECs on physiologic measures of auditory brainstem activity, and; 2) central auditory changes may correlate with depressive features reported in elderly patients with sensorineural hearing loss.

669.8

●PLASMA MHPG IN MAJOR DEPRESSIVE DISORDER:DIFFERENCES BETWEEN RESPONDERS AND NONRESPONDERS.
●K.MINE [a], M.OKADA [a], M.NIWA [b]* and M.FUJIWARA [a].
●Psychosom.Med.,Fac.of Med.,Kyushu Univ., Fukuoka, [a]Dep.of Physiol. and Pharmacol.,Fac.of Pharmac.Sci.,Fukuoka Univ., [b]Dep.of Pharmacol., Fac.of Med.,Nagasaki,JAPAN.

The plasma levels of free(f-) and sulfo-conjugated(s-) forms of both norepinephrine(NE) and 3-methoxy-4-hydroxyphenylglycol(MHPG), both before and after treatments, were examined in patients with major depressive disorders without melancholia. In 11 patients showing a good response to treatment(responders), the plasma s-MHPG levels were significantly reduced after treatment, while no significant changes were seen in 9 patients showing no response to treatment(nonresponders). On the contrary, the plasma s-MHPG levels in 6 patients who had been suffering from somatic diseases tended to increase after treatment. The plasma f-MHPG levels showed no significant changes after treatment in the responders, the nonresponders and the somatic disease group. The plasma f- and s-NE levels revealed no significant differences between each group. The plasma s-MHPG levels may be related to the treatment outcome of major depressive disorder.

669.9

CEREBRAL CORTEX G-PROTEINS AND cAMP FORMATION IN BIPOLAR AFFECTIVE DISORDER. L.T. Young*, P.P. Li, S.J. Kish, K.P. Siu, A. Kamble, O. Hornykiewicz, and J.J. Warsh. Clarke Institute of Psychiatry, Toronto, Ontario, Canada, M5T 1R8.

Substantial data from experimental animal and peripheral blood cell studies have pointed to a disturbance in transmembrane signal transduction at the level of the guanine nucleotide regulatory (G) protein in bipolar affective disorder which may be corrected with lithium treatment. Given the potential relevance of such disturbances in this disorder, we estimated brain G-protein levels and adenylyl cyclase activity directly in various brain regions from subjects with bipolar affective disorder. Membrane G-protein ($G_s\alpha$, $G_{i(1\&2)}\alpha$, $G_o\alpha$ and $G\beta$) immunoreactivities were estimated by Western blotting in postmortem brain regions obtained from 10 patients with bipolar affective disorder and 10 age and sex-matched controls. To examine whether there were functional correlates to the observed elevated $G_s\alpha$ levels, basal, GTPγS- and forskolin-stimulated cAMP production were determined by radioimmunoassay. Compared with control samples, $G_s\alpha$ (52 kDa species) immunoreactivity was significantly ($p<0.05$) elevated in prefrontal (+38%), occipital (+80%) and temporal (+62%) cortex but not in hippocampus (+24%), thalamus (-23%) or cerebellum (+22%). In contrast, no significant differences were found in the other G-protein subunits measured. Forskolin-stimulated cAMP production was significantly increased in temporal (+31%) and occipital (+96%) cortex. GTPγS-stimulated cAMP production was increased to a similar extent but failed to reach statistical significance. A significant correlation ($r=0.60$) was observed between forskolin-stimulated cAMP formation and $G_s\alpha$ (52 kDa) immunoreactivity when examined across these cortical regions. The findings of increased cerebral cortical $G_s\alpha$ subunit levels and adenylyl cyclase activity point to an important disturbance at the level of the G-protein in bipolar affective disorder which may explain the pathophysiology of this condition as well as the mechanism of action of lithium treatment.

669.10

PHASE DELAY AND DISORDERING OF NORADRENERGIC DIURNAL PATTERNS IN DEPRESSION. E.M. DeMet*, J. Piletz, H. Gwirtsman, and A. Halaris. Depts. Psychiatry, Univ. Calif. Irvine, CA 92717, Case Western Reserve Univ., Cleveland, OH 44109, and NIMH, Washington D.C., 20852.

Diurnal patterns of the noradrenergic metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) were studied in the plasma of 43 depressed patients and 12 controls. Daily rhythms were adequately fit by a sinusoidal function (24 hr fixed period) in both groups although the unexplained variance in patients was significantly greater than in controls. Disordered rhythms, while characteristic of the depressed patients, showed no evidence of state dependency. Peak MHPG levels, in controls, occurred at 2:54 PM. Patient values peaked 1.81 hr earlier than controls and this difference was significant. No group differences were found in either the time independent average levels or sinusoidal amplitudes of the MHPG rhythms. The effects of chronic desipramine treatment on diurnal patterns were examined in a subset of patients. No significant change was found in any variable after treatment regardless of the degree of clinical response. The results suggest that depressed patients typically have aberrant circadian noradrenergic regulation and to a lesser extent either an increased zeitgeber sensitivity or decreased circadian period. None of these abnormalities, however, appear to be directly linked to changes in mood state.

669.11

DISCRIMINATORY CAPABILITY OF PET MEASUREMENTS OF REGIONAL BLOOD FLOW IN FAMILIAL PURE DEPRESSIVE DISEASE. W.C.Drevets*, E.L. Spitznagel, A.K.MacLeod, M.E. Raichle. Washington Univ. Sch. of Med., St. Louis, MO 63110.

We reported increased blood flow (BF) in the left prefrontal cortex, amygdala, and medial thalamus, and decreased BF in the l. medial caudate in familial pure depressive disease (FPDD), a subtype of unipolar major depression (Drevets et al., J Neurosci., 1992). This and other evidence suggested a circuit involving the prefrontal cortex, amygdala, and related parts of the striatum, pallidum, and thalamus is involved in the pathophysiology of FPDD. Discriminant analysis of the covariance of activity in these structures correctly categorized FPDD subjects (n=9) in 89% of cases and controls (n=24) in 92% of cases (Wilks' lambda=.42, F=9.5, p<.0001). Of 4 additional subjects with FPDD (not previously studied) this covariance matrix classified 3 as FPDD. Of 8 bipolar depressives one was classifed as FPDD and 7 as normal. However, 3 of 6 normals scanned while thinking sad thoughts were incorrectly classified in the FPDD category. In the "sad thoughts" relative to the "rest" scan, BF increased in the l.prefrontal cortex and medial thalamus, but decreased in the amygdala (Wilks' lambda=.000012, F=35, p<.0001. This contrasts with FPDD, where amygdala BF is increased. If this experimental task reflects the cognitive and emotional state in FPDD, a possible explanation for these data is that abnormal modulation of the amygdala exists in FPDD.

669.12

PRENATAL AMPHETAMINE EXPOSURE INCREASES THE NUMBER OF NEURONS POSITIVE FOR NITRIC OXIDE SYNTHASE (NOS) IN THE LATERODORSAL TEGMENTAL NUCLEUS OF THE RAT. M. Nielsen, M. Lyon,* and W. O. McClure. Dept. of Biological Sciences, Univ. Southern California, Los Angeles CA 90089-2520, and *Dept. Psychiatry and Behavioral Sciences, Univ. of Arkansas for Medical Sciences, Little Rock AR 72205.

A substantial amount of evidence suggests that at least some of the schizophrenia(s) are related to errors in fetal development. To test the fetal development hypothesis we have exposed rat pups *in utero* to dopaminergic agonists and have examined the behavior and neuroanatomy of these animals when they mature to young adulthood. NOS-positive cells in the laterodorsal tegmental nucleus (LDT) were of interest, since these control phases of sleep which are deficient in schizophrenics. Twenty-three pregnant Wistar rats were injected on d 11-14 of pregnancy with d-amphetamine sulfate (5 mg/kg) or saline. Pups at 45-50 days of age were sacrificed by cardiac perfusion, after which cells of the LDT of selected horizontal sections were stained for NOS using NADPH and nitroblue tetrazolium. The number of NOS-positive cells observed in a field of 6.93×10^4 μm^2 increased by 12% in animals treated with amphetamine (control = 42.92 +/- 4.48, n=7; treated = 48.14 +/- 1.89, n=7; t=2.84(12), p=0.015). This increase agrees in direction but is smaller than that reported for schizophrenics (Karson et al. Psych. Res: Neuroimag. (1991) 40, 31-48). The data suggest that certain aspects of the fetal development hypothesis of human schizophrenia can be demonstrated in an animal model. (Supported by NIH and the Hedco Foundation).

669.13

THE EFFECT OF ELECTROCONVULSIVE STIMULATION ON HIPPOCAMPAL LONG TERM POTENTIATION *IN VIVO*. C.A. Stewart, I.C. Reid and L.J. Whalley*. Dept. of Psychiatry, Univ. of Edinburgh, Edinburgh EH8 9JZ, and *Dept. of Mental Health, Univ. of Aberdeen, Aberdeen, Scotland, UK.

Although electroconvulsive therapy (ECT) has been used as an effective treatment for severe depressive illness for 50 years, its mode of action, remains unknown. Apparent side effects of ECT include disruption to memory processes which have been documented in the medical literature. A recent study has suggested that electroconvulsive stimulation (ECS) in rats has profound effects on the induction of Long Term Potentiation (LTP) recorded from a hippocampal slice preparation (Anwyl et al, 1987, Brain Res.). Given the putative role of LTP in learning and memory, this finding provides a possible mechanism/explanation for the memory impairment associated with ECT.

In this study, the effects of repeated ECS on the induction and single ECS on the maintenance of LTP were examined in intact rats. Seizures were induced transcranially via ear-clip electrodes. Field potentials were recorded from the hilus of the dentate gyrus during low frequency stimulation of the perforant path in anaesthetised animals. A series of 10 ECS spaced over 20 days impaired LTP induction as measured by the excitatory post synaptic potential (EPSP) slope function (control 24.6 +/- 5.2%, ECS 11.1 +/- 2.8%) and the population spike height (control 337 +/- 75.2%, ECS 1.9 +/- 5.09%). Examination of the absolute EPSP slope and population spike values pre-tetanus indicated that the reduction in the amount of LTP obtained may have been due to the system being partially saturated. Preliminary experiments looking at the effect of a single ECS induced 25 minutes after tetanus suggest that the seizure reverses previously established LTP as assessed by EPSP slope and population spike. Further experiments are in progress to determine the precise time course of these effects and their behavioural consequences.

Supported by the Stewart Sim Bequest, Royal College of Physicians, Edinburgh.

669.14

PLATELET 3-H-PAROXETINE AND I-125-LSD BINDING IN OBSESSIVE COMPULSIVE DISORDER. W.A. Hewlett* and F. Ching. Department of Psychiatry, Stanford University School of Medicine, Stanford, CA. 94305

Serotonergic agents have been used to treat patients with Obsessive Compulsive Disorder and abnormalities in serotonergic functioning have been reported in this disorder. To study characteristics of the serotonin uptake carrier and the serotonin-2 receptor in OCD patients, we compared the binding kinetics of 3-H-Paroxetine and I-125-LSD in platelets obtained from untreated patients and controls subjects. Patients were treated for 6 weeks with either clomipramine or clonazepam. Binding characteristics were then compared in the treated and untreated conditions for these subjects. There was no difference for patients and control subjects in Bmax or Kd for either radioligand. In addition, there were no differences in these parameters for subjects treated with clonazepam. There was an apparent difference in Kd for 3H-paroxetine when subjects were treated with clomipramine, but there was no difference in the Bmax for this ligand, and no differences in I-125-LSD binding characteristics. These results provide no evidence for abnormal functioning of the serotonin uptake carrier or the serotonin-2 receptor in OCD patients.

669.15

Treatment Implications of Baseline Anger and of Anger Response to MCPP in Generalized Anxiety Disorder (GAD). M. Germine, M.D., A.W. Goddard, M.D.*, D.E. Sholomskas, Ph.D., G.R. Heninger, M.D., D.S. Charney, M.D., and S.W. Woods, M.D. Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06519

In a group of 10 patients with GAD a marked anger response to the serotonergic agonist MCPP has been noted, which appears to show relative specificity to GAD. Baseline anger is predictive of net anger response ($r=0.78$, $p=0.008$) and of net MCPP change in Panic Attack Symptom Score (PASS) ($r=0.83$, $p=0.003$). No significant correlations were noted between baseline anxiety and any of these measures. After the MCPP challenges and baseline ratings conducted over 3 weeks off medication, a clinical trial of open-label buspirone was conducted on 8 of the patients. One patient dropped out during the first week due to side effects. Four patients were judged to be clinical responders and three non-responders by a research psychiatrist blind to the anger data. The responders had a mean net anger response to MCPP of 46 ± 13mm (visual analog scale or VAS) compared to 0 ± 1mm in the non-responders ($p=0.002$) and VAS baseline anger scores of 4.5 ± 4.1mm vs 0.7 ± 1.2 ($p=0.12$). No significant realationship was noted between net VAS anxiety response to MCPP and response to buspirone. Together these results suggest a relationship between baseline anger, anger response to MCPP, and buspirone response in GAD. Although the neurobiological mechanisms underlying these relationships are not immediately apparent, these observations do suggest that baseline anger and/or anger response to MCPP may be useful predictors of clinical outcome, at least in GAD. Replication with larger samples is required.

669.16

ELECTROCONVULSIVE SHOCK (ECS) INDUCES LONG-TERM CHANGES IN REGIONAL BRAIN CYTOCHROME OXIDASE ACTIVITY. J.N. Nobrega *, R. Raymond and W.M. Burnham. Neuroimaging Research Section, Clarke Institute of Psychiatry, and Pharmacology Department, University of Toronto, Toronto, Ont., Canada.

Electroconvulsive therapy has clearly beneficial effects in severe depression. However, questions remain concerning its mechanisms of action and possible long-term deleterious effects on brain function. The present study used cytochrome oxidase (CO) histochemistry to examine long-term effects of ECS on regional brain metabolic activity. CO is a mitochondrial enzyme whose activity is tightly coupled to neuronal function (Wong-Riley, *TINS*, 1989, *12*, 94). Rats received a course of 8 ECS (once every 48 hr, 150 mA, .2 sec) and were sacrificed either 24 hr or 28 days after the last ECS or sham treatment. CO activity was quantitated in 99 different brain areas using calibrated densitometric standards. Twenty-four hr after the last seizure CO activity was generally elevated in ECS-treated brains, but in none of the regions examined did ECS vs. control differences reach statistical significance. In contrast, at 28 days CO activity in ECS brains was significantly increased in the bed n. of the stria terminalis (+25%), basolateral (+14%) and medial amygdala (+12%), dorsomedial (+20%) and ventromedial (+12%) hypothalamus, ventromedial thalamus (+9%), intrapeduncular n. (+20%), mammillary n. (+14%) and pontine n. (+16%). The observed general tendency for *increased* CO activity after ECS is consistent with an absence of gross pathology. The observations at 28 days suggest that ECS induces progressive metabolic changes which seem to develop independently of additional stimulation. Finally, the fact that changes were seen predominantly in limbic structures suggest a possible involvement of these areas in the therapeutic effects of ECT.

669.17

ANTERIOR CINGULATE GYRUS METABOLISM CORRELATES WITH RESPONSE TO BEHAVIORAL TREATMENT FOR OCD. J.M. Schwartz*, K.M. Martin, L.R. Baxter. UCLA School of Medicine, Los Angeles, CA 90024

Prior work by us using PET demonstrated that obsessive-compulsive disorder (OCD) patients who respond to either drug or behavioral treatment show a decrease ($p<.01$) in the metabolic rate (MR) of the head of their right (R) caudate nuc. (Cd) relative to the ipsilateral hemisphere (Hem) (Cd/Hem). Only drug responders showed a change in anterior cingulate gyrus (Cing) MR, with a decrease ($p=.03$) in R Cing/Hem (Baxter et al, Arch Gen Psychiatry, 1992).

Further data analysis in this ongoing study shows a significant rank order correlation between the percentage change in the Yale-Brown OC scale after drug-free behavioral treatment and the percentage change in left (L) Cing/Hem ($n=8$; $tau=-.62$, $p=.03$); this correlation was not significant for R Cing/Hem ($p=.38$). Drug treatment data for Cing/Hem did not approach significance on this correlation ($p>.45$, R & L).

Both drug and behavior treatment response may relate to alterations in the gating of cortical inputs by the Cd; behavioral treatment response may also involve activation of Cing, related to the work of behavioral self-control efforts.

670.1

SEROTONIN AND BETA-ADRENERGIC RECEPTOR BINDING IN FRONTAL CORTEX AND HIPPOCAMPUS OF SUICIDE VICTIMS. C.A. STOCKMEIER*, Y. ZHANG, H.Y. MELTZER, J. OVERHOLSER, P.R. ERNSBERGER, P.A. THOMPSON AND L. KHAITAN. Dept. of Psychiatry, Case Western Reserve University, Cleveland, OH 44106

Abnormalities in serotonin (5HT) and β-adrenergic receptor binding have been reported in frontal cortex of suicide victims. Brain samples were collected at autopsy from 12 violent suicide victims (gunshot or hanging) and 12 age and sex matched controls dying of natural or accidental causes or homicide. Psychological autopsy examinations revealed that 6 suicide victims had a history of depression. We used quantitative receptor autoradiography to measure the binding of [^{3}H]ketanserin, [^{3}H]8-OH-DPAT and [^{125}I]pindolol to 5HT-2, 5HT-1A and β-adrenergic receptors. In all samples, age was inversely correlated with 5HT-2 and 5HT-1A receptor binding in subregions of frontal cortex (area 8,9) and the hippocampus. Levels of 5HT or norepinephrine in frontal cortex were inversely correlated with postmortem delay. Postmortem delay had little effect on receptor binding. There were no significant differences between matched pairs of controls and all suicides or matched controls and suicides with a depressive illness for monoamine content or 5HT-1A, 5-HT-2 or β-adrenergic receptor binding in frontal cortex, or 5HT-1A or 5-HT-2 receptor binding in hippocampus. Further studies with larger sample size and attention to effects of drug treatment and prior depression are needed. Supported by PHS Grants MH45488 and MH41684.

670.2

MEASURES OF SEROTONIN FUNCTION AFTER SLEEP DEPRIVATION IN DEPRESSION. R.M. SALOMON, P.L. DELGADO, J. LICINIO, H.L. MILLER, G.R. HENINGER,* D.S. CHARNEY. VAMC Affective Disorders Program --116-A, Yale Univ. Sch. of Med., 950 Campbell Ave.,West Haven, CT 06516.

Sleep deprivation (SD) temporarily improves mood in 50% of medication-free depressed patients. The neurobiological mechanisms responsible for this improvement of mood are not currently understood. Most antidepressant medications are thought to enhance brain serotonin (5HT) function. This study assesses 5HT function during SD in depression. **Method**: 7 drug-free depressed patients received intravenous *l*-tryptophan (L-TRP) (100 mg/kg) after a night of undisturbed sleep (US) and again after a night of SD in a random sequence, rater-blind protocol. Repeated ratings of mood (Hamilton Depression Scale (HAM-D)) and blood for prolactin (PRL), cortisol, and growth hormone were collected at baseline prior to the L-TRP infusion and at 30, 60, 90, 120, 150, and 180 minutes after the infusion. **Results:** SD resulted in an improvement of mood (≥ 10 pt decrease in total HAM-D) in 4/7 (57%) of these drug-free depressed patients. The mean decrease in total Ham-D score for the 7 patients was 10 after SD but only 5 after US. Hormone data is available for 4 of the 7 patients. PRL response to L-TRP infusion was greater after SD than after US in 3 of 4 subjects. The fourth subject showed no appreciable prolactin rise after either of the tryptophan infusions. The mean ΔPRL (peak minus baseline) for the 4 subjects was 2.7 ng/ml after US and 9.6 ng/ml after SD. **Implications**: SD improved mood in about 50% of these depressed patients, consistent with previous literature. Preliminary data suggest that as with other AD treatments, SD may cause an enhanced prolactin response to L-TRP infusion. Data on the relationship between ΔPRL response to L-TRP infusion and the improvement of mood are currently being obtained.

670.3

INVOLVEMENT OF SEROTONIN$_2$-RECEPTORS IN THE LEARNED HELPLESSNESS MODEL OF DEPRESSION. S.C. Pandey*, M.P. Dubey, J. Sagen, J.M. Davis, and G.N. Pandey. College of Medicine, University of Illinois, Chicago, IL, 60612.

It has been shown that chronic treatment with most antidepressant drugs causes down regulation of serotonin-2 ($5HT_2$) receptors in rat brain. It has been also shown that $5HT_2$ receptors are upregulated in the frontal cortex of suicide victims and platelets of depressed and suicidal patients. To examine if the changes in $5HT_2$ receptors are similar both in brains and platelets during depression, we determined the $5HT_2$ receptors in cerebral cortex and platelets of learned helplessness (L.H) and control rats. Rats received uncontrollable shocks (2.5 mA) and were then tested for shock escape (L.H). Control rats received no uncontrollable shocks. After 10 days of training, rats were decapitated and brain regions were dissected out for determination of $5HT_2$ receptors by receptor binding technique using ^{125}I-lysergic acid diethylamide (LSD) as ligand. We observed that B_{max} of ^{125}I-LSD binding to $5HT_2$ receptors was significantly increased, while there was no change in K_D values in cortex and platelets of L.H rats as compared to control rats. These results thus suggest that upregulation of $5HT_2$ receptors in brain and in platelets is associated with learned helplessness behavior. Since $5HT_2$ receptors are increased both in brain and platelets, this again suggests that platelet $5HT_2$ receptors can be used as a biological marker for depression.

670.4

SEROTONIN 5-HT$_{1C}$ BUT NOT 5-HT$_2$ RECEPTOR NUMBER IS INCREASED IN HIPPOCAMPUS OF SUICIDE VICTIMS. *R.A. Henteleff*, V. Arango, and J.J. Mann.* Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

We have previously reported increased ^{125}I-LSD binding in the prefrontal cortex of suicide victims (Arango *et al., Arch Gen Psychiatry, 1990*). In order to determine whether this increase involves 5-HT$_2$ or 5-HT$_{1C}$ sites we determined the proportion of 5-HT$_2$ and 5-HT$_{1C}$ receptors in human cortex, choroid plexus and hippocampus. We assayed 5-HT$_2$ and 5-HT$_{1C}$ receptors by saturation binding isotherms using ^{125}I-LSD. Nonspecific binding was determined by 10μM mianserine. 5-HT$_2$ binding was masked by 3.2nM cisapride in order to determine 5-HT$_{1C}$ binding. We compared 5 pairs of suicide victims and controls matched for age, sex, postmortem delay and season of death. All subjects had clear toxicological screens. ^{125}I-LSD binding kinetics in both cortex and choroid plexus conformed to a single site model. Competition and saturation binding kinetics characterized the cortical site as the 5-HT$_2$ receptor and the choroid plexus binding site as the 5-HT$_{1C}$ receptor. No detectable 5-HT$_{1C}$ receptors were present in cortex. 5-HT$_{1C}$ receptor number (B_{max}) was significantly greater in the hippocampus of suicide victims compared to controls (54.9 ± 17.7 *vs.* 28.7 ± 10.3) fmol/mg protein, p=0.043, 2-tailed Wilcoxon matched pairs). In contrast, 5-HT$_2$ receptor number did not differ in suicides and controls (972.1 ± 122.9 *vs.* 1000.1 ± 348.5 fmol/mg, p=0.9). The affinity (K_D) of both receptor populations did not differ between groups. This study indicates that the increase in ^{125}I-LSD binding in the cortex of suicide victims is unrelated to 5-HT$_{1C}$ receptors and provides further evidence for the biochemical specificity and regional localization of the alteration in serotonin function. Supported by MH40210 and MH46745.

670.5

REDUCTION IN CORTICAL SEROTONIN TRANSPORTER SITE NUMBER IN SUICIDE VICTIMS IN THE ABSENCE OF ALTERED LEVELS OF SEROTONIN, ITS PRECURSORS OR METABOLITE. *T.F. Lagattuta*, R.A. Henteleff, V. Arango and J.J. Mann.* Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

A reduction in the number of serotonin (5-HT) transporter binding sites has been reported in the cortex suicide victims (Stanley *et al., Science, 1982;* Henteleff *et al., Soc. Neurosci. Abstr., 1991*). The 5-HT transporter is an index of the integrity of the 5-HT terminals. Levels of 5-HT, its precursors tryptophan (TRY) and 5-hydroxytryptophan (5-HTP), and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) are also indices of serotonin neuron function and yet have not been reported concurrently with 5-HT transporter binding kinetics in the cortex of suicide victims. We therefore, assayed 5-HT transporter binding kinetics in prefrontal (PFC) and temporal cortex (TC) of suicide victims and controls using ^{3}H-paroxetine (^{3}H-parox), and measured levels of TRY, 5-HTP, 5-HT and 5-HIAA in the same brain areas by HPLC-ECD. Suicides (S) and controls (C) with clear toxicological screens (N=16 pairs) were matched for postmortem interval (PMI), sex, age and season of death and assayed in pairs. ^{3}H-parox binding (B_{max}), but not affinity (K_D) was lower in the suicide group in both areas (PFC: S = 102±12 fmol/mg protein, C = 157±25 p=0.022; TC: S = 136±12, C = 186±18, p=0.004), while 5-HT, 5-HIAA, 5-HTP or TRY did not differ between groups (p>0.05). No correlations were found between B_{max} or K_D and levels of 5-HT, 5-HIAA, TRY or 5-HTP. Levels of 5-HT, its precursors or metabolite did not correlate with age, but 5-HIAA and TRY correlated positively with PMI (range 4-24h) in the suicide group but not in controls. Females (N=12) had lower levels of 5-HIAA (p=0.065), 5-HTP (p=0.096) and TRY (p=0.076) than males (N=20) in PFC but not in TC. We conclude that fewer 5-HT transporter sites are a more sensitive indicator of 5-HT nerve terminal alterations in suicide than levels of 5-HT, its precursors or metabolites. (Supported by PHS grants MH40210 and MH46745.)

670.6

SEASON SELECTIVELY AFFECTS BLUNTED PLATELET 5-HT$_2$ RECEPTOR SIGNAL TRANSDUCTION IN DEPRESSED PATIENTS. *B.W. Rigatti*, K.M. Malone, D.M. Abbondanza, G.L. Haas, T.M. Kelly, J.A. Sweeney, T.A. Mieczkowski, J.J. Mann.* Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

Seasonal effects on some serotonin (5-HT) indices in brain, platelet and CSF have been reported. Serotonergic abnormalities have been associated with depressive disorders and seasonal effects may be associated with the onset of episodes of depression. To determine the biochemical specificity of these effects, we studied the seasonal effects on the platelet 5-HT$_2$ receptor- and epinephrine-stimulated phosphoinositide (PI) hydrolysis second messenger system in healthy controls and patients with affective disorders.

Across all seasons, serotonin (0.2 mM) stimulated a 60.4 ± 5.1% increase in PI turnover in controls compared to 41.2 ± 3.5% in patients (p = 0.004). Epinephrine (EPI; 0.1 mM) associated increase in PI turnover was 34.3 ± 5.3% in controls compared to 17.6 ± 1.2% in patients (p = 0.01).

Seasonal effects were significant for 5-HT stimulated PI turnover for patients (p = 0.011) and healthy controls (p = 0.003). In patients, 5-HT stimulated PI turnover peaked during Summer (57.8 ± 6.6%) which was significantly higher than all other seasons. There was a 24.8% increase from Spring to Summer. Seasonal effects differed in controls (p = 0.005) because 5-HT stimulated PI responses were higher in Spring than Summer. No statistically significant seasonal effect was found for EPI-stimulated PI turnover in patients (p = 0.075).

We conclude: (1) PI hydrolysis signal transduction is blunted in depressed patients; (2) Season selectively affects 5-HT activation of PI hydrolysis in patients compared to EPI activation of PI hydrolysis; (3) Platelet 5-HT$_2$ and EPI stimulated PI hydrolysis in patients are maximally divergent from control values in Spring *versus* Summer. This finding may be related to the increased rate of depression and suicidal behavior in Spring. Supported by MH46745, MH48514 and MH40695.

670.7

EFFECT OF ALCOHOL-DEPENDENCE ON 5-HT$_{1A}$ BINDING IN SUICIDE.
V. Arango, M.L. Miller, M.D. Underwood, R.W. Smith, P.J. McDevitt, T.M. Kelly and J.J. Mann.* Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

Serotonin has been implicated in the regulation of alcohol preference and intake. Serotonin alterations have been associated with suicidal behavior. Using quantitative autoradiography we sought to determine whether postsynaptic 5-HT$_{1A}$ binding is altered in the prefrontal cortex of alcohol-dependent suicide victims by studying nine Brodmann areas (8, 9, 11, 12, 24, 32, 45, 46 and 47) of alcohol-dependent suicide victims and nonalcoholic controls with negative toxicological screens for other drugs. Alcohol-dependence was determined by conducting a psychological autopsy. Subjects were matched for postmortem delay, age and gender (N=4 pairs). In addition, a group of nonalcoholic suicide victims and matched controls were also studied (N=14 pairs). Slide-mounted coronal sections (20μm) from the right hemicerebrum were incubated with 2nM ^{3}H-8-OH-DPAT as described previously (Arango *et al., Soc. Neurosci. Abstr.*, 1991). 5-HT$_{1A}$ receptor distribution across cortical layers was similar in all groups, forming five isodensity bands corresponding to layer I, layer II, outer layer III, layers III-IV, and layers V-VI. Layer II had the highest level of binding. The nonalcoholic suicide group had 20% more 5-HT$_{1A}$ binding than the controls in area 46, but not in the other areas (p = 0.024). The alcoholic suicide group had elevated binding compared to controls in areas 46, 8, 9, 11, 12, 24 and 32. The binding in both suicide groups and controls was negatively correlated with with postmortem delay. Females had greater 5-HT$_{1A}$ binding than males. We conclude that: 1. 5-HT$_{1A}$ binding is increased in some brain regions of suicide victims; and 2. alcoholism and suicide was associated with a more widespread alteration of 5-HT$_{1A}$ binding. This study provides further evidence for an association of serotonin and both alcoholism and suicide. Further studies should investigate other brain areas and other 5-HT$_1$ receptor subtypes. (Supported by PHS grants AA09004, MH40210 and MH46745.)

670.8

ALTERATIONS IN ^{3}H-KETANSERIN BINDING IN ALCOHOLIC SUICIDES.
M.L. Miller, M.D. Underwood, R.W. Smith, T.M. Kelly, J.J. Mann and V. Arango.* Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

Serotonin alterations are associated with alcohol-dependence. We recently reported that 5-HT$_{1A}$ binding alterations in the prefrontal cortex are more widespread in alcoholic suicides than in nonalcoholic suicides (Arango *et al., Soc. Neurosci. Abstr.*, 1992). Using quantitative autoradiography we sought to determine whether similar alterations in ^{3}H-ketanserin (^{3}H-KET) binding were present in alcohol-dependent suicide victims. Coronal sections of the right hemicerebrum at a level anterior to the genu of the corpus callosum (20μm) were taken from alcohol-dependent suicide victims and nonalcoholic controls who had negative toxicological screens for all other drugs. Each coronal section at this level included Brodmann areas 8, 9, 46, 45, 47, 11, 12, 32 and 24. Alcohol-dependence was determined through a psychological autopsy. Subjects were matched for postmortem delay, age, gender, race and season (N=5 pairs). Slide-mounted sections were incubated with 2nM ^{3}H-KET and nonspecific binding was defined by 1μM mianserin. Binding to nonserotonergic sites was masked by 1μM prazosin and 1μM tetrabenazine. The distribution of ^{3}H-KET binding across cortical layers was similar in both groups, appearing as 5 isodensity bands corresponding to layers I-II, layer III, layer IV, layer V and layer VI. Layers III, IV and V had the highest level of binding. The alcoholic suicide group had decreased binding compared to controls in Brodmann area 47 (Suicides: 23.2 fmol/mg tissue; Controls: 33.8 fmol/mg tissue, p=0.016). The binding in both groups was positively correlated with postmortem delay in some brain areas. Binding in males and females did not differ. We conclude that ^{3}H-KET binding is decreased in some brain regions of alcoholic suicide victims, an effect that differs from suicide victims that are not alcohol-dependent. These preliminary results provide further evidence for the involvement of serotonin in alcohol-dependence. (Supported by PHS grants AA09004 and MH46745.)

670.9

^{3}H-CYANOIMIPRAMINE BINDING IS REDUCED IN SUICIDE VICTIMS.
R.W. Smith, V. Arango, M.L. Miller, M.D. Underwood and J.J. Mann.* Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

We previously reported decreased ^{3}H-paroxetine binding to the serotonin transporter in membrane homogenates from suicide victims compared to controls (Henteleff *et al., Soc. Neurosci. Abstr.*, 1991). Conflicting reports exist in the literature as to whether binding to the serotonin transporter on serotonin nerve terminals is reduced in brain cortical regions of suicide victims (Arango and Mann, *Int. Rev. Psychiatry*, 1992). Using the selective radioligand ^{3}H-cyanoimipramine (^{3}H-CNIMI), we sought to determine whether binding is altered in slide-mounted sections (20μm) from the prefrontal cortex (Brodmann areas 8, 9, 46, 45, 47, 11, 12, 24 and 32) of suicide victims compared to controls. All cases had negative toxicological screens. Subjects were matched for postmortem delay, age, gender, and where possible, race and season (N=13 pairs). Tissue was preincubated in 50mM Tris-HCl buffer with 130mM NaCl, 5mM KCl (pH 7.4, 30 min, 23°C) and then incubated (24 h, 4°C) in the same buffer containing 30μM PMSF and 0.4nM ^{3}H-CNIMI. Nonspecific binding was determined by 10μM sertraline. After a 17 week exposure, films were developed and quantified by image analysis. The highest level of binding in both groups was localized to the outer layers of the anterior cingulate gyrus (Brodmann area 24), sixfold higher than the dorsal prefrontal cortex (areas 8 and 9). In area 46 (lateral aspect of the hemisphere) the suicide group had a 42% reduction in binding, compared to controls (Suicides: 5.36 fmol/mg of tissue; Controls: 9.50 fmol/mg of tissue, p=0.031). Binding did not differ between groups in any of the other areas examined. Binding was positively correlated with postmortem delay (p<0.05) but not with freezer storage, age or sex (p>0.05).

We conclude that ^{3}H-CNIMI binding sites: 1) have a specific distribution in prefrontal cortex; 2) are reduced in a specific area of the prefrontal cortex in suicide victims; 3) correlate positively with postmortem delay; and 3) are not different in males and females. The reduction in binding is consistent with the hypothesis of a serotonergic deficiency associated with suicide. (MH40210 and MH46745.)

NEUROTOXICITY: BIOLOGICAL

671.1

PATHOLOGIC CHANGES IN THALAMUS, HIPPOCAMPUS AND CORTEX OF RAT FOLLOWING THIAMINE DEFICIENCY INDUCED SEIZURES.
Shuxing Zhang* and P.J. Langlais. Dept. of Psychology, SDSU, and VA Med. Ctr., San Diego, CA 92182.

Rats recovered from an acute bout of pyrithiamine induced thiamine deficiency (PTD) display learning and memory deficits and severe destruction of medial thalamus and mammillary bodies. Milder damage to limbic and cortical areas has been difficult to detect because of long recovery periods (3-6 months). In the present study male Sprague-Dawley rats were treated daily with a thiamine free diet and pyrithiamine hydrobromide (0.25 mg/kg, i.p.). Separate groups were given a large dose of thiamine (10 mg/kg, i.p.) 5, 6, or 8 hrs following onset of seizures, placed on regular diet for one week, and the brains perfused transcardially. In animals reversed after 5 hrs of seizures, neuronal loss and gliosis was limited to anteromedial, paracentral and posterior nuclei of thalamus. Gliosis without neuronal loss was present in medial mammilary body (MB) nuclei. Dark, pyknotic and vacuolated pyramidal neurons were evident in hippocampal CA 3-4 and to a lesser extent in CA 1-2 sectors. In animals reversed after 6 hrs of seizures, the increase in damage was remarkably greater within thalamus than in MB or hippocampus. Animals reversed after 8 hrs of seizures had near total destruction of thalamus, hemorrhagic lesions within MB but no appreciable increase in severity of hippocampal changes. Animals reversed after 5 hrs of seizures demonstrated dense concentration of degenerating fibers within cortical layers 3 and 4 of frontal and parietal areas, and CA3-4 hippocampal sectors. Supported by NIH grant NS2948101 and VA Merit Program Award to PJL.

671.2

INCREASED EXTRACELLULAR GLUTAMATE IN THALAMUS AND HIPPOCAMPUS OF ACUTELY THIAMINE DEFICIENT RATS.
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An excitotoxic basis for thiamine deficiency encephalopathy has been suggested by the demonstration that MK-801 prevents thalamic lesions in pyrithiamine treated (PTD) rats (Langlais & Mair, *J. Neurosci.*, 10:1664-1674, 1990). The current study measured extracellular fluid levels of excitatory amino acids (EAAs) before and during the onset of PTD induced seizures and pathologic lesions. Male Spraque-Dawley rats were treated with daily pyrithiamine (0.25 mg/kg, i.p.) and thiamine deficient diet. Microdialysates were simultaneously collected from probes acutely inserted via guide cannula into right medial thalamus and left hippocampus. Hourly samples were collected during separate 5-7 hour sessions from unanesthetized and freely moving animals. Basal levels were obtained at a pre-lesion stage (9-11 days of PTD treatment). No significant change from basal levels were observed in a second set of dialysates collected later in PTD treatment (11-12 days) and prior to onset of seizures or pathologic change. In samples collected during the onset of seizures (days 14-15), the levels of glutamate and aspartate in dialysates began to rise within 1-2 hrs and from 4-5 hrs after onset of seizures were elevated to 300-700% of basal levels in medial thalamus and 200-300% in hippocampus. In dialysates collected 8-10 hrs after seizure onset, levels of the EAAs fell to 10-30% of basal levels. Neuronal loss within thalamus containing the probe was noticeably less severe than on the undialyzed side, presumably due to the removal of EAAs. Supported by NIH grant NS2948101 and VA Merit Program Award to PJL.

671.3

EFFECTS OF NICOTINE ON RETINAL DEVELOPMENT.
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Department of Pathology, USC School of Medicine, Los Angeles CA 90033

Prenatal exposure of rats to high doses of nicotine via maternal infusion can impair nervous system development in association with decreased fetal viability and growth. Persistent alterations in certain parameters of neural cell development also occur at lower doses of nicotine which do not impair intrauterine growth. The retina serves as an excellent model for developing nervous tissue because of its ease of access and well-described developmental sequence.

Timed pregnant rats were implanted on embryonic day (E)15 with minipumps containing nicotine bitartrate or sodium bitartrate (control). Serum levels of nicotine in experimental animals averaged 200 ng/ml. Fetuses were sacrificed at days E18 and E21. Eyes were dissected, fixed in 4% paraformaldehyde and embedded into glycol resin for morphologic examination. Animals from experimental groups showed atrophy and vacuolization of the nerve fiber layer of the retina when compared to controls. These changes were mild at E18 and prominent at E2l. Ganglion cell number did not appear to be affected.

Cultures of retinoblastoma cells (Y79), and primary cultures of human astrocytes and retinal pigment epithelium were tested for viability, proliferation and cell number in the presence of varying amounts of nicotine. There was no effect noted at levels of nicotine below 250 μg/ml.

Chick retinas from embryonic stage 34 of development were cultured on membranes for 72 hours in the presence of varying amounts of nicotine. Explant cultures showed normal development and maintenance of outer retinal structure at levels up to 320 ng/ml. Neurite outgrowth was assessed by culturing stage 34 dissociated chick retinal cells on poly-L-lysine coated plastic. Although the number of cells extending neurites was similar, nicotine treatment at 100 ng/ml and above resulted in significant shortening of neurites.

These experiments suggest that nicotine is acting on retinal tissues by affecting differentiated neuronal functions such as neurite outgrowth.

671.4

BRAIN EDEMA IN HEPATIC ENCEPHALOPATHY (HE) AND HYPERAMMONEMIA. W. Hilgier and J.E. Olson*.
Wright State Univ. Sch. Med., Dayton, OH 45401.

Brain edema in HE has been associated with circulating ammonia which is metabolized to glutamine in the brain. We measured alterations in blood chemistry and brain regional specific gravity and osmolyte contents in models of simple hyperammonemia and liver failure induced by daily administrations of ammonium acetate (AAc) or thioacetamide (TAA), respectively. Serum and brain ammonia increased to similar levels (≈200% and 70% of control, respectively) in both experimental groups. GOT and GPT activities increased 10 fold in TAA but not AAc model. In both groups, specific gravity decreased in white matter, gray matter and basal ganglia indicating edema formation. Moreover, we observed a concomitant decrease in potassium, glutamate, and taurine contents, whereas glutamine was elevated in all brain regions. In AAc injected rats, gray matter specific gravity and potassium and taurine contents returned to control levels 24 hours after the third injection. In HE, ammonia-induced changes of brain amino acids may result in brain edema while potassium loss represents a mechanism of water homeostasis. Supported by NS 23218 and Kettering Medical Centre, Medical Education.

671.5

CENTRAL EFFECTS OF T-2 TOXIN, A TRICHOTHECENE MYCOTOXIN. J. Wang, J.R. Wilson* and D.W. Fitzpatrick. Departments of Foods and Nutrition and Psychology, University of Manitoba, Winnipeg MB R3T 2N2 Canada.

The effect of T-2 toxin on the brain was examined. To examine the dose-dependent effect of T-2 on neurotransmitter, rats were orally dosed with T-2 @ 0.1, 1.0 or 2.5 mg kg^{-1} BW; killed 2, 6 and 10 hrs post dosing; and brain nuclei were analyzed. T-2 treatment increased 5-hydroxy-3-indoleacetic acid (HIAA) and serotonin (HT) throughout the brain, produced transient increases in nucleus raphe magnus (NRM) norepinephrine (NE) and a decrease in substantia nigra (SN) NE. No regional changes in epinephrine (E), dopamine (DA) or dihydroxyphenylacetic acid (DOPAC) were observed. Few treatment differences were observed, with 0.1 mg kg^{-1} T-2, 2% of the LD_{50}, affecting brain monoamines. To investigate the effect of dietary T-2, rats were fed diet containing 2.5 or 10 ppm T-2; killed after 7 or 14 days; and brain nuclei were analyzed. NRM's HT, HIAA and NE increased in a dose dependent manner and a transient DA increase was observed. In the SN, animals fed 10 ppm T-2 had increased E after 7 days, and decreased NE after 14 days. Paraventricular nucleus of the hypothalamus and medial forebrain bundle, DOPAC concentration were lower in T-2 animals. To examine the effect of T-2 on the blood brain barrier, rats were dosed with T-2 @ 0.2 and 1 mg kg^{-1} BW intraperitoneally and 2 hrs post dosing, permeability was determined using C^{14}-mannitol and C^{14}-dextran with H^3-water the diffusible reference. In all brain regions examined, permeability increases were observed for mannitol, a small molecular weight saccharide, but not dextran. The observed affect of T-2 on the blood brain barrier, brain monoamines and the resulting neurochemical imbalance may account for the physiological and behavioral manifestation of trichothecene intoxication.

671.6

ALCOHOL REDUCES NERVE GROWTH FACTOR RECEPTOR IMMUNOREACTIVITY IN NEONATAL RAT CEREBELLUM. D.P. DOHRMAN, J.R. WEST, C.R. GOODLETT and N.J. PANTAZIS*. Dept. of Anatomy, Univ. of Iowa Medical School, Iowa City, IA 52242.

Nerve growth factor receptor (NGFR) is expressed during a restricted time (postnatal days 6-20) in the rat cerebellum. NGF, a potent neurotrophic agent, may play a critical role in the development of cerebellar Purkinje cells. Alcohol exposure during development depletes cerebellar Purkinje cells in the rat, with the most severe depletion occurring when alcohol is administered at a time which approximates NGFR expression on Purkinje cells. This raises the possibility that alcohol depletes Purkinje cells by disrupting the interaction between these cells and neurotrophic factors, such as NGF. Since NGF activity is mediated via NGFR, this study examined whether alcohol alters the expression of NGFR in the cerebellum.

Rat pups (4 day) were divided into 3 groups. The alcohol group was artificially reared and fed ethanol via a gastrostomy tube from days 4-10. The gastrostomy control group was artifically reared, but fed no alcohol. The suckle control group was reared normally and received no alcohol. At day 10, all animals were perfused and cerebellar sections were immunostained for NGFR with 192-IgG antibody.

The two control groups had identical results and revealed that Purkinje cells expressed NGFR, with the greatest expression at this age present in more posterior cerebellar lobules. Purkinje cells in more mature areas of the cerebellum displayed prominent NGFR in their dendritic fields, whereas less developed cells had NGFR on their somas and dendrites. The external granule layer also expressed NGFR, but only in the proliferative zone; no NGFR was evident in the premigratory zone.

Alcohol reduced NGFR immunoreactivity in the Purkinje cell layer and dendrites in the molecular layer. The data support the hypothesis that alcohol reduces neuronal cell numbers by interfering with expression of NGFR, thereby disrupting neurotrophic support of neuronal cells. (Support: NIH AA05523; AA07313)

671.7

ALCOHOL INHIBITS PROLIFERATION OF NEURONAL-LIKE CELLS (PHEOCHROMOCYTOMA, PC12) IN CULTURE. J. LUO, C.R. GOODLETT, J.R. WEST* and N.J. PANTAZIS. Dept. of Anatomy, Univ. of Iowa Medical School, Iowa City, IA 52242.

Animal studies have shown that fetal alcohol exposure depletes selected populations of neuronal cells in the CNS. Although killing of cells by alcohol has often been suggested as a potential mechanism for this loss, alcohol can also reduce cell numbers by inhibiting (slowing) cell proliferation. This latter possibility was the focus of this study. Because it is difficult to determine mechanisms of alcohol action in animals, an *in vitro* neuronal cell model (PC12) was used, incorporating a novel approach (synchronized cultures of PC12 cells) to examine alcohol's effect on cell proliferation.

PC12 cells were synchronized by feeding the cells with media (RPMI) containing low serum (0.08% fetal calf serum, FCS; 0.17% horse serum, HS). This low serum treatment growth-arrested the cells and the cells accumulated in the G1 phase of the cell cycle. Three days later, growth-arrested cells were fed normal serum (5% FCS, 10% HS) which stimulated synchronized growth of the cells (i.e., the vast majority of cells were at the same point in the cell cycle and progressed synchronously through the cycle). The alcohol group also received ethanol (400 mg/dl) along with the normal serum, whereas the control group received no alcohol. The alcohol concentration in the culture media was maintained by placing the cultures in sealed containers which contained an alcohol bath. Cell numbers were determined by hemacytometer at selected time points after addition of normal serum.

Results show a steep rise in cell division in these synchronized cultures which begins approximately 30 hours after addition of normal serum. Alcohol treatment delays this rise in cell division by several hours. This study suggests that inhibition of proliferation is one potential mechanism by which alcohol can reduce cell numbers in proliferating cell populations. (Support: NIH AA05523; AA07313)

671.8

EFFECT OF NICOTINE ON NEURAL CREST CELL MOTILITY AND NEURITE ELONGATION IN VITRO T.X. Jiang, C.K. He, D.R. Hinton and C.M. Chuong* Dept. Pathology, Univ. Southern California, Los Angeles, CA 90033.

It has been shown that prenatal exposure to nicotine influences the development of the nervous system. To analyze the specific effects and mechanism of nicotine (10 ng - 10 ug/ml) on neural development, we used primary neural crest cell cultures and dorsal root ganglion explants for the evaluation of the effect of nicotine. In trunk neural crest explant cultures, crest cell were plump, spindle shaped, with long axis of cells randomly arranged, and the advance margin was very uneven, suggesting active cell motility. In contrast, in the presence of nicotine, crest cells were flat, polygonal in shape, arranged regularly in palisades and the advance margin was even, suggesting lower cell motility. Time lapse video confirmed the difference in cell motility. In dorsal root ganglion cultures (chicken embryo stage 32), nicotine showed significant inhibition of neurite length around 100 ng/ml. At 10 ug/ml, there was complete inhibition. However, the number of dying cells and the degree of fasciculation were similar between control and nicotine treated explants. There is no apparent difference in immunofluorescent staining patterns of adhesion molecules N-CAM and Ng-CAM. This suggests that nicotine is specifically inhibiting neurite elongation. The results suggest that nicotine has inhibitory effect on neural cell motility and neurite elongation in vitro. We are currently studying the mechanism of these effects and evaluating the effect of nicotine on neural development in vivo.

671.9

NEUROANATOMICAL ANALYSIS OF CEREBELLA FROM MARIJUANA-EXPOSED RHESUS MONKEYS. M.Halks-Miller*, Megan Yao, & Gordon Pryor. Dept. of Neuroscience, SRI International, Menlo Park, CA 94025.

Several investigators have reported neuroanatomical changes in both rat and primate brains after exposure to either THC or marijuana (MJ) smoke. Our studies were designed to use unbiased stereologic methods to assess the putative changes in primate brain after long-term exposure to MJ smoke. Fifteen male rhesus monkeys were divided into three groups and exposed to either placebo smoke (P), moderate MJ smoke (M), or high MJ smoke (H). These subjects were implanted with an array of intracerebral glass electrodes before MJ exposure. Three untreated control monkeys were also included for study. After completion of the behavioral and electrophysiologic testing, all animals were sacrificed by per cardiac infusion with aldehyde fixatives. The cerebellum was chosen for initial study because of the high concentration of cannabinoid receptors in its molecular layer. The cerebella were sagittally sectioned, weighed, and blocked for light and electron microscopy. One cerebellar hemisphere was used for analysis of the volume compartments: molecular layer, granular layer and white matter. Within the molecular layer three volume compartments (vessels, large dendrites, and neuropil) were also evaluated. Synaptic number within the neuropil was estimated using the unbiased dissector method. Purkinje cell number was similarly evaluated. Synapse number was then normalized to number of Purkinje cells. The three test and the untreated control groups were compared by analysis-of-variance (ANOVA) methods for possible marijuana-induced changes in the neuroanatomical parameters described above. These tests failed to reveal any differences among the marijuana-treated and control groups. These studies do not support the premise that chronic exposure to marijuana smoke leads to permanent structural changes in primate cerebellum.

671.10

BOTULINUM NEUROTOXIN SEROTYPES A, B AND E EXHIBIT PROTEOLYTIC ACTIVITY. B. R. DasGupta* and W. Tepp. Food Research Institute, Madison, WI 53706

Botulinum neurotoxin (NT) serotypes A-G are encoded by genes found either in the chromosome, plasmid or a phage hosted by Clostridium botulinum. The ~150 kDa single chain NT after posttranslational proteolytic processing (nicking) is a dichain protein made of ~50 kDa L- and ~100 kDa H chains that correspond to the N- and C-terminal segments of the parent protein; -S-S- bond(s) links L and H chains. The NT binds via H chain to the presynaptic membrane at the neuromuscular junctions. The L chain after entering the secretory cells inhibits neurotransmitter release by an activity presumed to be enzymatic, the substrate of which is unknown. We report that the NT types A, B and E incubated at pH 5-8 show self-digestion--evidence is time dependent discrete bands in SDS-PAGE. The self-digestion is promoted following nicking and DTT reduction. The dichain type E NT cleaved actin (rabbit muscle) into discrete fragments. The L chain of type E NT, separated from the H chain, digested actin more effectively than the parent NT. These new data indicate, consistent with earlier reports (Biochime 71, 1193, '89; J. Physiol. (Paris) 84, 220, '90; Soc. for Neuroscience Abst. 16, 609, '90; 17, 1526, '91; FASEB J. 6, A227, '92; J. Neurochem. 57, 1413, '91) that the NT is a protease and suggest that the NT types C and D represent phage encoded proteases. The peptide bonds cleaved by the proteolytic activities of the three NT types are under study. Funded by NS17742.

671.11

TETANUS TOXIN ENTERS NEURONS THROUGH ACIDIC ENDOSOMES. L.C. Williamson*, W. Y. Clarke, S.C. Fitzgerald and E. A. Neale. Laboratory of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892.

Tetanus toxin (TnTx) acts preferentially on inhibitory synapses to block neurotransmitter release. In murine spinal cord cell cultures, TnTx inhibits Ca^{++}-dependent evoked release of glycine. To test whether TnTx enters neuronal cytoplasm via acidic endosomes, toxin action was assayed in cultures pretreated with monensin to neutralize acidic compartments. Monensin (0.5 μM) completely blocked the effect of TnTx, presumably by interfering with its movement from endosomes into the cytoplasm. Monensin effects were reversible and the drug had no effect on toxin binding. If an acid environment were required for toxin translocation across endosomal membranes, lowering the external pH should allow the entry of toxin directly through the cell membrane, by-passing the endosome. Cultures were exposed to TnTx at 4°C to achieve toxin binding without endocytosis, and then were pulsed for 6 min in the cold with medium at pH 7.25 or pH 4.9. Monensin was added and the cultures warmed. Inhibition of evoked glycine release was observed in toxin-exposed cultures in the presence of monensin when cultures were pulsed at pH 4.9. However, there was no inhibition of evoked glycine release in cultures pulsed at pH 7.25. These findings indicate that TnTx requires an acid environment to penetrate cellular membranes. 3-(2,4-Dinitroanilino)-3'amino-N-methyldipropylamine (DAMP) accumulates in acidic compartments and can be visualized by immunohistochemistry as punctate staining in neurons and glia in spinal cord cultures. Monensin reduced DAMP accumulation in neurons and glia. If, in the presence of acid, TnTx forms pores in membranes, it could disrupt the pH gradient in endosomes and likewise prevent DAMP accumulation. In cultures exposed to toxin, DAMP immunoreactivity was reduced in neurons although immunostaining in glia was similar to controls. These data suggest that TnTx enters the neuronal cytoplasm through an endosome pathway.

671.12

CYTOTOXIC ACTION OF PALYTOXIN IN AORTIC SMOOTH MUSCLE CELLS IN CULTURE. R.E. Sheridan*, B.F. Doxzon and S.S. Deshpande. Neurotoxicology Branch, Pathophysiology Division, USAMRICD, Aberdeen Proving Ground, Maryland 21010-5425, USA.

Palytoxin (PTX) produced by coelenterate species (genus *Palythoa*) is a potent marine toxin which depolarizes neurons, skeletal, smooth and cardiac muscles. In addition to direct myotoxicity, PTX induces intense vasoconstriction and hemorrhage which contribute to lethality (Toxicon,12, 427, 1974). A7r5 clonal cells derived from fetal rat aorta have many characteristics of smooth muscle (Exp Cell Res. 98, 349, 1976). We have used the A7r5 cell line to further delineate the mechanism of toxic action of PTX. A7r5 cells were grown in 35 mm dishes to confluency (4-10 days) in DMEM supplemented with 5% fetal bovine serum under standard conditions. Exposure of cells to PTX (10^{-8}M) for 15 min at room temperature followed by wash and incubation in culture medium at 37°C (≥30 min) led to swelling, clumping of cytoplasm, vacuolation and shrinking of heterochromatin. These cells were nonviable as confirmed by inclusion of trypan blue and release of LDH. Concentration-response determination for PTX (3 X 10^{-9} to 4 X 10^{-8}M) using the vital dye assay gave an EC50 value of 7.1 X 10^{-9}M. PTX-induced cytotoxicity could not be reversed by washing. Prior incubation of cells with ouabain (10^{-4}M) or verapamil (10^{-5}M) for 30 min reduced the cytotoxic effects produced by PTX. Whole-cell patch clamp recording from single A7r5 cells showed that PTX (3 X10^{-9} to 10^{-8}M, 25°C, 15 min) depolarized cells and increased membrane conductance to Na^+ and K^+ (>5-fold) as we previously reported in guinea pig ileum cells (Soc. Neurosci. Abst. 17, 1522, 1991). The precise mechanism responsible for the cytotoxic effects of PTX on A7r5 cells is presently unclear, but seems to involve Na^+ and/or Ca^{2+} overload. In light of the involvement of smooth muscle in PTX poisoning, A7r5 cells could serve as a useful model to test specific drugs for protection against PTX toxicity.

671.13

A NEW FOOD-RELATED NEUROTOXIN: OKADAIC ACID. M. T. Fernandez, V. Zitko, S. Gascón and A. Novelli*. Dept. Functional Biol., Biochem. and Mol. Biol. Section, Oviedo Univ., Sch. of Med., 33006 Oviedo, Spain; Marine Chem. Div., Biol. Station St. Andrews, N.B. E0G2X0, Canada.

Okadaic acid (OKA), a marine toxin of algal origin, is a potent inhibitor of protein phosphatases 1 and 2A, and in humans is responsible for the *diarrhetic shellfish poisoning*. We have recently reported that OKA at concentrations as low as 0.5 nM produced neurotoxicity in primary cultures of cerebellar neurons (Fernandez et al., Life Sci.1991,49,PL-157). It has been also reported that okadaic acid may affect a subtype of excitatory amino acid (EAA) receptor (Wang et al., Science 1991,253,1132). We now present further evidence indicating that neurotoxicity by OKA occurs independently from the activation of EAA receptors. Thus, changes in the neurotoxic effect of these two types of toxins with neuronal age in culture were not parallel, and EAA receptor antagonists did not protect against neurotoxicity by OKA. OKA neurotoxicity was not enhanced by glucose-deprivation, as it occurred for EAAs, and OKA did not potentiate neurotoxicity by EAAs. Biochemically, OKA did not stimulate cGMP increase nor did enhance stimulation of cGMP by EAAs.

672.1

BRAIN CONTENT OF GLYCOSPHINGOLIPIDS AFTER ORAL ADMINISTRATION OF MONOSIALOGANGLIOSIDES (GM1) DERIVATIVES. A. Polo, G. Kirschner[1], A. Guidotti and E. Costa*. Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ. Med. School, Washington, DC 20007 and Fidia Research Laboratories, Abano Terme, Italy

Natural (GM1, GD1a, GD1b, and GT1b) and semisynthetic [GM1 with [N-acetyl sphingosine (LIGA4), and GM1 with N-dichloroacetyl sphingosine (LIGA20)] glycosphingolipids protected against glutamate-induced neuronal death when added to primary neuronal cultures or when given parenterally to rats exposed to hypoxic brain lesion. Glycosphingolipids also prevented intraneuronal destabilization of $[Ca^{2+}]_i$ homeostasis without blocking the function of glutamate receptors (FASEB J., 1990, 4: 2789). Thus, a protracted ganglioside treatment could be attempted as a therapeutic strategy to prevent neuronal damage in chronic neurodegenerative diseases (e.g. Alzheimer's, epilepsy, Parkinson's) in which glutamate could be neurotoxic. To develop such a strategy, sphingolipids, absorbed orally, are needed. To this end, we have administered p.o. to rats 70 μmol/kg of glycosphingolipids tritium labeled in the 3C position of sphingosine: GM1; LIGA4; LIGA20; GM1 inner ester (AGF2); GM1 isopropyl ester (AGF44). Six hours after oral administration the increase in the content of authentic glycosphingolipids in brain measured after tetrahydrofuran extraction and separation on HPTLC was as follows: AGF2 0.012 μM < AGF44 0.032 μM < GM1 0.050 μM < LIGA4 0.55 μM < LIGA20 0.86 μM. The brain concentration remained elevated for at least 12-24 hrs, whereas the concentration in plasma declined with time. Because LIGA4 and LIGA20 are readily absorbed and slowly metabolized, repeated oral administration of these glycosphingolipids produced brain concentrations between 1 and 4 μM which can protect against glutamate neurotoxicity. In contrast, AGF2 and AGF44, even after 7 days of treatment, were able to accumulate in brain to concentration of only 0.2 to 0.4 μM.

672.2

TEMPERATURE DECREASE MAY MEDIATE PROTECTION BY MK-801 AGAINST SEROTONERGIC TOXICITY. G.M. Farfel* and L.S.Seiden. The University of Chicago, Dept. of Pharm. and Physiol., Chicago, IL 60637.

Several amphetamine analogues, when administered in high dose regimens, have been shown to cause long-lasting depletion of CNS serotonin (5-HT) which can be attenuated pharmacologically or by lowering ambient temperature. We have found MK-801 (MK) blocks depletion of 5-HT induced by methamphetamine (MA) and MDMA, but not p-chloroamphetamine (PCA) or d-fenfluramine (FEN). MK, when combined with MA, has been observed to cause hypothermia which is not seen with either drug alone. The purpose of this study was to assess whether MK protects against 5-HT toxicity by induction of hypothermia. Our results indicate that MK is more potent at inducing hypothermia when combined with MA or MDMA than with PCA or FEN, indicating hypothermia may be the basis for its neuroprotection. Single-housed male Sprague-Dawley rats (n=4-6/group) were given saline (SAL; 1 ml/kg IP) or MK (2.5 mg/kg IP) followed 15 min later by SAL (1 ml/kg IP or SC), MA (10 mg/kg IP in 4 injections 1 h apart), MDMA (40 mg/kg SC), PCA (10 mg/kg IP) or FEN (12.5 mg/kg IP as 1 injection or 2 injections 1 h apart). Some rats also received a second injection of SAL or MK. Core body temperature (TEMP) was measured every 15 min for at least 4 h, and ambient temperature was maintained at 20-22 ° C. Neither SAL alone nor MK + SAL produced significant effects on TEMP (± 1 °C). MA + SAL caused a significant increase in TEMP which peaked 2.6 °C above baseline after the fourth MA injection. MDMA + SAL caused a decrease in TEMP (-1.3 °C) at 30 min which returned to baseline by 90 min. PCA + SAL caused a TEMP increase of less than 1 °C. Co-administration of MK with MA or MDMA caused TEMP to decrease 3.6 and 3.5 °C, respectively, within 2 h of the first injections and lasting more than 3 h. MK + PCA caused a 2.2 °C decrease in TEMP 1 h after the injections which returned to baseline within 2 h, while MK + FEN caused a 2.1 °C decrease in TEMP 3 h after the first injection which also lasted less than 2 h. Since MK protects against serotonergic toxicity induced by MA and MDMA but not PCA nor FEN, these data suggest that protection against serotonergic toxicity by MK is mediated by a decrease in TEMP of more than 2.5 °C which lasts for at least 3 h. (Supported by DA-00085).

672.3

RELEASE OF GLUTAMATE FROM CULTURED STRIATAL GLIA: EFFECTS OF GLUTAMINE, KYNURENATE AND KAINATE EXPOSURE. J.F. Bowyer[1*], G.W. Lipe[1] and D.L. Davies[2]. [1]Div. of Neurotoxicol., NCTR/FDA, Jefferson, AR 72079-9502 and [2]Dept. of Anat., Univ. of Arkansas Med. Sciences, Little Rock, AR 72205.

In striatal slice preparations, the release of glutamate can be evoked by application of exogenous glutamine; potential sources for this glutamate release include neuronal glutamate terminals and glia. In an endeavor to assess the role of glia in this release, glutamate release from neuron-free glial cultures derived from 3-day-old rat striatum was measured by HPLC. The present study examined the interaction of exogenous glutamine with kynurenate, kainate and hypotonic buffer. For analysis of glutamate release, cultures were washed twice with Krebs-Ringer bicarbonate buffer, and then incubated for 30 min at 37°C. Inclusion of 500 μM glutamine in the buffer increased glutamate levels (from <0.2 μM to 0.44 μM). In the presence of kynurenate (1 or 2 mM) and 500 μM glutamine, the [glutamate] rose to 0.75 μM while kynurenate alone yielded no change. However, when cultures were pre-incubated (4 hr) in 50 μM kainate glutamate release was slightly decreased with or without exogenous glutamine. Hypotonic buffer significantly elevated glutamate release, and 500 μM glutamine potentiated this effect. These effects in glial cultures are similar to those previously observed in striatal slices, and they indicate that under some circumstances, glia may contribute to glutamate release in striatum.

672.4

NIACINAMIDE BLOCKS THE TOXICITY OF 3-ACETYLPYRIDINE IN CEREBELLAR GRANULE NEURONS IN VITRO. M. Weller, A. M. Marini and S. M. Paul*. Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, MD 20892.

3-Acetylpyridine (3AP) is a potent experimental neurotoxin which preferentially damages the inferior olivary nucleus when administered in vivo. To our knowledge its effects on neuronal viability have not been investigated in vitro. Cerebellar granule cells in vitro are susceptible to 3AP toxicity with an ED50 of 220 μM after 90 hours of exposure. Higher concentrations of 3AP result in a more rapid toxicity. Neuronal cell loss is completely prevented by prior application of niacinamide at concentrations ten times lower than 3AP. The toxicity of 3AP is enhanced by pretreatment with N-methyl-D-aspartate (NMDA) but not significantly attenuated by the NMDA receptor antagonists MK-801 and APV-5. Deprenyl, mazindol, and tetrahydrofolic acid have no influence on 3AP-induced neuronal death. This characteristic pharmacological profile of 3AP is probably due to the erroneous substitution of 3AP for niacinamide in the formation of niacinamide adenine dinucleotide phosphate (NADP) by neuronal cytosolic nucleosidases.

672.5

BLOOD-BRAIN BARRIER BREAKDOWN, DEMYELINATION AND REMYELINATION AFTER NMDA-INDUCED LESIONS OF RAT LATERAL HYPOTHALAMUS. H. Brace, M. Latimer and P. Winn (SPON: Brain Research Association) Dept. Psychol., Univ. St Andrews, Fife, Scotland KY16 9JU

Excitotoxic lesions of the rat lateral hypothalamus (LH) promote demyelination of fibers *en passant* (Stellar et al. Brain Res. 1991, 541:29) but whether this involves blood-brain barrier breakdown (Coffey et al. Neurosci. 1990, 35:121), the time course of events, and the extent to which the myelination recovers are all uncertain. Rats received unilateral NMDA lesions of the LH (1.0ul 0.09M NMDA, pH 7.3, phosphate buffer vehicle) and were sacrificed at various times after surgery. 20um sections were cut and stained with cresyl violet or Gallyas silver stain for myelin. Lesions were characterised by cell loss in the LH and adjacent structures. At 7 days lesions were large and gliosis was evenly spread but not dense. Vascular cuffing and large cells with acentric nuclei were present in the damaged tissue. These cells were not present after 14 days. Gliosis increased further by 21-28 days and crystals, thought to contain calcium, could be seen. Demyelination was found after 7 days, contiguous with but not outside the area of neuronal loss. At the edges of the damaged area myelin was abnormal: myelinated fibers had a "braided" appearance and some had swollen into large, balloon-shaped droplets. At 14 days the edges of the demyelinated area were better defined: myelin was still braided but there were fewer (and smaller) swellings. After 21 days small dots of myelin appeared in the damaged area, often clustered around blood vessels. Remyelination continued and by 2 months strands could be seen passing through the LH. These data suggest that NMDA lesions of the rat LH promote neuronal loss and demyelination; that at 7 days after surgery the blood-brain barrier is absent locally; and that remyelination occurs over time.

672.6

INTRASTRIATAL INJECTIONS OF QUINOLINIC ACID CAUSE SPATIAL LEARNING DEFICITS IN RATS. C.L. Curtis, G.J.Ross III, E.A. Hyde, R.E. Szymanski, J.S. Hull and G.L. Dunbar*. Brain Research Laboratory, Dept. of Psychology, Central Michigan University, Mt. Pleasant, MI. 48858.

Intrastriatal injections of quinolinic acid (QA) cause increases in locomotor and seizure activity. We examined if these QA lesions also would affect spatial learning abilities. Rats were given intrastriatal injections (1 μl) of either 200 nmole QA (n=16) or saline (n=8). Spontaneous motor activity of each rat was observed for 1 hr at 2- and 24-hr after surgery, and for 10 mins on postoperative days 5 and 10. All rats were tested in the Morris water maze on postoperative days 5-15. Body weight was recorded daily and amount of food and water consumed was calculated on postoperative days 1-5 and 10. Half of the QA rats died within 48 hr after the injection. Half of the remaining QA rats required force feeding and all of the QA rats showed significant decreases in food and water consumed. All QA rats showed barrel rotations and tonic-clonic episodes, but no significant differences in spontaneous motor activity levels were observed after postoperative day 5. All QA rats were significantly impaired on every measure in the Morris water maze task. These results indicate that QA-induced cognitive impairments remain even after most locomotor abnormalities subside.

672.7

N-METHYL-D-ASPARTATE RECEPTOR-MEDIATED NEUROPROTECTION IN CEREBELLAR GRANULE CELLS REQUIRES NEW RNA AND PROTEIN SYNTHESIS. A.M. Marini*, P. Damschroder-Williams, and S.M. Paul. CNB, NIMH, Bethesda, Md 20895.

Cultured cerebellar granule cells are glutamatergic neurons which express all of the glutamate receptor subtypes. Previous studies have shown that cultured cerebellar granule cells are susceptible to the neurotoxic effects of the excitotoxin glutamate and the chemical neurotoxin, 1-methyl-4-phenylpyridinium (MPP^+). Although the toxicity of glutamate and MPP^+ are time- and concentration-dependent in cultured cerebellar granule cells, the mechanisms of toxicity are different. Whereas glutamate mediates its toxicity by the activation of N-methyl-D-aspartate (NMDA) receptors, MPP^+ kills neurons by intracellular uptake into vulnerable neurons. Paradoxically, preincubation of cultured granule cell neurons with subtoxic concentrations of NMDA or glutamate markedly antagonizes the neurotoxicity from subsequent exposure to toxic concentrations of either MPP^+ or glutamate. The neuroprotective effects of NMDA and glutamate against MPP^+ toxicity are observed at concentrations as low as 1 μM, blocked by specific NMDA receptor antagonists and require at least 30 min to fully develop. Preexposure of the neurons to subtoxic concentrations of NMDA also resulted in significant protection against toxic glutamate concentrations (50-1000 μM). Moreover, NMDA-receptor mediated neuroprotection is prevented by the RNA synthesis inhibitor, actinomycin D, or the protein synthesis inhibitor cycloheximide. Thus, activation of NMDA receptors by glutamate results in either neurotoxicity or neuroprotection, depending on the glutamate concentration and apparent degree of receptor stimulation. NMDA receptor-mediated neuroprotection in these neurons requires new RNA and protein synthesis and appears to be mediated by the expression of a neuroprotective protein(s). Taken together, these data demonstrate the presence of an active NMDA receptor-mediated and transcriptionally-directed neuroprotective mechanism in cerebellar granule cells.

672.8

COMPLEMENT mRNAs INCREASE IN THE RESPONSE TO NEUROTOXIC BRAIN LESIONS. I.Rozovsky, D. Willoughby, T.E.Morgan, G.M.Pasinetti, M.N. Dugich-Djordjevic, T.H. McNeil*, and C.E. Finch. Andrus Gerontology Center,University of Southern California, Los Angeles, CA 90089.

Recently complement receptor, activated complement products, regulatory complement proteins, and complement mRNAs were demonstrated in normal human and rat brains. We also showed up-regulation of complement mRNAs in AD brain in association with AD type lesions, and in experimental rat brain lesions. Data from our lab show that resident brain cells synthesize and regulate complement component mRNAs in response to brain injury. Kainic acid (KA) induced seizure activity resulting in eventual neuronal loss in CA3-CA4 hippocampal pyramidal neurons was used as a model of selective neurodegeneration. By *in situ* hybridization analysis the major increase of C1qB mRNA signal in CA3 hippocampal layer was observed 48 hours after KA induced seizure activity . Northern blot hybridization analysis of total RNA isolated from microdissected hippocampus showed elevation of C1qB mRNA signal 48 hours after the onset of KA induced seizure activity: 6 fold increase in CA3 layer, 4 fold - in dentate gyrus- CA4 layer, and less than 2 fold- in CA1 layer. This differential up-regulation of C1qB mRNA correlates with different sensitivity of hippocampal neurons to KA actions. Granule neurons of the dentate gyrus can be selectively destroyed by intrahippocampal injections of colchicine. By *in situ* hybridization C1qB and C4 mRNA signals increase in colchicine lesioned hippocampus (ipsilateral to the lesion). The highest increase of the signal was observed 10 day post lesion distributed mainly in areas adjacent to granule neurons layer, subiculum and outer molecular layer of CA1. These results suggest possible role of complement and complement mediated inflammation in association with local cell death. (Supported by AG 00093-10).

672.9

KAINATE INDUCED SEIZURES UPREGULATE EXPRESSION OF bFGF mRNAs IN THE RAT HIPPOCAMPUS. M. Khrestchatisky*, K. Bugra, H. Pollard, L. Ferhat, G. Charton, I. Moreau, A. Autour, A. Represa, D. Diabira, P. Chinestra, G. Barbin and Y. Ben Ari. Unité INSERM 29, 123 Bd de Port Royal 75014 Paris , France.

bFGF stimulates proliferation and subsequent differentiation of a variety of mesenchymal cell types. In vitro, this factor demonstrates neurotrophic activity. In the central nervous system bFGF, seems to be constitutively expressed, and in the rat hippocampus in situ hybridization reveals low levels of bFGF expression in field CA2. These findings support the notion that this potent neurotrophic factor may be involved in CNS development and maintenance and that it could act as a survival factor in the adult CNS. To test this hypothesis, we have studied the regulation of the bFGF gene following neuronal hyperactivity induced by kainic acid (KA) in vivo and in vitro. Our in vivo models include intraperitoneal and intra-amygdala injections of KA while the in vitro experiments were conducted with hippocampal slices. bFGF mRNA expression was monitored using in situ hybridization and reverse transcriptase coupled PCR. We have observed that three hours following seizure activity, bFGF mRNA expression was increased in fields CA1 and CA2 when compared to control animals and that this upregulation was transient.

672.10

ZINC ENHANCES KAINATE NEUROTOXICITY IN THE RAT HIPPOCAMPUS K. SHIRAISHI[1]*, S. NAKAZAWA[1], H. ITO[2]
Department of Neurosurgery[1], and Anatomy[2], Nippon Medical School, Tokyo, Japan

Zinc is one of the N-methyl-D-aspartate(NMDA) antagoniosts and has recently been known to potentiate non-NMDA currents in *Xenopus* oocytes expressing mRNA of a glutamate receptor clone(GluR3). Histochemical studies proved that the zinc distribution was localized in the hippocampus, CA3-4, where kainate binds specifically. We designed to examine whether the neuropharmacological effects of zinc could exist and to find areas where they would develop in the rat brain. S-D rats, weighing 300-320 gm, were anesthetized with chloral hydrate 350 mg/kg. The left external carotid artery was exposed, and a small catheter was introduced into the common carotid artery to inject zinc chloride to the internal carotid artery without disturbing the blood flow. Zinc chloride (7.0 mM, pH 6.50, volume 1.0 ml/kg) was injected for 10 minutes. One hour after the injection, *in vitro* quantitative autoradiography of kainate revealed an increase of binding of kainate in the left hippocapus CA3-4. In another rats, 1.0mg/kg kainate, which is not sufficient to induce neuronal damage, was intraperitoneally injected after the carotid infusion. One day after the insult, Nissl preparations showed neuronal damage in the left CA3-4. According to these results, we thought zinc enhanced the kainate neurotoxicity in the rat hippocampus.

672.11

KAINIC ACID LESION INDUCES DIFFERENTIAL RESPONSE OF [125I]IGF I AND [125I]IGF II RECEPTOR BINDING SITES IN THE RAT HIPPOCAMPAL FORMATION. D. Seto*, S. Kar and R. Quirion. Douglas Hospital Research Center, Depts. of Pharmacology and Therapeutics and Psychiatry, McGill Univ., Canada H4H 1R3.

The insulin-like growth factors I and II (IGF I and IGF II) are mitogenic polypeptides with structural and functional homologies to hormone insulin. Both peptides address a wide spectrum of neuronal and non-neuronal cells and their physiological response are presumed to be mediated by respective cell surface receptors i.e., IGF I and IGF II receptors. Besides their involvement in growth and development, evidence suggests that these growth factors in the adult nervous system act as maintenance factors in addition to modulation of hormone/neurotransmitter releases. In the present study following systemic injection of kainic acid to the adult rat (10 mg/kg; i.p.), [125I]IGF I and [125I]IGF II receptor binding sites were evaluated to investigate their possible differentiation in cellular localization and response to injury. [125I]IGF I receptor binding sites were found to be concentrated in the granular layer of the dentate gyrus and the pyramidal layer of the CA1-CA3 regions whereas the underlying strata showed only moderate to low density of labelling. Following kainic acid treatment, [125I]IGF I binding was substantially decreased in all layers of the CA1-CA3 regions whereas in the dentate gyrus, granular cell layer showed only a moderate decrease in the labelling. As for IGF I receptors, relatively high densities of [125I]IGF II sites were primarily localized in the pyramidal cell layer of the CA1-CA3 regions and in the granular cell layer of the dentate gyrus whereas other layers exhibited only low binding densities. Systemic adminstration of kainic acid induces a moderate decrease of [125I]IGF II binding especially in the pyramidal cell layer of the CA1 region. In the granular layer of the dentate gyrus however, no apparent alteration in the density of IGF II binding sites was noticed. Thus, it appears that IGF I and IGF II receptor binding sites in the hippocampal formation are differentially affected following peripheral kainic acid injection. This is most evident in the granular cell layer and provide further evidence for the differential localization of these two receptor classes in this region of the rat brain.(Supported by FRSQ and MRCC).

672.12

Oxidative Stress and Antioxidant Response in Rat Piriform Cortex Associated with Kainic Acid-Induced Seizures. M.E. Layton, J.K. Wagner, S.R. Nelson, F.E. Samson and T.L. Pazdernik*. Dept.of Pharm.,Tox. and Therapeutics and R. L.Smith Res. Ctr., Kansas University Medical Center, Kansas City, Kansas 67337 USA.

Seizures induced by kainic acid (KA; 12 mg/kg ip) cause extensive neuropathology in the piriform cortex. To measure oxidative stress in brain during KA-induced seizures, thiobarbituric acid-reactive substances (TBARS), a general index of lipid peroxidation, and the antioxidants ascorbic acid (AA) and uric acid (UA) in intracerebral microdialysates were analyzed. In controls (N=7) and in KA-treated rats (N=7), TBARS levels in the piriform cortex 90 minutes after injection were 22 ± 4.6 and 30 ± 3.6 ng/mg protein, respectively ($p < 0.03$). In contrast, TBARS levels in the frontoparietal cortex, a region of the brain not damaged by seizures, were not significantly different in controls (18 ± 6.7 ng/mg protein) versus KA-treated rats (20 ± 3.7 ng/mg protein; N=7). Control microdialysate AA levels in the piriform cortex were 0.9 ± 0.4 μM and increased dramatically to 15.9 ± 6.0 μM during KA-induced seizures ($p = 0.0001$, ANOVA with repeated measures; N=3). Control microdialysate UA levels of 2.4 ± 0.5 μM increased to 4.7 ± 0.5 μM during KA-induced seizures ($p = 0.0001$; N=3). Thus, KA-induced seizures produced oxidative stress in the form of increased lipid peroxidation in a brain region known to be extensively damaged by seizures, but not in an undamaged region. These data suggest that oxidative stress may contribute to seizure-associated neuropathology, whereas increased AA and UA in brain extracellular fluid may represent an antioxidant response to the oxidative stress. Supported by DAMD 17-90-C-0041, ES07079 and NIH 2 P30 HD02528-25A1.

672.13

SUBSTANCE P INJECTIONS AND POLYMER IMPLANTS PROTECT AGAINST EXCITOTOXIN-INDUCED D_1 DOPAMINE RECEPTOR LOSS IN RAT STRIATUM
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Recently, it was demonstrated by Pert & colleagues (O'Neill et al Soc.Neurosci.Abstr. 16,194,1990) that substance P (sub P) and a heptapeptide fragment potentially protected against NMDA-induced toxicity of hippocampal neurons *in vitro*, and NMDA-induced lethality *in vivo*. The present study examined whether sub P could protect against quinolinic acid(QA)-induced lesions of the striatum, as measured by a loss of striatal D_1 dopamine receptors.

Sub P (200 nmol/10 ul) was injected unilaterally into the lateral ventricle of rats. About 25 min. later, an intrastriatal injection of QA (100 nmol/ul) was given. Controls received vehicle only. Animals were sacrificed approx. 5 days later and the striata were frozen and examined for D_1 dopamine receptors. The D_1 receptors were assayed using ^{3}H-SCH23390, and saturation analysis determined Bmax and Kd. Results showed that QA alone induced about a 40% loss of D_1 receptors. Sub P alone produced no significant change, but sub P prevented the QA-induced loss of striatal D_1 receptors.

In another study, sub P was extruded into Evac polymer rods for slow release. One 4mm rod segment was implanted unilaterally into the striatum of each rat. One week later, animals received a stereotaxic injection of QA (50, 75 or 100 nmol/ul) 0.5 mm medial to the implanted rod. Controls received QA alone. Three weeks later, the striata were analyzed for D_1 dopamine receptors. Results showed a dose-dependent loss of D_1 receptors following QA alone. Sub P rods protected the striatum from QA-induced D_1 receptor loss at the time point studied. These results support the neuroprotection role of sub P on excitotoxicity.

NEUROTOXICITY: MISCELLANEOUS TOXINS

673.1

IMMUNOCYTOCHEMICAL LOCALIZATION OF CATALASE IN RAT BRAIN.
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Peroxisomes are cytoplasmic organelles first described in mouse proximal kidney tubules and now well characterized and known to be present in virtually every eukaryotic cell. They are responsible for the removal by catalase of hydrogen peroxide produced in the peroxisomal oxidative pathways and are involved in several metabolic processes, such as lipid ß-oxidation, aminoacid metabolism and biosynthesis of plasmalogens, major myelin constituents. The importance of peroxisomes in the nervous system is emphasized by numerous genetic diseases associated with impaired function of peroxisomes, or their numerical reduction, and neurological disturbances. Little is known, however, about neural peroxisomes (also called microperoxisomes, because they are smaller than those in kidney and liver cells). We have studied the distribution of peroxisomes in the adult rat brain by means of a new, extremely sensitive, immunocytochemical procedure (J.C. Adams, in press), utilizing an affinity-purified polyclonal antibody against catalase. Two main categories of neurons showed an intense positivity: (a) large neurons located in the mesencephalic nucleus of the trigeminal nerve, red nucleus, cerebellar and vestibular nuclei, the reticular formation, and all the cranial and spinal motor nuclei; (b) small interneurons of the cerebral cortex and the hippocampal formation, the cerebellar Golgi cells, and the principal neurons of the thalamic reticular nucleus. In both white and gray matter, most astrocytes and oligodendrocytes were distinctly immunostained. Ependymal cells showed a high staining intensity, while no reaction was evident in endothelial cells. A moderate degree of staining was found in most neurons throughout the brain. These data suggest that catalase expression is only partly correlated with cell size. High catalase content in certain small neurons and the macroglial cells may reflect particular cellular metabolic processes, such as those related to firing, myelin synthesis or metabolite transport.

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673.2

Benzylamines Reduce Hydrogen Peroxide Induced Neuronal Degeneration In Vitro: Correlation Between Structure and Cytoprotection.
C.A. Luttman, P.T. Keith, J.R. McCowan, J.H. Wikel, M.J. Yu and R.D. Saunders* Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285

Generation of reactive oxygen species plays a pivotal role in neuronal degeneration during ischemia/reperfusion and traumatic injury. During brain ischemia, H_2O_2 may accumulate to levels which are toxic to neurons. Agents which prevent phospholipid oxidation may prevent membrane degradation and subsequent neuron death. We tested the ability of a homologous series of 3,5-dialkoxy-4-hydroxy-benzylamines in preventing H_2O_2-induced neuronal degeneration in vitro. Acute exposure of neuronal cultures to 50 uM H_2O_2 for 15 min resulted in few morphological changes, but "delayed" widespread neuronal degeneration occurred during the ensuing 24 hours. Treatment of the cultures with benzylamines reduced the cytotoxic effect of H_2O_2. In general, increasing the length of the carbon side chain was associated with increased cytoprotection. However, substituent bulk (MR) and substituent length (L1) may represent independent parameters in the context of H_2O_2 toxicity- i.e. derivatives with equivalent L1 values were equally effective despite having different MR values. These compounds were only partially effective and may not address other mechanisms occurring in the cascade of H_2O_2 toxicity (i.e. enzyme inactivation). Our data provide evidence that agents which prevent oxidation of membrane phospholipids may prove beneficial during pathological conditions where hydrogen peroxide is produced in vivo.

673.3

A GLUTAMATE HYPOTHESIS FOR TARDIVE DYSKINESIA.
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Several animal models have been developed for the study of tardive dyskinesia (TD) based on hyperkinetic states induced in long-term neuroleptic-treated animals. These models for TD have been contrasted against models for Parkinson's disease (where an upregulation of dopamine transmission within certain brain areas is characteristic of TD, while a downregulation is linked with parkinsonism). This dualistic view of contrasting models has been presented for years, despite numerous reports from the clinic, claiming that TD and parkinsonism often occur together.

The GABA hypothesis for TD has been linked with decreased GABA activity in the internal segment of globus pallidus (GPi), substantia nigra reticulata (SNr) and the subthalamic nucleus (STN). Interestingly, a downregulation of GABA terminals has recently been demonstrated in these brain regions of the hypokinetic, parkinsonian brain. The changes within the GABA-system may thus reflect or sometimes antedate the occurrence of tardive parkinsonism (TP) rather than TD. The changes within the GABA neuron system may account for signs of TP, whereas the origin of TD must be sought elsewhere.

Studying an admittedly limited number of Cebus monkeys with TD we found a reduced deoxyglucose uptake both in GPi and its thalamic target area (though not in the SNr). The animals were treated with fluphenazine-decanoate for several years and sacrificed four months after the last depot injection. The reduced glucose utilization in these areas may reflect a reduced synapse activity, possibly resulting from glutamate excitotoxicity. The STN-fugal neurons terminating in GPi and SNr are known to be glutamatergic and this system of glutamate neurons is upregulated in the parkinsonian brain and also during long-term neuroleptic treatment. This chronic upregulation might have excitotoxic effects on the GABA pathways from GPi and SNr to the thalamus. A destruction of those thalamic afferents could result in a hyperkinetic state and may be an underlying mechanism explaining the choreic elements of TD. This would thus give rise to a glutamate hypothesis for TD.

673.4

LEVODOPA TOXICITY IN CELL CULTURE.
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Parkinson's disease continues to progress in patients treated with Levodopa (L-dopa), and this progression has been ascribed to the continued loss of dopaminergic neurons in the substantia nigra. It has been suggested that L-dopa and dopamine can generate reactive molecules that might contribute to the acceleration of the loss of dopaminergic neurons. Using cultured E15 rat mesencephalic neurons and the pheochromocytoma PC12 cell line as model systems, L-dopa was cytotoxic with $LD_{50's}$ of 140 and 400 μM in dopaminergic and GABAergic mesencephalic neurons, respectively, following a 2 day exposure and 60 μM in PC12 cells after a 4 day exposure. The mechanism of toxicity of L-dopa was investigated to see whether there was a correlation between cytotoxicity and autoxidation of L-dopa itself or dopamine formed from the L-dopa. Carbidopa, an inhibitor of aromatic L-amino acid decarboxylase, did not protect against L-dopa cytotoxicity in either PC12 cells or neurons, and clorgyline, an MAO-A inhibitor, did not enhance L-dopa toxicity in PC12 cells suggesting that cytotoxicity was not due to dopamine formed from L-dopa. Catalase or superoxide dismutase alone provided partial protection against L-dopa toxicity in PC12 cells. However, the combination of these enzymes totally protected against cytotoxicity. Therefore, L-dopa cytotoxicity in PC12 cells and mesencephalic neurons is most likely due to its autoxidation. Furthermore, these preliminary findings would suggest that although L-dopa is beneficial in the treatment of Parkinsonism, it could also be deleterious to neurons in the brain of patients because of its capability to be autoxidized, generating reactive molecules.

673.5

2'-AMINO SUBSTITUTED MPTP DEPLETES BRAIN SEROTONIN AND NOREPINEPHRINE WITHOUT AFFECTING DOPAMINE IN C57BL/6 MICE. A. M. Andrews, N. A. Garrick*, and D. L. Murphy. Lab. of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

Both 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 1-methyl-4-(2'-methylphenyl)-1,2,3,6-tetrahydropyridine (2'-CH_3-MPTP) are known dopaminergic neurotoxins. Experiments with another 2'-substituted derivative of MPTP, namely 1-methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydropyridine (2'-NH_2-MPTP), produced considerably different results following systemic administration (4 x 20 mg/kg i.p.) to C57BL/6 mice. Amine neurotransmitters and their metabolites were quantitated by HPLC-ECD in mice sacrificed 1 and 3 weeks post-treatment. While 2'-CH_3-MPTP (2 x 20 mg/kg) caused dopamine(DA) and its primary metabolites to become substantially reduced (~80%) in key brain areas such as striatum, 2'-NH_2-MPTP produced marked depletions (~70%) in serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and norepinephrine (NE) in frontal cortex and hippocampus. Smaller decreases in 5-HT, 5-HIAA, and NE were seen in brain stem and striatum as well. 2'-NH_2-MPTP had no effect on dopaminergic neurochemistry. Our current studies are investigating whether 2'-NH_2-MPTP or a metabolite has a different specificity than other MPTP analogs for aminergic transport systems.

673.6

NEW EVIDENCE FOR A LOSS OF SEROTONERGIC NERVE TERMINALS IN RATS TREATED WITH FENFLURAMINE. R.I.Westphalen and P.R.Dodd*
Clinical Research Centre, Royal Brisbane Hospital Foundation,
Bancroft Centre, Royal Brisbane Hospital, Herston Q 4029, AUSTRALIA.

Evidence to classify fenfluramine as a serotonergic neurotoxin comes from reports that show decreases in markers of serotonergic nerve terminals (vesicular 5-HT and 5-HT uptake sites on pre-synaptic nerve terminals) in rats treated with fenfluramine. However, suggestions that the antigen (5-HT) was repositioned by fenfluramine (fenfluramine-induced 5-HT release) rendered the immunocytochemical evidence questionable. Therfore, studies which used the 5-HT uptake sites as markers of serotonergic nerve terminals gained attention. These studies found decreased radio-ligand binding to 5-HT uptake sites, and a reduced rate to which synaptosomes from animals treated with fenfluramine take up radio-labelled 5-HT. However, the present study demonstrates that this evidence is also exhibited in an animal model of reduced 5-HT uptake site density on otherwise functional serotonergic nerve terminals (Rx), established by injecting male Wistar rats with an irreversible 5-HT uptake site antagonist, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). Therefore, a decrease in 5-HT uptake site density and maximum 5-HT uptake rate into synaptosomes may not portray a loss of 5-HT containing nerve terminals, highlighting the fact that it had not been demonstrated conclusively that fenfluramine administration leads to a loss of serotonergic nerve terminals.

When maximal loading of [^{14}C]5-HT into synaptosomes (α) was measured, it was found that animals treated with fenfluramine gave a significantly lower value than those in the Rx group ($p < 0.02$). Rx animals did not differ significantly from controls. Therefore the measurement of α allowed a distinction to be made between a loss of uptake sites on an intact neurotransmitter system, and a loss of uptake sites as a consequence of terminal loss.

It was concluded that the combination of decreased uptake site density (B_{MAX}) and/or uptake rate (V_{MAX}) with decreased synaptosomal loading of neurotransmitter (α) give evidence of a loss of pre-synaptic nerve terminals. The present study thus indicates fenfluramine-induced destruction of serotonergic nerve terminals.

673.7

DEXFENFLURAMINE (DFEN) REGIMENS THAT REDUCE BRAIN SEROTONIN (5HT) MARKERS DO NOT AFFECT EITHER GFAP OR MICROGLIA IN RAT FRONTAL CORTEX. N.E. Rowland, W.J. Streit, A. Kalehua, B.H. Li, S.L. Semple-Rowland* Depts Psychology & Neuroscience, Univ of Florida, Gainesville FL 32611.

Male Sprague-Dawley rats were administered DFEN (0-12 mg/kg, twice daily, per os) for 4 days and were sacrificed either 18 hr, 66 hr, or 2-3 weeks after the last injection. Higher doses of DFEN produced a marked and long-lasting depletion of brain 5HT content and uptake sites (by paroxetine binding). 5HT immunohistochemistry showed the previously-described swollen 5HT-reactive axons at short survival times in frontal cortex. As a measure of possible degenerative changes, we also studied responses of glial cells in the vicinity of swollen 5HT-reactive processes. No evidence for an astrocytic reaction was obtained in either sections or Western blots of tissue extracts immunostained for glial fibrillary acidic protein (GFAP). In double-stained sections, there was no GFAP reaction even adjacent to 5HT-immunoreactive swollen processes. Likewise, sections stained for reactive microglia (Griffonia simplicifolia) showed no change in the number or intensity of microglia at any time after DFEN.

Supported by a grant from IRIS (Servier).

673.8

A DIHYDROPYRAZOLE INSECTICIDE, RH 3421, BLOCKS VOLTAGE-DEPENDENT SODIUM CHANNELS IN PRIMARY CULTURES OF RAT DORSAL ROOT GANGLION NEURONS. J.M. Frey*, D. Highfield, S.M. Murray, and G .R. Christoph. Central Research and Development, E.I. Du Pont de Nemours and Company, Newark, DE 19714.

Certain dihydropyrazoles have potential utility as insecticides. Previous neurophysiological data on invertebrate preparations indicate that RH 3421, methyl 1-[*N*-(α,α,α-trifluoro-*p*-tolyl) carbamoyl-3-(4-chlorophenyl)-4-methyl-2-pyrazolin-4-ylcarboxylate, blocks voltage-dependent sodium channels (Salgado, V.L. Pestic. Sci. 1990, 28, 389-411). To characterize the potential neurotoxic effects of dihydropyrazoles in mammalian preparations, RH 3421 was independently synthesized and was applied by pressure ejection to individual neurons during tight-seal, whole-cell voltage clamp experiments. Sodium currents were isolated by including calcium and potassium channel blockers in the bath and pipet solutions. In order to de-inactivate sodium channels (50% inactivation voltage *ca.* -70 mV), a 5-sec conditioning prepulse to -110 mV was delivered prior to each depolarizing voltage step. Sodium currents recorded under these conditions had typical current-voltage characteristics and peak amplitudes of 4-10 nA elicited by voltage steps to -10 mV from holding potentials of -60 mV or -90 mV. RH 3421 (up to 10 μM) decreased the peak sodium current in a concentration-dependent manner, with greater effect at less negative holding potentials. Blockade was not reversed by washing during the 45-min duration of the typical experiment. Membrane hyperpolarization, however, did partially reverse compound-induced sodium current blockade. These results directly support Salgado's conclusion that dihydropyrazole insecticides cause voltage-dependent blockade of sodium channels and extend the findings to mammalian neurons.

673.9

SOMAN INDUCED CHANGES IN THE CAT VISUAL SYSTEM FOLLOWING PYRIDOSTIGMINE. A.W. Kirby*, A.T. Townsend, C. Pope, G.evans, R. Murhpy and R. Collins. USAARL, Fort Rucker, AL 36362.

Soman is an organophosphorus compound, an irreversible inhibitor of cholinesterase (ChE), and a potential nerve agent. Pyridostigmine (pyrido) is a carbamate, a reversible inhibitor of ChE, and the pretreatment drug of choice for protection against nerve agent exposure. Last year at this meeting we reported changes in visual function following soman. These studies were done to evaluate the protection to the visual system when pyrido pretreatment is used.

Adult cats were prepared surgically under halothane anesthesia and maintained throughout the experiment with urethane. Following base line visual evoked response (VER) collection, enough pyrido was given to inhibit 30-80% of blood ChE. Soman (4 μg/kg) then was given.

This dose of soman resulted in about 80% inhibition of blood ChE and 80-90% VER reduction with no recovery over the next 24 hours. A 60% inhibition of blood ChE with pyrido did not alter the VER. Following soman, reduction of the VER was about 30% compared to 80% without pyrido, and blood ChE returned to about 60% within several hours. Even with less pyrido pretreatment, significant recovery occurs within several hours.

673.10

COMPARISON OF ORGANOPHOSPHATE INTERACTIONS WITH ACHE AND MUSCARINIC RECEPTORS. T.R. Ward, D.J. Ferris, H.A. Tilson, S. Padilla* and W.R. Mundy. Neurotoxicology Division, U.S. EPA, Research Triangle Park, NC 27711

Toxicity of organophosphate compounds (OPs) has long been associated with their ability to inhibit acetylcholinesterase (AChE). Recently reports have surfaced indicating direct interaction of OPs with muscarinic acetylcholine receptors in the CNS. We investigated the sensitivity of muscarinic receptor binding to [3H]quinuclidinyl benzilate (QNB) and [3H]cis-methyldioxolane (CD) to competition with a series of OPs in washed homogenates of frontal cortex from adult male Long Evans rats. The OPs' IC50s for competition with radiolabeled ligands were compared to the IC50s for the inhibition of AChE activity. The OPs studied were diisopropylfluorophosphate (DFP) and echothiopate (EC), the insecticides parathion (PT), malathion (MT) and disulfoton (DI), and their activated forms paraoxon (PO), malaoxon (MO) and demeton S (DE). QNB (a muscarinic antagonist which binds to all receptor subtypes) binding was not affected by these compounds at concentrations under approximately 100 μM. CD (a muscarinic agonist which binds to only 8-10% of the total muscarinic sites) binding was inhibited with IC50s of: PT >100 μM, PO 0.021 μM, MT 91 μM, MO 0.74 μM, DI 55 μM, DE 16 μM, DFP 4.3 μM, EC 0.011 μM. AChE activity was inhibited with IC50s of: PT >100 μM, PO 0.004 μM, MT >100 μM, MO 0.029 μM, DI >100 μM, DE 2.3 μM, DFP 0.42 μM, EC 0.005 μM. The ability of OPs to inhibit AChE activity correlated well with their ability to compete with CD binding (r=.99). These results suggest that OPs can interact directly with a subset of muscarinic cholinergic receptors in the CNS at biologically relevant concentrations.

673.11

CHANGES IN CELLULAR CALCIUM HOMEOSTASIS AND TOXICITY OF 2,2'-DICHLOROBIPHENYL IN RAT CEREBELLAR GRANULE CELLS. P.R.S. Kodavanti, D. Shin, H.A. Tilson* and G.J. Harry. Neurotoxicology Division, HERL, U.S.E.P.A and DART/STB, NIEHS, Research Triangle Park, NC 27711.

Some polychlorinated biphenyls (PCB) affect locomotor activity and brain dopamine function in laboratory animals. The exact mechanism of these effects, however, is not known. Since neurotransmitter uptake and release are dependent on the maintenance of normal calcium homeostasis of the nerve cell, we have studied the effects of a potentially neurotoxic PCB congener, 2,2'-dichlorobiphenyl (DCBP), on Ca^{2+}-homeostasis using cerebellar granule cells. Perturbations in calcium homeostasis have also been closely associated with cell death caused by a variety of toxicants. Free Ca^{2+} measurements (fluo-3 fluorescence) and cytotoxicity (LDH leakage) assays were performed in cerebellar granule cells (10-14 DIV). Ca^{2+} sequestration was studied in mitochondria and microsomes, whereas Ca^{2+} extrusion was studied in synaptosomes, prepared from adult rat cerebellum. In cultured cerebellar granule cells, DCBP was cytotoxic at 200 μM and higher concentrations after 1 hr of exposure. However, 50 μM DCBP increased cerebellar granule cell free Ca^{2+} two-fold following 20 min incubation. This increase was not transient, and a steady rise was observed with time. DCBP was also very potent in inhibiting ^{45}Ca-uptake by mitochondria (IC50 = 6.21 μM) and microsomes (IC50 = 7.68 μM). Synaptosomal Ca^{2+}-ATPase was also inhibited by DCBP (IC50 = 81 μM). These results indicate that at concentrations where cytotoxicity is not observed, DCBP increases intracellular free Ca^{2+} and inhibits Ca^{2+} sequestration by intracellular organelles as well as Ca^{2+} pumps in synaptic plasma membrane. Since DCBP is very potent in inhibiting these events, the possible neurotoxicity of this PCB might be associated with perturbations in cellular calcium homeostasis. (Supported by EPA award CR 818550-01-0).

673.12

EFFECTS OF NEONATAL MONOSODIUM GLUTAMATE ON PEPTIDE mRNA EXPRESSION IN RAT ENDOCRINE AND VISUAL CNS STRUCTURES. S. E. Bachus*, W. S. Young, III, & M. Palkovits. Lab of Cell Biology, NIMH, Bethesda, MD 20892.

Since we found that electrolytic arcuate-premammillary lesions modulate levels of mRNA for galanin (GAL), vasopressin (VP), angiotensin (AII) and cholecystokinin (CCK) in hypothalamic magnocellular neurons (Neurosci. Abst. 17:1186), we investigated whether arcuate lesions caused by neonatal monosodium glutamate (nMSG) produce similar effects. In addition, because nMSG causes optic nerve atrophy, we measured CCK and substance P (SP) mRNA levels in the visual system structures, the dorsal nucleus of the lateral geniculate (DLG) and superficial layers of the superior colliculus (SCs). Rat pups received 4 mg/kg MSG, or 0.9% saline, s.c., on days 0, 2, 4, 6 and 8, and were sacrificed on day 35. Peptide mRNAs were measured with hybridization histochemistry using ^{35}S-oligodeoxynucleotide probes (Meth. Enzym. 169:702).

Arcuate levels of mRNAs for neuropeptide Y (NPY), GAL, and neurokinin B were depleted by nMSG, and levels for proopiomelanocortin (POMC) were reduced to 30% of control ($p<.0005$). (Similar results using immunohistochemistry for NPY, GAL and POMC gene products were reported by Meister et al., Exp. Brain Res. 76:343). GAL mRNA was doubled in the paraventricular nucleus ($p<.0005$) and increased by 17% in the supraoptic nucleus ($p<.05$) by nMSG. Magnocellular CCK, VP, and AII mRNA levels were not affected. In the SCs, while the density of label was comparable, the area was smaller after nMSG, so that the total label was reduced to 30% of control for SP ($p<.001$) and to 28% for CCK ($p<.001$). In the DLG, nMSG increased the density of CCK mRNA by 21% ($p<.005$), but because the area was reduced, the total label was reduced to 66% of control ($p<.001$). Thus, in addition to alterations of peptide gene expression in hypothalamic nuclei, the nMSG-induced damage to optic fibers affected peptide gene expression in subcortical visual areas.

673.13

DIMETHYL SULFIDE AS A PHARMACOLOGICAL ANALOGUE FOR HYDROGEN SULFIDE. R.J. Reiffenstein*, A.F. Almeida and S.H. Roth. Department of Pharmacology, University of Alberta, Edmonton, AB, Canada T6G 2H7, and University of Calgary, Calgary, AB, Canada T2N 4N1.

Despite the extensive use of rats to study the toxic effects of hydrogen sulfide, deficiencies exist. Coma, which is a common symptom of humans exposed to hydrogen sulfide is not often seen in rats because the dose curves for coma and lethality are very steep. Though other animal models are used these problems remain. In an attempt to resolve them, a comparison of hydrogen sulfide has been made with dimethyl sulfide (DMS) which is chemically similar but less potent and can produce coma. To reduce the animals used, the cumulative method of Reed and Muench (Am.J.Hyg. 27, 493,1938) was used to derive ED50 and LD50 values.

The intraperitoneal injection of DMS elicits a comatose state in Sprague Dawley rats (200-300g body weight) that is dose dependent with an ED50 of 813 mg/kg. The period coma lasted less than 25 minutes and the animals appeared to recover fully. Animals that died did so several hours later of relatively abrupt respiratory failure. Using a 24-hour post injection time mark, the LD50 for DMS is 537 mg/kg showing a decreased toxicity of greater than thirty fold as compared with hydrogen sulfide. It is noted that the LD50 is lower than the ED50 under these conditions.

Agents purported to have antidotal effects for sulfide poisoning, namely dithiothreitol (DTT) and sodium nitrite were studied for their ability to reverse coma and to prevent death. Sodium nitrite, 75 mg/kg administered at onset of coma decreases duration of coma, but DTT (50mg/kg) had no effect whether administered at onset of coma or 20 minutes prior to DMS. Pretreatment with DTT caused a small reduction in lethality (LD50-661 mg/kg). Supported by Medical Research Council of Canada.

673.14

N-BUTYL BENZENESULPHONAMIDE TOXICITY IN PRIMARY NEURONAL CULTURES. I. Wakayama, V.R. Nerurkar and R.M. Garruto*. Laboratory of Central Nervous System Studies, NINDS, National Institutes of Health, Bethesda, MD 20892

We previously demonstrated that N-butyl benzenesulphonamide (NBBS) is neurotoxic both *in vivo* and *in vitro*. *In vivo*, NBBS-inoculated rabbits develop a specific myelopathy with axonal and dendritic changes in spinal motor neurons after monthly intracisternal inoculations of a 100 µg dose for up to 12 months. *In vitro*, using continuous cell lines of neuronal (Neuro-2a) and glial (C6-glioma) origin, we demonstrated that, at a concentration of 10 µM and 50 µM NBBS respectively, cell growth and DNA synthesis were inhibited.

To further determine the mechanisms of NBBS neurotoxicity, particularly on cytoskeletal derangement, we studied the toxicity of NBBS on primary hippocampal cultures. Ten days after plating when neurofilament and other cytoskeletal proteins were fully expressed in the perikarya and in neurites, dissociated mouse hippocampal neurons were exposed to NBBS at different concentrations (10 µM, 25 µM and 50 µM) for 2 to 4 days. Immunostaining using antibodies against phosphorylated neurofilament and α-tubulin revealed that staining intensity of both neurofilament and α-tubulin was markedly reduced in distal neurites, while staining in the perikarya and proximal neurites was preserved. Neuritic outgrowth was also inhibited in NBBS-treated neurons compared to untreated neurons. These findings suggest that cytoskeletal derangement and redistribution in neurites begin distally and eventually lead to structural changes and neuronal death.

NEUROTOXICITY: METALS

674.1

EARLY PRENATAL AND POSTNATAL LEAD EXPOSURE INDUCES ASTROGLIAL REACTIVITY. A. Selvin-Testa, C.F. Loidl, J.J. López and J. Pecci-Saavedra*. Inst. de Biología Celular. F. de Medicina, U.B.A. Buenos Aires, 1121, R. Argentina.

In previous results we observed that 3 months of lead exposure -started when pups were 7 days old- caused hypertrophic astrocytes in the rat hippocampus. The aim of the present study was to compare the astroglial response in the cerebellum and the hippocampus, two regions with late postnatal development. Experiments were done with rats whose parents were lead intoxicated during 4 months (1g% lead acetate solution -*subclinical dose*). Lead administration was continued during conception, gestation, the early postnatal life, and following weaning, for 3 months. Immunohistochemistry methods were used for identification of the cytoskeletal intermediate filaments, GFAP and vimentin. Reactive vimentin and GFAP immunostaining astrocytes were observed in the different layers of the hippocampus. They showed an increase in size, changes in shape, and a great increment in number and in the immunoreaction. Besides, vimentin-hypertrophic-hyperplastic astrocytes only appeared in the cerebellar white matter. Lead exposure during the period of rapid brain growth could modify the complex neuron-glia interaction altering the neuronal microenvironment and so disturbing the brain function. (Work supported by the CONICET and U.B.A, Agentina).

674.2

LEAD POISONING AND DMSA: EFFICACY OF CHELATION TO ATTENUATE LEAD-INDUCED BEHAVIORAL CHANGES. P. W. Stewart, J. D Goldblatt, M. E. Lennon, R. G. Burright, P. J. Donovick*. Psychol. Dept., S.U.N.Y. Binghamton, Binghamton, N.Y. 13902.

We investigated the effect of 2,3 dimercaptosuccinic acid (DMSA), a relatively new chelating agent for lead poisoning, on activity, aggression and blood-lead levels in non-lead and lead-exposed male, young adult, Binghamton Heterogeneous Stock mice. The effect of water temperature (5C - extreme stress vs. 25C - moderate stress) in a water swim test was examined to determine how it might interact with lead and DMSA to affect behavior. Literature suggests lead-induced behavioral changes more reliably occur in aversive conditions, we therefore hypothesized that lead exposure would result in hyperactivity in mice relative to controls, but only in the extreme stress condition. Lead-exposed mice were also predicted to be more aggressive in aggression tests relative to controls - a prediction consistent with clinical literature associating lead exposure with aggression in young children. DMSA was expected to reduce blood-lead, and thus might be expected to attenuate lead induced behavioral changes. Results indicated that 5C water stress caused all animals, regardless of lead or chelation status, to reach an apparent behavioral asymptote (ceiling) in the activity chamber. Surprisingly, the effect of lead and DMSA on swim activity actually appeared additive in the 25C water stress chamber. Isolation-induced intermale aggression testing showed that lead-exposed pairs of mice were less aggressive than control pairs. However, a three-way interaction indicated that the non-lead, non-chelation group had significantly elevated aggression, but only for those pairs with a history of exposure to the 25C water swim condition. Although DMSA failed to attenuate lead-induced behavioral changes in this study, results should be weighed relative to the clinical benefits that DMSA reportedly provides.

674.3

DIFFERENTIAL EFFECTS OF EARLY CHRONIC LEAD (Pb) EXPOSURE ON NEONATAL RAT BRAIN NMDA, PCP AND ADENOSINE A_1 RECEPTORS. M.F. Jarvis*, W. J. Brooks., J. C. Leboutillier, J. N. Nobrega, T. L. Petit. Dept. of Psychology, Univ. of Toronto, Ontario and Rhone-Poulenc Rorer Cent. Res. Collegeville, PA 19426.

In order to further characterize the neurotoxic effects of chronic Pb exposure, autoradiographic techniques were used to examine the effects of early Pb exposure on NMDA, PCP, and adenosine A_1 receptors in neonatal rat brain. Rat pups nursed mothers exposed to 4% $PbCO_3$ in thier diet, or a Na_2CO_3 control diet from postnatal day 1 (P1) to P25. At P25, rats were sacrificed and the distributions of [^{3}H]CGP 39653 binding to NMDA receptors, [^{3}H]TCP to PCP receptiors and [^{3}H]CHA to adenosine A_1 receptors in brain were assessed. Chronic Pb exposure was found to produce an increase (+ 20-30%) in the density of ligand binding to NMDA and PCP receptors in specific regions of the hippocampus. However, [^{3}H]CHA binding to A_1 receptors was found to be generally decreased (20-60%) throughout the neonatal rat brain. The present observations are consistent with recent behavioral and electrophysiological data indicating that Pb may directly alter the NMDA/PCP receptor complex in the rat forebrain and may also suggest a direct effect on inhibitory neuromodulation via adenosine A_1 receptors.

674.4

LEAD ELEVATES THE INTRACELLULAR CALCIUM ION CONCENTRATION OF NEURONS: A F-19 NUCLEAR MAGNETIC RESONANCE STUDY OF NEURONS IN MICROCARRIER CULTURE. F.A.X.Schanne, D.K.Batter* and J.A.Kessler. Depts. of Pediatrics, Pathology, Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, NY 10461

Exposure to lead (Pb) early in development leads to significant neurobehavioral deficits in humans and animals. The objective of this study was to determine the effect of Pb on the intracellular calcium ion concentration $[Ca^{2+}]_i$ of developing neurons. Neurons were isolated from the cortex and hippocampus of fetal rat brains on day 18 of gestation. Neurons were attached to Cytodex #3 microcarriers and maintained in Eagle's Minimal Essential Medium supplemented with 10% fetal calf serum at 37°C, 5% CO_2. Neurons were loaded with the divalent cation indicator 1,2-bis(2-amino-5-fluorophenoxy)ethane-N,N,N',N'-tetraacetic acid (5F-BAPTA) and the $[Ca^{2+}]_i$ was measured using ^{19}F NMR. This method provides for the simultaneous identification and measurement of Ca^{2+} and a variety of heavy metals, including Pb^{2+}. The untreated or baseline $[Ca^{2+}]_i$ of neurons measure on days 4 to 6 *in vitro* ranged from 128 to 184 nM. Upon treatment with Pb^{2+} (5μM) the $[Ca^{2+}]_i$ rose 50 to 60% within 1 hr in cortical neurons and greater than 80% in hippocampal neurons. These data indicate that Pb^{2+} elevates $[Ca^{2+}]_i$ in developing neurons in culture. Given the importance of $[Ca^{2+}]_i$ in the expression of neuronal structure and activity we propose that these changes in $[Ca^{2+}]_i$ may contribute to Pb-induced alterations in neural morphology, plasticity and responses to neurotransmitters.

674.5

SELECTIVE TOXICITY TO CENTRAL NORADRENERGIC NERVOUS SYSTEM IN POSTNATALLY LEAD EXPOSED RATS. S.G. Song, D.O. Seo, J.H. Cheong, C.Y. Shin, M-U. Choi*° and K.H. Ko. Department of Pharmacology, College of Pharmacy and Department of Chemistry, College of Natural Science, Seoul National University, Seoul 151, Korea.

Possibility whether postnatal lead ingestion can cause selective toxicity to central noradrenergic nervous system in rats was tested. Three groups of wistar rats; 1) Control, 2) Low dose and 3) High dose groups, were prepared. Right after parturition from dams rat pups received drinking water containing either 0% (control), 0.05% (low dose) or 0.2% (high dose) of lead acetate. At 2, 4, 6 and 8 weeks of age, dopamine β-hydroxylase (DBH) activity and Na-K ATPase activity were measured in 5 areas of rat brain; Telencephalon, Diencephalon, Midbrain, Pons/Medulla and Cerebellum. DBH activities were assayed by modified method of Coyle and Axelrod (1972) using S-adenosyl-[^{14}C]-methionine as substrate. DBH activity was determined as a criterion of lead poisoning to central noradrenergic nervous system and ATPase activity as a criterion of nonspecific lead poisoning to any kinds of tissues. In lead exposed rats, DBH activities were higher but Na-K ATPase activities were lower than those observed in age-matched control animals. Selective toxicity of lead poisoning to central noradrenergic nervous system was evaluated by the changes of DBH activities without concomitant changes of ATPase activities. Brain areas where selective toxicity of lead seems to be induced were telencephalon and pons/medulla (2 weeks of age) and telencephalon, diencephalon and pons/medulla (4 weeks of age), midbrain and pons/medulla (6 weeks of age), cerebellum (8 weeks of age) in low dose group, and midbrain (6 weeks of age), cerebellum (8 weeks of age) in high dose group.

674.6

LEAD-INDUCED ALTERATIONS IN CYTOSOLIC PROTEINS BY CULTURED ASTROCYTES EXPOSED TO LEAD. L. Shemancik, D. Cory-Slechta, B. Weiss,* and J. Finkelstein. Env. Health Sci. Center and Strong Children's Res. Center, Univ. of Rochester, Rochester, NY 14642

Astroglial cells accumulate Pb and are less sensitive to detrimental effects than neurons. A possible mechanism by which astrocytes adapt to Pb may be upregulating the expression of particular genes. We have previously demonstrated that exogenously added Pb resulted in enhanced synthesis of a 23kD acidic cytosolic protein by astrocytes. To begin to address the question of the mechanisms underlying the regulation of the 23kd protein by Pb, astrocytes were cultured in actinomycin D prior to the addition of the metal. Astrocytes, both pretreated or untreated, were exposed to 0, 5, and 50μM Pb acetate for 24 hours and pulse-labeled [^{3}H]-leucine. Proteins were separated by SDS-PAGE and subsequently analyzed by fluorography. Treatment with actinomycin D precluded synthesis of the 23kD protein. The differences in size and charge of the cytosolic proteins in Pb-exposed and untreated astrocytes were futher characterized by two-dimensional electrophoresis. After a 24 hour treatment period with 0 or 50μM Pb acetate, cells were pulse-labeled and cytosolic proteins were analyzed by two-dimensional electrophoresis and fluorography. The 23kD protein was resolved into 7 different charged species in Pb treated astrocytes. The untreated control revealed faint but definite spots corresponding to all but the most acidic of the 23kD proteins. To determine the relative abundance of the various proteins in Pb-treated and untreated astrocytes, cytosolic proteins were also analyzed by silver stain. Two spots corresponding to the 23kD proteins were notably more abundant in Pb-treated cells and an acidic trail of proteins ranging from 5.2-5.9pI was apparent in the same molecular weight region, thereby suggesting that the 23kD protein may accumulate and undergo post-translational modifications in response to Pb. These results suggest that Pb may affect both transcriptional and translational events in astrocytes.

674.7

COMPARISON OF FE(III) AND PB(II) SIGNATURES IN CULTURED ASTROGLIA: A NEW ASSAY FOR SCREENING NEUROTOXINS. **M. E. Legare, W. H. Hanneman, R. Barhoumi, R. C. Burghardt, and E. Tiffany-Castiglioni*. Dept. Vet. Anat. and Public Health, Texas A&M University, College Station, TX 77843.**

A battery of in vitro assays was developed to detect the presence of neurotoxic metals, particularly Pb, in cultured astroglia. In order to produce such a battery, it was necessary to distinguish unique neurotoxic effects of Pb (a Pb signature) from the toxic effects of other compounds. The Pb signature we obtained was compared to the signature produced in astroglia by the epileptogen Fe(III). Cellular responses to metal exposure were quantitated by the use of vital fluorescent probes and interactive laser cytometry. We found distinct signatures for Pb and Fe. Whereas Pb stimulated an increase in intracellular glutathione content on day 6 of treatment, Fe had no effect. Furthermore, cell-cell communication via gap junctions, which was unaffected by Pb treatment, was increased in Fe-treated cells. In addition mitochondrial membrane potential was decreased in Pb treated cells but increased in Fe-treated astroglia. We are also comparing metal effects on Ca metabolism.

674.8

ONTOGENETIC ALTERATIONS OF ODC ACTIVITY IN DEVELOPING BRAIN REGIONS AFTER POSTNATAL EXPOSURE TO LEAD-ACETATE. N.H. Zawia*and G. J. Harry, DART/STB/NIEHS, P.O. Box 12233, RTP, NC 27709.

Ornithine decarboxylase (ODC), the rate limiting enzyme of the polyamine pathway, can serve as an important tool to examine the effects of toxicants on early developmental phases of the nervous system. ODC activity follows a distinct ontogenetic pattern in individual brain regions, peaking at periods of maximal cell proliferation. We have studied the effects of inorganic lead on characteristic developmental profiles of ODC activity in the cerebellum, neocortex and hippocampus. Two exposure models were used with Long Evans hooded rats: 1) lactational exposure via 0.2% lead-acetate in the drinking water of the dam from PND1 to PND20 (23 mg/kg daily dam dose); 2) daily dosing of pups by gavage (600 mg lead acetate/kg of body weight) from PND2 to PND5. Animals were killed on PND 5,10,15 and 20, and distinct brain regions were dissected, and then frozen at -70^{0}C until assayed for ODC activity. Lactational exposure to Pb-acetate resulted in a dramatic (2 fold) stimulation of cerebellar ODC activity which was maintained up to PND 10 but dropped to control levels by PND 15. This induction of ODC activity was only observed in the cerebellum. In contrast to animals receiving low doses of Pb-acetate (lactational exposure), animals on direct Pb-acetate dosing exhibited an inhibition of ODC activity in all the brain regions examined. This loss of activity is suspected to be associated with cell loss due to high lead dosing. These data suggest that low lead exposure can interfere with proliferative events in the postnatally developing cerebellum and suggest that lead may produce generalized perturbations in neuronal development.

674.9

HEMOGLOBIN NEUROTOXICITY IN CORTICAL CELL CULTURE IS ATTENUATED BY NIMODIPINE. R.F. Regan*, S.S. Panter, and J.R. Hess Letterman Army Institute of Research, Presidio of San Francisco, CA 94129-6800.

The neurotoxicity of human oxyhemoglobin (HbAo) was investigated in murine neuronal and glial cell cultures. A twenty-four hour exposure to 100.0 μM HbAo, approximately 1.0% of the hemoglobin concentration present in whole blood (calculated on the basis of heme concentration), resulted in death of over 80% of neurons. Glia were not injured. Neuronal death was blocked by the ferric iron chelator deferoxamine (10.0 μM). Nimodipine was neuroprotective in a concentration-dependent fashion between 1.0-30.0 μM. Thirty micromolar nimodipine reduced cell injury about 90% compared with control untreated cultures. The related dihydropyridine nifedipine was also effective but was significantly less potent. Neuronal death was reduced about 50% by 100.0 μM nifedipine. The benzothiazipine calcium channel antagonist diltiazem, however, was ineffective at its maximum non-toxic concentration (30.0 μM). Both nifedipine and nimodipine significantly inhibited iron-dependent brain lipid oxidation, as measured by the generation of thiobarbituric acid reactive substances; however, nimodipine was a significantly less potent inhibitor of lipid oxidation than nifedipine. These observations suggest that hemoglobin is a potent neurotoxin in cortical cell culture. This neurotoxicity is dependent on iron and may be mediated, in part, by mechanisms sensitive to dihydropyridines.

674.10

Alteration in the ^{3}H dopamine uptake by synaptosomes exposed to ascorbic acid and Fe^{2+} is associated with a decrease in membrane fluidity and α-tocopherol level. Prevention by a Ginkgo biloba extract (EGb 761). C.RAMASSAMY*[c], J.PINCEMAIL[a], C.DEBY[a], Y.CHRISTEN[b] and J.COSTENTIN[c] [a] Centre Pathologie de l'Oxygène 4000 Sart-Tilman, Belgium. [b] IPSEN 30 rue Cambronne 75015 Paris cedex 15, France. [c] Neuropsycho pharmacologie U.R.A. 1170 C.N.R.S. 76803 St-Etienne Rouvray, France.

After 1h incubation in a Krebs Ringer medium (95% O2, 5%CO2) synaptosomes prepared from mice striata or cortex loss their ability to uptake respectively ^{3}H dopamine or ^{3}H serotonin. This effect was depending on the simultaneous presence of ascorbic acid (from 1μM) and Fe^{2+}(1μM). This decrease appeared as a consequence of a lipid peroxidation induced by the couple ascorbic acid/Fe^{2+} (Asc/Fe^{2+}) : it was associated with a decrease (≈70%) in the level of α-tocopherol (measured by HPLC), which constitutes an index of lipoperoxidation. It was also associated with a decrease in membrane fluidity (measured by fluorescence polarization of the probe 1,6-diphenyl-1,3,5-hexatriene) that might also result from a lipoperoxidation process. In the presence of EGb 761 (10μg/ml) or quercetin (which structure looks like that of some components present in the EGb 761 extract) all these alterations were completely prevented. We conclude that the synaptosomal lipoperoxidation elicited by (Asc/Fe^{2+}) through a decrease in membrane fluidity alter the activity of the amine uptake complexes. These alterations were prevented by EGb 761 which operated either by its free radical scavengers or its ferrous ions chelating properties.

674.11

THE EFFECTS OF VARIOUS LEVELS OF ASCORBIC ACID ON THE RESPONSE OF THE ODS RAT TO TRIMETHYLTIN. **A.W. Bannon, A.J. Verlangieri, and M.C. Wilson*. University of Mississippi, Dept. of Pharmacology, School of Pharmacy, University, MS 38677.**

Trimethyltin (TMT) is an organotin that can produce specific damage to the hippocampus. Behavioral deficits (e.g., memory) observed following TMT administration are often correlated with damage to the hippocampus. The influence of ascorbic acid (AA) levels on the response to TMT was investigated in ODS rats. Like man, this strain of rat does not synthesize AA. Rats were maintained on three levels of ascorbic acid: (1) 0.1 mg/ml (Low AA); (2) 0.2 mg/ml (Basal AA); and (3) 1.0 mg/ml (High AA). Following a determination of baseline performance in a radial arm maze (RAM) task and measuring locomotor activity, all rats received a single oral dose of 7.5 mg/kg TMT chloride. The rats were then allowed a 14-day recovery period. After this period, rats were retested in the RAM and activity levels were reevaluated. By the end of baseline testing in the RAM, performances of all AA groups were similar. During baseline, the mean activity score of the High AA group was significantly lower than the other groups. Following TMT dosing, fewer acute effects of TMT (e.g., aggressiveness) were observed in the Low AA group, as compared to the Basal and High AA groups. As for RAM testing following TMT, all groups were affected, with the greatest initial deficits being observed in the High AA group. Also, compared to baseline locomotor activity, the High AA group displayed a significant increase in locomotor activity. Significant group differences were not found in the histological data, but the trend was that rats maintained on "high" levels of AA exhibited more hippocampal damage than rats maintained on "low" levels of AA. This trend was consistent with the behavioral results. (Supported in part by the Res. Inst. Pharm. Sci.)

674.12

HYDERGINE TREATMENT REDUCES THE ENHANCED CHOLINESTERASE STAINING OBSERVED IN THE DENTATE GYRUS OF TRIMETHYLTIN EXPOSED RATS R.L. Cannon*, R.C. Hamdy +, and M.L. Woodruff Depts. of Anat., and Int. Med. +, J.H. Quillen Sch. of Med. East Tenn. St. Univ. Johnson City, TN 37614

Trimethyltin (TMT), is a neurotoxin that destroys cells in the hippocampus including those that receive cholinergic (ACh) input. This destruction models that found in Alzheimer's disease (AD). Increased acetylcholinesterase (AChE) staining and choline acetyltransferase (ChAT) activity in the outer molecular layer (OML) of the dentate gyrus have been reported in TMT treated rats. Hydergine (HYG) is used to attenuate some of the cognitive and behavioral deficits observed in conditions such as AD. HYG has been shown to effect the ACh system of the hippocampus. The purpose of this study was to examine the effect of HYG on (1) TMT induced hyperactivity and (2) on AChE staining in the hippocampus. Forty eight adult male Long-Evans rats were assigned to 8 groups. Six groups were orally gavaged with 6mg/kg TMT chloride, three of these received one of 3 doses of HYG, 0.2, 1.2 or 3.0 mg/kg in an ethanol vehicle by gavage daily for 28 days. One was given vehicle, one handled and one TMT group untouched. The remaining rats served as controls with half being gavaged with vehicle. The animals were then observed in an open field for ten minutes a day for 3 days. Line crossings and rearings were noted. The rats were sacrificed and their brains sectioned at 20 μm for thionin or 40 μm for AChE stain. Statistical analysis of behavior and densitometry results indicate HYG reduced open field activity, and AChE stain density. These findings suggest that Hydergine may be effective in treating damage by neurotoxins.

674.13

TRIMETHYLTIN-INDUCED c-fos EXPRESSION: ADOLESCENT vs NEONATAL RAT HIPPOCAMPUS. G.J. Harry* and N.H. Zawia, DART/STB, NIEHS, P.O. Box 1233, RTP, NC 27709.

In the adult animal, the immediate early genes (IEG) such as c-fos, c-jun, and other activity-dependent genes such as ornithine decarboxylase (ODC) are induced within minutes to hours in response to perturbations to the cellular environment. We have examined the induction of these genes following acute exposure to a known neurotoxicant, trimethyltin (TMT). TMT-induced alterations, morphological, physiological, and biochemical, in selective neuronal populations in the hippocampus have previously been observed within 16-24 hours following an acute exposure. Using Northern blot analysis, we have examined the induction of these genes within the hippocampus, frontal cortex, and the cerebellum at 0.5, 1.5, and 5 hours following acute exposure to TMT (4 mg/kg, s.c.). In the neonatal (PND 4) rat hippocampus, the basal expression of c-fos, c-jun, and ODC was high and appeared to be unaltered at all time points examined following TMT exposure. However, in the adolescent (PND 35) rat, TMT exposure produced a dramatic induction of c-fos mRNA in the hippocampus within half an hour. No changes in the expression of this gene were seen in the cerebellum and the frontal cortex at either age. Expression of actin mRNA was not altered by TMT exposure at either age. The age-dependent immediate early gene response to TMT may be due to a differential regulation of these genes, however, it may be representative of a localization to the hippocampal granule cells of the facia dentata which are relatively immature at PND 4. These results suggest that immediate early genes may be involved in the response of the brain to TMT in an age-dependent and region-specific manner.

674.14

NEURODEGENERATIVE EFFECTS OF TRIMETHYL TIN INVOLVES SEVERAL TRANSMITTERS AND NEUROTROPHINS. H. Lindström, C. Wetmore, J. Luthman*, E. Lindqvist and L. Olson. Department of Histology and Neurobiology, Karolinska Institute, 104 01 Stockholm, Sweden.

Acute exposure to trimethyl tin (TMT) in adult rats induces a behavioral syndrome which includes aggression, hyperirritability, tremor, convulsive episodes, hyperactivity and learning deficiencies. TMT (8 mg/kg i.p.) was injected in adult Sprague-Dawley male rats and previous findings of a selective hippocampal lesion were confirmed. Two weeks after TMT treatment a marked loss of CA3 pyramidal neurons was seen in hippocampus, concomitant with a pronounced gliosis as demonstrated by GFAP and vimentin immunoreactivity (IR). NGF mRNA was found to be expressed in neurons scattered throughout the pyramidal cell layer and in the hilar region of the dentate gyrus; BDNF protein IR and mRNA were abundantly expressed in pyramidal neurons as well as granule neurons of the dentate gyrus, as visualized using polyclonal antipeptide antibodies and oligonucleotide probes. Decreases in the BDNF protein IR and mRNA were seen after 4 hours and 2 weeks; NGF mRNA was decreased only following 2 weeks. A minor decrease in fibers with acetylcholinesterase IR was seen in the CA3 region. In addition, alterations in the GABAergic, tyrosine hydroxylase and serotonin IR were observed at various time points after the TMT injection (1, 2 or 12 weeks post-injection). Also, endogenous monoamine levels were affected, in particular regional 5-HT levels. Treatment with MK-801, a non-competitive NMDA receptor antagonist, tended to reduce the loss of acetylcholinesterase fibers and the gliosis. TMT has previously been shown to induce overflow of various neurotransmitters, including glutamate. It is therefore possible that at least one component of the neurodegenerative effect of TMT is similar to other neurodegenerative conditions that may involve increased extracellular glutamate levels and overstimulation of excitatory amino acid receptor. However, the present findings do not indicate that the NMDA receptor subtype is critically involved in the neurodegenerative actions of TMT.

674.15

IN VITRO NEUROTOXICOLOGY OF ORGANOTIN COMPOUNDS T.A. Thompson, S.M. Toggas, W.B. Severs* and M.L. Billingsley. Dept. of Pharmacology, Pennsylvania State University College of Medicine, Hershey, PA 17033

Organotin compounds induce neurotoxic changes; trimethyltin (TMT) causes selective patterns of neuronal destruction whereas triethyltin (TET) causes degeneration of myelin. In order to characterize mechanisms of TMT and TET toxicity, human SMS-KCNR neuroblastoma, HTB-14 glioma and mouse 3T3 fibroblast cell lines were exposed to graded concentrations of TET (0-200 μM), TMT (0-200 μM) and SnCl2 for 48 hrs. Cell viability was determined using the fluorescent dyes calcein-AM and ethidium homodimer. TMT caused a dose-related loss of cell viability in SMS-KCNR cells (LD50= 25 μM); HTB-14 and 3T3 cells were resistant to TMT. In contrast, TET was toxic to SMS-KCNR (LD50= 7 μM) and HTB-14 cells (LD50= 10 μM). All cells tested were resistant to SnCl2. Previous experiments coupling subtractive hybridization and molecular cloning demonstrate that a novel 88 residue protein, termed stannin, is expressed in TMT-sensitive cells. Immunoblot analysis indicated that SMS-KCNR cells and hippocampus expressed stannin, while HTB-14 cells gave a background signal, suggesting that stannin expression correlates with TMT sensitivity. Transfection experiments which cause stable heterologous expression of stannin in resistant cell lines are currently in progress.

674.16

MERCURY TOXICITY: BAND 3 PROTEIN MEDIATED TRANSPORT. K. Kiningham, E.J. Kasarskis*. Grad Ctr for Toxicology and Dept Neurology, VAMC and Univ. Kentucky, Lexington, KY 40536.

Inorganic mercury (Hg^{+2}) is a recognized cytotoxicant, however, the mechanism by which Hg^{+2} enters the cell has not been defined. Recently it has been reported that cations such as zinc, cadmium, and lithium are transported across cell membranes via the band 3 anion channel protein. Band 3 is a ubiquitous protein that mediates the exchange of anions, maintains acid-base balance, and provides a cytosolic binding site for glycolytic enzymes. By using erythrocytes as a model, we have shown that DIDS, a specific band 3 inhibitor, blocks Hg^{+2} uptake.

Erythrocytes were incubated at 37°C for 10 minutes in a solution containing 10 mM HEPES buffer, pH 7.4, ^{203}Hg, increasing concentrations of $HgCl_2$ (.01 μM to 100 μM) and a range of inhibitor concentrations (10 μM to 200 μM). Hg^{+2} uptake was inhibited in a dose-dependent manner with increasing concentrations of DIDS. At a concentration of 10 μM $HgCl_2$ and 200 μM DIDS, the percentage of ^{203}Hg associated with the cells was decreased by more than 50%. The results indicate that the band 3 protein is a transporter of Hg^{+2}, perhaps in the form of an anionic species such as $HgCl_4^{-2}$. The presence of band 3-like proteins in brain might represent the site through which essential and toxic elements enter cells in the central nervous system.

674.17

EFFECTS OF METHYLMERCURY ON RESPIRATORY ACTIVITY OF IMMATURE CEREBRAL CORTICAL NEURONS, CEREBELLAR GRANULE CELLS, PURKINJE CELLS, OLIGODENDROCYTES AND ASTROCYTES OF PRENATAL AND NEONATAL RAT IN-VITRO. S. Yee and B. H. Choi*. Div. of Neuropathology, Univ. of California, Irvine, Irvine, CA 92717

The sensitivity of the CNS to toxic effects of methylmercury (MeHg) has been well documented. However, the molecular events underlying cellular damage in various CNS cell types following MeHg poisoning remain unclear. To study the effects of MeHg on the respiratory activity of different CNS cell types, homogeneous cultures of astrocytes, oligodendrocytes, immature cerebral cortical neurons, cerebellar granule cells and Purkinje cells established from prenatal and neonatal rats were used to examine the oxygen uptake following exposures to various concentrations of MeHg (1-10 μM) in basic salt solution (MEM) using polarographic technique. Under control conditions, the respiratory rate of oligodendrocytes was relatively higher (6.70% O_2/sec/μg protein x 10^{-5}) than other cell types (5.45% O_2/sec/μg protein x 10^{-5}). MeHg caused significant reduction in the respiratory rate at all concentrations in all cell types within short periods of time. However, the most profound effect in the reduction of respiratory rate and of the time to reach cessation of the oxygen uptake was noted in oligodendrocytes, followed by cerebellar granule cells, immature cortical neurons, astrocytes and Purkinje cells, in successive order. Exposures to various concentrations of metal ions including tin and iron showed no significant effect in the respiratory rate. These results indicate that MeHg affects respiratory activity of the CNS cells in a specific but differential manner, and provide a significant insight into the molecular mechanism of MeHg neurotoxicity. (Supported by NIH grant ES 02928)

674.18

METHYLMERCURY-INDUCED DISRUPTION OF RAT HIPPOCAMPAL SYNAPTIC TRANSMISSION AND LONG-TERM POTENTIATION. **Y.Yuan and W.D. Atchison*.Dept. of Pharmacol./Toxicol. and Neurosci. Prgm. Michigan State University, E. Lansing, MI 48824.**

Using extracellular microelectrode recording techniques, population spikes (PS) and long-term potentiation (LTP) were recorded to examine the effects of the neurotoxicant methylmercury (MeHg) on synaptic transmission in isolated hippocampal slices. PSs were induced in the CA1 region by stimulating (0.25 Hz) schaffer collaterals and LTP was induced by applying a brief high-frequency stimulation (HFS, 15 trains of 4 stimuli at 100 Hz in 100ms intervals). MeHg was applied to slices acutely by perfusion with artificial cerebrospinal fluid (ACSF). At 20-500 μM, MeHg first increased PS amplitude by 20-50 % and then decreased and blocked the PS completely. Time to increase and time to block PS were both concentration-dependent. In the absence of MeHg, application of HFS increased PS amplitude by 50-100 %, an effect which lasted for at least 2 hours. With simultaneous application of 20-100 μM MeHg and HFS, the PS amplitude was increased further by 20-50 % based on the already elevated PS amplitude by HFS. Subsequently, the PS amplitude was reduced and finally blocked in a similar way to that produced by MeHg on PSs recorded without HFS. If MeHg (100μM) was applied for 20 min before HFS, PS amplitude was decreased below the control level. However, LTP was still induced by HFS, suggesting that MeHg may not affect induction of LTP even though it did suppress the maintenance of LTP. Reversibility of effects of MeHg on PS and LTP were examined by washing slices with MeHg-free ACSF for 60-90 min. No recovery or only partial recoveries were observed. Thus, bath application of MeHg caused a biphasic irreversible effect on central synaptic transmission, as well as long-lasting increased central synaptic transmission. **Supported by NIH grant ES03299 and RCDA ES00178 to WDA.**

674.19

CUMULATIVE DOSE RESPONSE FUNCTIONS FOR LEAD ACETATE, TRIETHYLLEAD, AND METHYLMERCURY IN THE RAT HIPPOCAMPAL SLICE. S.B. Fountain* and J.D. Rowan. Dept. of Psychology, Kent State Univ., Kent, OH 44242.

The present study assessed the effects of lead acetate (PbAc), triethyllead (TEL) chloride, and methylmercury (MeHg) chloride on rat hippocampal slice excitability using a cumulative dose response function (cDRF) procedure. Slices were prepared using standard techniques and maintained at the interface of a pool of artificial CSF and an O_2/CO_2 atmosphere. Stimulating and recording electrodes were positioned in the Schaffer collaterals and CA1 cell body layer, respectively. When a stable waveform was recorded, an input/output (I/O) profile was obtained as a baseline measure by administering a series of increasing stimulus intensities. Exposure was accomplished by switching from normal to agent-bearing medium reservoirs supplying the slice chamber. Tests of excitatory and inhibitory systems in area CA1 of the hippocampal slice were conducted using a paired-pulse technique (25 msec interpulse delay) every 5 min over a period of 2 hr postexposure. During the monitoring period, the cDRF procedure monitored changes in excitatory and inhibitory systems beginning with a low dose of an agent, then successively higher doses were introduced every 30 min. Slices were exposed to 0.1, 1, 10, and 100 μM PbAc, TEL, or MeHg in successive 30-min periods. Slices were exposed to only one agent. Significant suppression of excitability was observed at the 10 μM dose of TEL and at the 100 μM dose of PbAc and MeHg. In addition, MeHg produced significantly greater suppression than PbAc at the 100 μM dose. The results favor the view that the hippocampal slice preparation may prove to be a valid method of neurotoxicity screening. (Supported by the Johns Hopkins Center for Alternatives to Animal Testing.)

675.1

EFFECTS OF ALUMINUM ON CALCIUM SEQUESTRATION MECHANISMS IN 7 DAY AND ADULT RAT BRAIN. W.R. Mundy*, P.R.S. Kodavanti, V. Dulchinos and H.A. Tilson. Neurotoxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Aluminum (AL) is a neurotoxicant which acts on a number of targets in the central nervous system. Recent studies from our laboratory indicated that AL chloride interferes with Ca^{2+} homeostasis in the cerebellum. We have further examined the effect of AL on cellular Ca^{2+}-homeostasis in the developing and adult rat brain. ^{45}Ca-uptake was examined in mitochondria (M) and microsomes (ER), and Ca^{2+}-ATPase actvity examined in synaptosomes (SYN) isolated from the frontal cortex, hippocampus, and cerebellum of 7 day (developing) and 5 month (adult) male, Long-Evans rats. ^{45}Ca-uptake was greater in the hippocampus and cerebellum of adult rats compared to developing rats, but was similar in the cortex. AL (50-800 μM) inhibited ^{45}Ca-uptake in M and ER at both ages. Developing rats were more sensitive to AL inhibition of ^{45}Ca-uptake (IC_{50} 425-620 μM) compared to adult rats (IC_{50} 600-1100 μM) in M. AL inhibition of ^{45}Ca-uptake was similar for both ages in ER (IC_{50} 775-1500 μM). Ca^{2+}-ATPase activity was greater in SYN from all three brain regions in adult compared to developing rats. In contrast to ^{45}Ca-uptake, AL stimulated Ca^{2+}-ATPase activity. In the cortex and hippocampus, AL stimulated Ca^{2+}-ATPase activity to a greater extent (2-3 times) in developing rats than adult rats. However, AL-induced stimulation of Ca^{2+}-ATPase activity in the cerebellum was similar for both ages. These results suggest that some mechanisms of Ca^{2+}-homeostasis are not fully developed in the 7 day old rat, and that developing rats are differentially sensitive to the effects of AL compared to adults.

675.2

WIDESPREAD AUTOFLUORESCENT GRANULOMAS IN THE RABBIT CENTRAL NERVOUS SYSTEM (CNS) FOLLOWING INTRANASAL EXPOSURE TO A LIPOPHILIC ALUMINUM-CONTAINING COMPOUND (ALUMINUM FLAVONOL). R.N.Katz[1], P.F.Good[1], A.A.Kaputsin[2], A.Hsu[2], and D.P.Perl[1,2]* 1. Fishberg Research Center for Neurobiology, 2. Department of Pathology. Mt. Sinai Medical Center, New York, NY 10029.

We have previously shown that intranasal exposure to aluminum compounds may lead to uptake into the CNS along olfactory pathways (Perl and Good, Lancet 1:1028,1987). In those experiments it was noted that aluminum-organic compounds were more extensively distributed in the brain than $AlCl_3$. We now report that aluminum flavonol ($AlFl_3$) a lipophilic autofluorescent aluminum chelate compound, is widely distributed following intranasal exposure. A stable suspension of $Al(Fl)_3$ (75mM, 0.25 ml) was introduced directly into the nasal recess of 6 adult New Zealand albino rabbits, two animals each exposed for 2, 4 and 8 weeks. The rabbits remained asymptomatic throughout the experiment. In all animals, granulomas consisting of macrophages, lymphocytes and giant cells were encountered in pyriform cortex, basal forebrain, dorsal neocortex and hippocampus. Some animals also showed lesions in the midbrain and spinal cord. Neurofibrillary degeneration was not encountered in any of the animals although aluminum was detected in macrophages of the granulomas using laser microprobe analysis. We examined unstained tissue sections from the exposed animals using fluorescent microscopy and demonstrated prominent autofluorescence of the granulomas indicating the presence of $AlFl_3$. We conclude that aluminum compounds, particularly this unique lipophilic complex, may be rapidly taken up and transported throughout the CNS following intranasal exposure. These findings may have important implications to the potential risks posed by certain airborne neurotoxins. Supported by the American Health Assistance Foundation.

675.3

ALUMINUM-INDUCED INHIBITION OF CHOLINE UPTAKE INTO RAT BRAIN SYNAPTOSOMES. M.L. Caspers*, M.J. Fu and M.J. Dow. Dept. of Chemistry, Univ. of Detroit Mercy, Detroit, MI 48219.

Aluminum is a toxin which has been linked to several neurological disorders. The high affinity uptake of choline is the rate-limiting step in acetylcholine biosynthesis. Pretreatment of synaptosomes with $AlCl_3$ for 1 min prior to addition of [^{3}H]choline gives maximum inhibition of choline uptake. $AlCl_3$ can inhibit the high affinity uptake of choline in a dose-dependent manner, with 8.11% inhibition ($P<0.03$) noted at 1μM $AlCl_3$ and 15.69% ($P<0.02$) inhibition at 100μM $AlCl_3$. Double reciprocal plots of high affinity choline uptake versus [^{3}H]choline concentration indicate that 100μM $AlCl_3$ reduces the maximum rate of choline uptake from basal levels of 253 fmol/min/mg to 124 fmol/min/mg ($P<0.033$) whereas no change in the apparent K_M of the choline transporter is noted. Sodium citrate in ten-fold molar excess of $AlCl_3$ is unable to reverse this inhibition. Our data suggest that low concentrations of $AlCl_3$ can interfere with high affinity choline uptake and thus acetylcholine biosynthesis. (Supported by Alzheimer's Disease Research of the Amer. Health Assist. Found. and a gift from J. Rose.)

675.4

EXCESSIVE *IN VIVO* PHOSPHORYLATION OF NEUROFILAMENT SUBUNIT PROTEINS IN ALUMINUM NEUROTOXICITY. M.J. Strong*, D.M. Jakowec. Dept. Clin. Neurol. Sci., University of Western Ontario, Canada, N6A 5A5.

Aluminum-inoculated New Zealand white rabbits develop intraneuronal inclusions of phosphorylated neurofilament proteins (NF). To determine if these are aggregates of excessively phosphorylated NF, littermates of New Zealand white rabbits, age 5-6 weeks, were inoculated with either 1000, 750, 500, 250 or 100 μg $AlCl_3$ in 0.9% NaCl or 0.9% NaCl alone, killed 48 hours later and the NF-enriched fraction (Triton X-100 insoluble) from spinal cord isolated . Adjacent sections were formalin-fixed, serially sectioned and either silver stained or immunostained (SMI 31; 1,10,000). Time course dephosphorylation studies with alkaline phosphatase (E. Coli.; enzyme: substrate 1:50) and two dimensional electrophoresis were performed.

Inclusions did not occur with inoculums of 100 or 250 μg $AlCl_3$, but thereafter developed in a dosage-dependant manner. Immunoreactivity on Western blots to phosphorylated NF-H epitopes was abolished by 20 minutes incubation of purified NF with alkaline phosphatase in the control, 100 μg and 250 μg inoculums and by 30 minutes for 500 μg, but remained present at 90 minutes for both the 750 and 1000 μg inoculums. On two dimensional electrophoresis, the NF-H isolated from rabbits with spinal pathology demonstrated a loss of immunoreactivity to the poorly phosphorylated tail region (pI 6.1, @170 kda) and the transitional band to the higher molecular weight, highly phosphorylated isoform (pI 5.8, 210 kda). These results demonstrate a dose-dependant induction of NF inclusions in spinal motor neurons in which NF-H and NF-M subunit proteins are highly phosphorylated. Whether this reflects phosphorylation of all carboxy-terminal KSVP sequences, or of phosphorylation at novel sites, and whether this process is integral to the induction of inclusions is the subject of current experiments.

KEY WORD INDEX

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